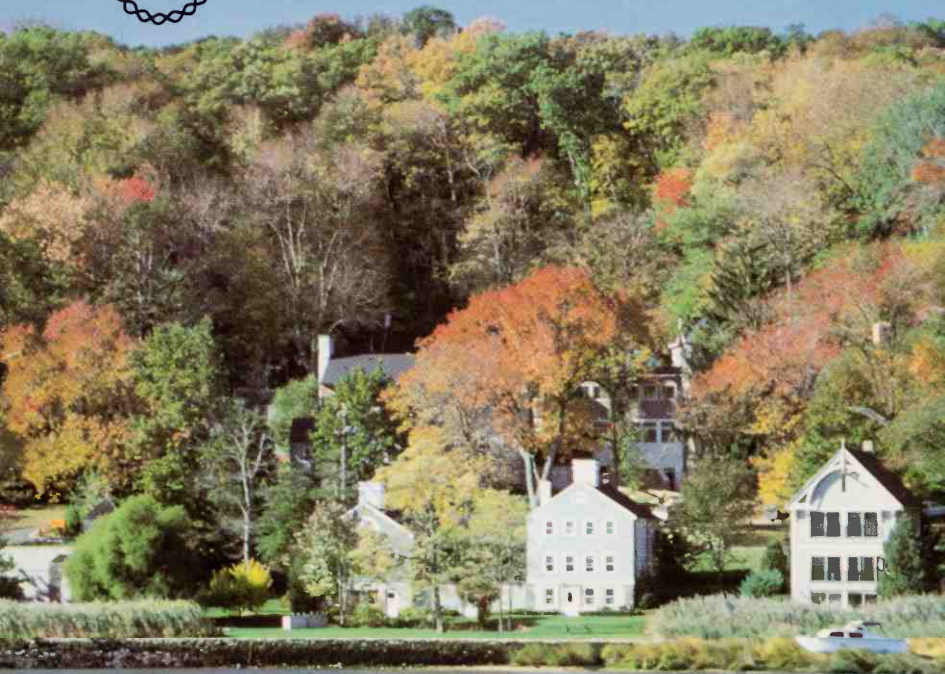
ANNUAL REPORT 1984

COLD SPRING HARBOR LABORATORY



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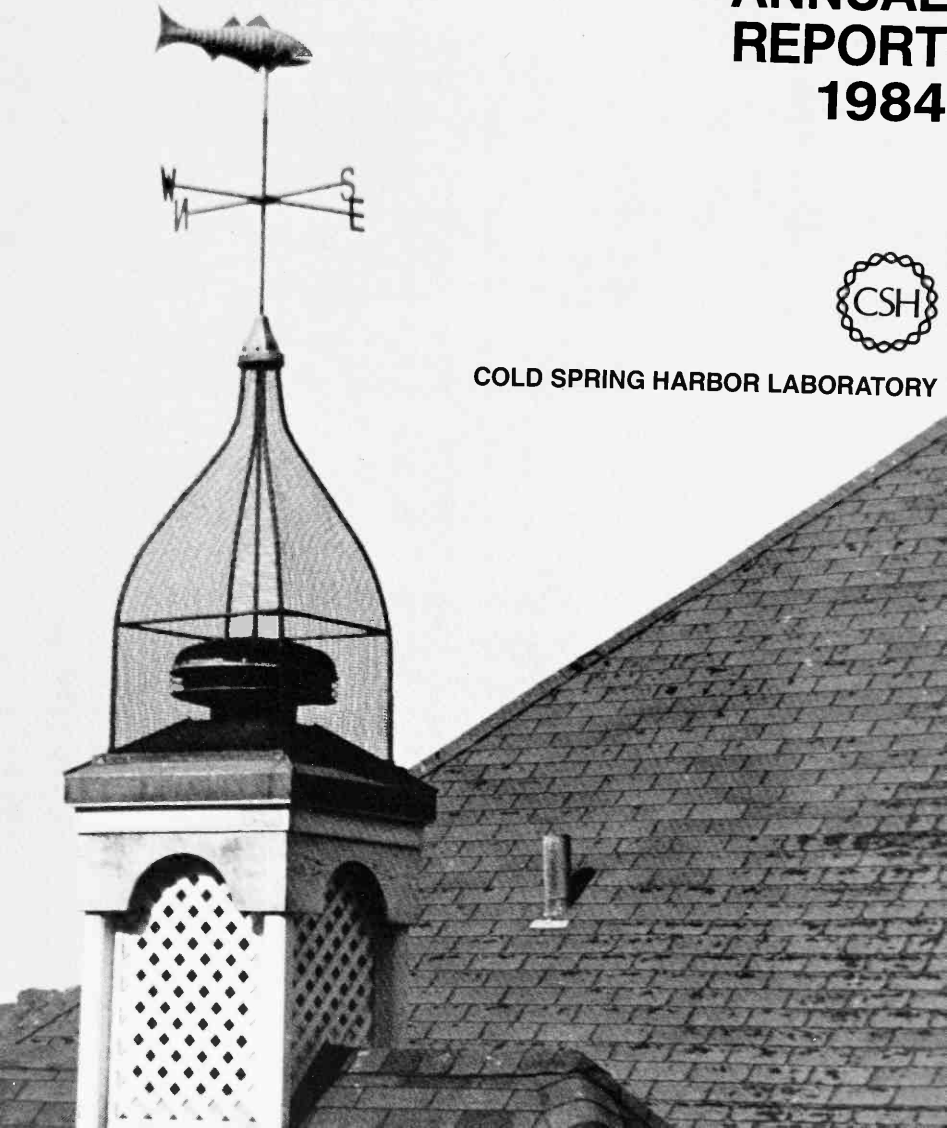

COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1984

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

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Cover photo by David Micklos

Recto: Weathervane atop Jones laboratory, which was erected in 1893 by the Laboratory's founder, John D. Jones.

Contents

(*Front row:*) E. Pulling, T.J. Knight, Mrs. C. Dolan, W.S. Page, Mrs. H. Harris, Jr., Mrs. S. Hatch; (*back row:*) B. Magasanik, R. Cummings, N.D. Zinder, M. Scharff, B. Clarkson, J. Klingenstein, T. Whipple, J.D. Watson, R. Webster, W.S. Robertson, S. Strickland

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The Laboratory is governed by a 25-member Board of Trustees which meets three or four times a year. Authority to act for the Board of Trustees between meetings is vested in the Executive Committee of the Board of Trustees. The Executive Committee is composed of the Officers of the Board plus any other members who may be elected to the Executive Committee by the Board of Trustees. Additional standing and ad hoc committees are appointed by the Board of Trustees to provide guidance and advice in specific areas of the Laboratory's operations.

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

Institutional Trustees were brought onto the Board during the 1960s—a difficult period when the Laboratory was undergoing reorganization as an independent entity. In addition to supplying scientific leadership to the governing body, participating institutions also provided emergency funds to help keep the Laboratory afloat during this crucial phase of development. Although participating institutions now give only token financial support, their Trustees continue to help steer the course of the Laboratory's scientific and administrative policies.

Also represented as participating institutions are the Wawepex Society and the Long Island Biological Association (LIBA). The Wawepex Society was formed in the mid-nineteenth century as a philanthropic arm of the Jones family, who supported the formation of the Laboratory. LIBA's origins began in 1890 when members of the local community became involved as friends and with membership on the Board of Managers of the Biological Station in Cold Spring Harbor, under the aegis of the Brooklyn Academy of Arts and Sciences. When the Brooklyn Academy withdrew its support of the Biological Station, community leaders, in 1924, organized their own association that actually administered the Laboratory until the Laboratory's reorganization as an independent unit in 1962. Today, LIBA remains a nonprofit organization organized under the Department of Social Welfare of the State of New York and represents a growing constituency of "friends of the Laboratory." Its 450 member families support the Laboratory through annual contributions and participation in fund drives to raise money for major construction projects.

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

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DIRECTOR'S REPORT

Much more than it realizes, our nation now faces the choice of how to deal with the great scientific revolutions whose consequences increasingly dominate the quality of human life. Whether we use or misuse this new science will determine whether our now old and venerable Western Civilization can be maintained or whether it will collapse into a form of modern feudalism marked by conformity to arbitrary ideals and a reluctance to question ideas and customs already in use. Past history provides no example of a civilization that did not eventually dig its own grave and great achievements like the Taj Mahal have occurred when the tide was already turning.

In particular, we must face the fact that the human brain is probably no more effective than it was at the time of the American Revolution, or for that matter during the reign of the Pharaohs. Yet the world's affairs move incredibly faster, with the implications of single human acts having infinitely greater long-term consequences. So we have to develop institutions that allow us to react better to the rapid changes of the moment without stifling our capacity for self-expression and innovation. Today, our most perplexing concerns arise from the consequences of modern physics and the development of nuclear weapons. Their use on even a modest scale would destroy the essence of Western Civilization, leaving whatever population remained so mesmerized by fear of a recurrence that it would be likely to trade off freedom as we know it today in the West for easy assurances, no matter how hollow, that the holocaust would never be repeated.

It is just 40 years since Hiroshima entered our vocabularies, and yet the immediate horror of the apocalypse that it epitomized is now, more often than not, replaced with an uneasy satisfaction that the threat of mutual annihilation will keep the peace. Initially I would have thought that the possession of just a few dozen atomic bombs and perhaps only one or two hydrogen bombs would scare off even the most fearless. Today, however, we find ourselves and our putative enemy, the Russians, with tens of thousands of nuclear weapons, all too many of which are encased in delivery vehicles that have reduced the time between decision to strike and indescribable destruction to mere minutes. So as each year passes, the chance of our respective military establishments knowing how to act and react during any nuclear exchange becomes more and more remote. But like all too many cancers, we know not how to remove the nuclear triggers from our lives.

It is thus natural to want to believe that modern science, having first given us nuclear weapons, would someday be able to provide the magic bullets that would flick them off course to explode harmlessly in the desolation of outer space. Today, in fact, we are told that given some 50–1000 billion dollars and the active participation of our nation's brightest, we might some 15–20 years from

now throw up a shield of powerful laser beams that would knock down the majority of intruders and let us retain the capacity to get back at any power so insane as to believe that we can be defeated. Conceivably, this modern form of Maginot Line might actually work as planned, giving us the capacity to retaliate against any evil force that would enslave us.

But before devoting such a large fraction of our national creative talent to this purpose, we must ask what level of contingency planning does this Star Wars response represent. In my opinion, we are reacting to a very, very low probability event, merely because if it were to happen, it would be so catastrophic. That the collective Russian leadership would actually ever believe that it could win a nuclear exchange, no matter how one-sided, strikes me as a most improbable event. Much more likely, though also a very improbable event, would be the failure of our nation's soundest bank, the Morgan Bank, followed by a complete collapse of the world's financial institutions. Yet today I suspect that not one treasurer of a major world corporation is devoting any of his real resources to the consequences of such a total banking collapse. Assuming the worst possible outcome is not the way successful individuals, banks, or nations run their daily lives. If they have the money, they take out just enough insurance (weapons) to survive a finitely calculable misfortune. Most certainly I would be thought silly, if not insane, to spend most of my funds on life insurance, leaving virtually no money to feed, house, educate, or medically protect my family.

The counter arguments, of course, can be made that as biologists we are too inexperienced to think in a military way and, even more important, that we are too small a lobby ever to influence major national priorities. So, no matter how we feel, we should sit tight, not unnecessarily make enemies in the wrong places, and leave to others the task of controlling the arms race. This is a position I cannot subscribe to as long as I feel that Star Wars money could be spent much more wisely to prevent tragedies that we know will actually happen.

Now that we at last have the means to finally understand the molecular nature of cancer, it would be at best perverse for us not to find the added monies necessary to deploy this knowledge for the human good within the lifetimes of those we love. Today, not 20 years distant, is the time to find out, say, the molecular defects that make a breast cell cancerous. Yet at this moment, even though cancer research is still better funded than all other parts of biology (but not physics!), there is not enough money for even our major laboratories to push ahead full speed. We are now half-throttled back, often spending more time worrying about money than our science. Instead, our recent great successes in the isolation and molecular dissection of oncogenes should be followed up by a national commitment, expressed by our President himself, to go forward with cancer research even faster. By so behaving as a nation, we would be living up to our greatest potential as humans, the capacity for accumulating the self-knowledge that will let us live even more meaningful lives.

Until such a turnabout in national priorities occurs, I fear that an ever-increasing malaise will spread through our lives as biologists. Up till now, our scientific system has worked well because we could treat our younger scientists with the respect they deserve. It is they who do most of the experiments that our more senior scientists talk about. So when they succeed, they should know that they, like their bosses at the moment, will soon be able to set up their own labs and test out their own ideas. Today, however, even our most talented younger scientists live with the fear that they will not get research grants of a size sufficient to let them do effective science. This situation cannot go on too much longer with-

out destroying the civility of the peer review system that up till now has worked so well to support our country's science. Without some form of major upward financial readjustment, it will be all too clear that one's success in getting a grant will be at the expense of another equally talented scientist's potential for doing science. So, hopefully, the greatly enlarged funds available to the Howard Hughes Medical Research Institute can very soon be used to help the essential fabric of biology and medical research temporarily survive while our nation reassesses our national priorities.

Even the Howard Hughes monies, however, will not be enough to let today's biologists do all the jobs of which they are now capable. I would guess that we shall need at least a fifty percent increase in total funds (toward 10 billion dollars) to generate the new facts that will let us not only handle current, immediate challenges like AIDS, but also respond to the fact that as our nation ages, we must produce the medical advances that will allow many more of our over-65 population to continue to work productively. What thus represents the self-interests of today's biologists and medical research communities should serve equally well the long-term interests of our nation as a whole.

We thus must not be alarmed that an ever-growing part of our national wealth must go not only to biological research, but also to the other forms of science whose futures look equally bright. Instead, we would have every reason to worry if we thought that our national scientific effort could stay constant while the growing impact of the world's vast population increase continues to present us with dilemmas, both practical and ethical, which at a minimum must be called daunting. In the long run, the effective limit for our scientific efforts is likely to be the educational aspirations of our population. With the world changing so fast, we have no reason to believe that our nation will remain a leading power if the peoples of other countries have a greater desire to learn the facts necessary to take up the new technologies of the future. There is no reason at all to believe that if we lose the will to move ahead, then other people will drop out at the same time.

Our only sensible course is to look as far into the future as possible, make intelligent guesses as to what will and will not happen, and act accordingly. Those nations who so arrange their affairs will have the best chance to have long-term destinies for the civilizations they promote.

HIGHLIGHTS OF THE YEAR

Using Yeast Cells to Understand Human Cancer

Over the past several years, Mike Wigler and his research group have focused much of their research on a group of three human *ras* genes, mutations in which lead to the cancerous phenotype. Each of these *ras* genes codes for one of a closely related set of small (189 amino acids) proteins that associate with the inner surfaces of the outer cytoplasmic membrane of cells and that bind GTP (GDP). These two characteristics suggested that the *ras* proteins might be functionally related to the so-called "G proteins" that mediate the transmission of signals from cell-surface receptors to the membrane-bound enzyme adenyl cyclase which converts ATP to cyclic AMP (cAMP), the key intracellular regulator. Because the *ras* proteins are present in relatively small amounts in cells, the effective purification and characterization of these proteins from cancer cells have proved very difficult, and the working out of how they function at the molecular level has not been straightforward.

Wigler's group has thus recently directed much of its attention to the two *RAS* proteins of yeast whose structures are very similar to those of mammalian cells and whose functioning is required for yeast cell growth. Most importantly, the powerful new recombinant DNA procedures for introducing genetic information into yeast allow a genetic analysis far more subtle than would ever be possible with mammalian cells. Through such experiments they have shown that the same amino acid replacements that generate cancer-producing *ras* proteins in mammalian cells lead to metabolically out-of-control yeast cells that overproduce cAMP. Further evidence favoring regulation of cAMP production by the *RAS* proteins comes from analysis of a special class of yeast mutants that compensate for the absence of functional *RAS* genes. These genes that effectively suppress the *RAS* phenotype have been shown by Matsumoto in Japan to code for the regulatory subunit of the protein kinase normally controlled by cAMP. Thus, mutants that lead to the absence of the regulatory subunit need not require cAMP for kinase activation. Hence, they have the ability to survive in the absence of the *RAS* proteins whose presence is normally required to make cAMP. These very important experiments confirm the now almost forgotten mania of some 12 years ago when the cAMP levels of many cancer cells were found to be abnormally high and the essence of cancer was proclaimed to be an excess of cAMP. Though this hypothesis clearly understates the true complexity of cancer, it is now clear that one must think about cAMP levels if we are to have a serious understanding of the metabolic uniqueness of many forms of cancer.

William R. Udry

The Loss of a Superb Administrator

It was with sadness that we bid farewell to Administrative Director Bill Udry at the end of the year. Bill came to us in late 1970, after serving as Chief Executive Officer of the Eye Research Institute of Bethesda, Maryland. A skillful administrator, he introduced professional management to our then financially uinsophisticated institution. His advice at many levels, from the receipt of the first Cancer Center grant in 1971, through the renovation of our once highly decrepit physical plant, to initiating construction on the Grace Auditorium, was a crucial factor in the development of this Laboratory over the past 15 years.

Bill's departure created a large gap in our administrative infrastructure, which was exacerbated by my own slow recovery from injuries sustained in a wretched car accident in December. It is a tribute to Bill that the senior administrative staff selected and trained during his tenure pitched in admirably to close the breach during this difficult period.

Changes in Our Scientific Staff

The Laboratory's great success in recent years has been built upon a steady influx of young scientists, to whom we give virtually unlimited intellectual freedom and few administrative duties. Under this system, the Laboratory has nurtured the early careers of many molecular biologists who have since risen to prominence. Although we benefit greatly from the intellectual fervor of youth, it is a sad fact that we cannot permanently absorb a large number of mature scientists.

Due to a lack of endowed research appointments, we often cannot offer the security—and, increasingly, the salaries—offered by major universities and companies. Thus, we are forced to contend with a high turnover in our staff. Though we have come to accept this fact of life, we are always saddened to face the

departure of long-valued staff members. This is tempered by the knowledge that they have accepted positions at some of the foremost universities and companies in the country.

Tom Broker and Louise Chow left their Senior Scientist positions in July to establish a new electron microscopy unit at the University of Rochester. Tom and Louise arrived in 1975, after serving post- and predoctoral periods, respectively, in Norm Davidson's lab at Caltech. Even before coming here, they had established themselves as leaders in the electron microscopy of nucleic acids. In 1977, their superb photos did much to first establish the phenomenon of RNA splicing.

Jeff Strathern left his Senior Scientist position in June to head up a research group at the Frederick Cancer Research Facility. Jeff arrived in 1977 from Ira Herskowitz's lab to organize, with Jim Hicks, the Delbrück (then Davenport) Yeast Group. In 1979, Jeff, Jim Hicks, Amar Klar, and Jim Broach firmly established yeast as a major system for studying development when they showed at the molecular level that mating-type switches in *Saccharomyces cerevisiae* are achieved through the movement of genetic cassettes.

Also leaving for the Frederick Cancer Research Facility was Steve Hughes. After serving a postdoctoral period with the Bishop-Varmus group in San Francisco, Steve came to the Laboratory in 1979 as a Senior Staff Investigator. Our first neurobiology appointment in 1978, Birgit Zipser left in December from her position as a Senior Staff Investigator for a visiting professorship at the National Institutes of Health. Senior Staff Investigator Ron McKay, a tumor virologist turned neurobiologist, left in July to accept an associate professorship at MIT. In 1980, Ron and Birgit helped revolutionize neurobiology when they developed cell fusion techniques to produce monoclonal antibodies against leech neural antigens.

After ten years at the Laboratory, Bill Topp accepted a position as President of Otisville Biotech, Inc., in upstate New York. Bill came to us in 1974 as a postdoctoral fellow in Bob Pollack's mammalian cell genetics unit and advanced in 1978 to Senior Staff Investigator working in James lab. Leaving in October for an assistant professorship at Princeton was Lee Silver, who came to us as a Senior Staff Investigator in 1981 to begin our effort in mouse embryology. After scoring a major coup by showing that two oncogenic functions are required to transform primary cells, Earl Ruley left his Senior Staff Investigator position for an assistant professorship appointment at MIT.

Accepting a position at the College of Physicians & Surgeons of Columbia University was Staff Investigator Mitch Goldfarb, who worked with Mike Wigler over the last four years and played a key role in isolating the *ras* oncogenes. Jim Lin, a Staff Investigator in McClintock who developed many of the first monoclonal antibodies against cytoskeletal proteins, left in February for an assistant professorship at Iowa State University. A native of Italy, Staff Investigator Mara Rossini accepted a position with the biotechnology firm SCLAVO in Siena. Returning to Exxon Research and Engineering Company in Clinton, New Jersey, following joint appointments at Cold Spring Harbor, were Susan Bonitz and Dan Lundell.

Visiting scientists Pei-Mao Lin and Guang-Yun Cai returned to the People's Republic of China, while Jin-Zhao Li took a position at Downstate Medical Center in Brooklyn. Kristen Frederiksen relocated to MIT with Ron McKay's research group.

Leaving after their postdoctoral periods were Bill Huse to become an assistant professor at Yale in Chuck Stevens' neurobiology program; Prasad Koka to the

Louise Chow and Tom Broker

Jeff Strathern

Center for Cancer Research at MIT; Nita Maihle to work with Steve Hughes at the Frederick Cancer Research Facility; Paul Mains to the Department of Molecular, Cellular and Developmental Biology at the University of Colorado; Kazuo Maruyama to MIT with Earl Ruley; Olof Sundin to the MRC Laboratory in Cambridge; Elizabeth (B.J.) Taparowski to continue postdoctoral work at the University of Virginia; Stuart Weisbrod to pursue a MBA degree at Columbia Business School; and Bob Weiss to the University of Utah.

Joining the Laboratory in 1984 as Senior Staff Investigator was Clive Slaughter. Clive came to us from the University of Texas Health Science Center at Dallas to reestablish a major focus in protein chemistry in the new Demerec extension. Pablo Scolnick, formerly a research associate at the University of Chicago, also set up a laboratory in the Demerec extension. Pablo studies gene expression and carotenoid biosynthesis in *Rhodopseudomonas* and tomatoes.

During the year, both Russell Malmberg and David Beach were promoted to Senior Staff Investigator from Staff Investigator positions. Russell studies the role of polyamines in tobacco flower development, and David's studies focus on cell-cycle control in fission yeast. Receiving promotions from Postdoctoral Fellow to Staff Investigator were Steve Dellaporta, Bob Franza, Doug Hanahan, Winship Herr, and Bill Welch. After completing his dissertation under our joint Ph.D. program in genetics with Stony Brook, Rich Kostriken began a postdoctoral fellowship with Mark Zoller.

Jim Feramisco Appointed to Rolling Five Position

In April, the Trustees of the Laboratory approved the promotion of Jim Feramisco to Senior Scientist in recognition of his work in focusing the efforts of McClintock lab on the cell biochemistry of oncogenic proteins. Jim came to Cold Spring Harbor in 1978 as a postdoctoral fellow in our Cell Biology Group. With Guenter Albrecht-Buehler's departure in 1982, Jim assumed leadership of the cell biology effort.

Our Senior Scientist rank carries with it a "rolling" five-year appointment, which means that the position will be funded continuously for a minimum of five years from any point in time. Our closest approximation to tenure for almost all our senior staff, the rolling five falls short of the job security offered by universities and industry. Thus, over the next several years, we must work hard to establish several tenured research professorships funded by hard money if we are to remain competitive for the top echelon of mature scientists.

Joe Sambrook F.R.S.

In March of this year, the Fellows of the Royal Society, meeting in London, elected into their Fellowship Joe Sambrook for his distinguished contributions to the understanding of tumor viruses at the molecular level. That Joe would receive this most coveted honor for a British scientist was long expected since his record of involvement in important discoveries about DNA tumor viruses dates back to the late 1960s when he was at the Salk Institute. Since coming here, he has attracted an extraordinarily talented group of co-workers who, with him, have made this Laboratory into a major, internationally recognized site for research on DNA tumor viruses. In doing so, they have given James lab a reputation for intellectual intensity of which we are most proud.

Joint Agreement with Monsanto to Study Mouse Genetics

In October, we signed a five-year, $2.1 million agreement with Monsanto Company to conduct a cooperative research program on the use of gene transfer in the study of gene expression during mammalian development. Under the terms of the agreement, Monsanto has the option for an exclusive license to develop for commercial use inventions arising from research covered by the agreement. Cold Spring Harbor will retain ownership of any patents and will receive royalties on sales resulting from technology developed here. Also, up to two Monsanto scientists will be trained for three-month periods at Cold Spring Harbor.

The program's main goal is to shed light on one of the great unanswered questions in modern biology: How does an organism develop from a single, undifferentiated cell into a complex system of many different types of cells, tissues, and organs? Current theory holds that differentiation is achieved by "tissue-specific" expression of genes—the selective turning on and off of specific genes in specific tissues during different phases of development.

One major approach to this problem is the microinjection of a solution of foreign DNA directly into fertilized mouse eggs. The eggs are then reinserted into the oviducts of pseudopregnant female mice and allowed to develop. Approximately six percent of all microinjected eggs give rise to live "transgenic" mice in which the injected DNA has become incorporated into the host DNA. Because the injected DNA can code for one or more novel proteins not found in normal mice, expression of the foreign DNA in various tissues of transgenic mice can be detected by looking for the telltale proteins.

Early in 1985, Doug Hanahan obtained a remarkable research result using this technique. Following microinjection of a fusion gene that linked the insulin promoter to the SV40 large-T antigen, he found that the T antigen is expressed only in the insulin-producing beta cells of the pancreas. Now that he has achieved correct tissue-specific expression, regulatory DNA sequences within the insulin promoters which are responsible for turning the transplanted genes on and off at the proper stages in development can be isolated.

Our current foray into the world of mouse genetics dates to the appointment of Lee Silver in 1981, who came to study the *T* locus. With the completion of the Harris building in 1982, we had a first-class facility for rearing mice, and in 1983 we introduced a course on the Molecular Embryology of the Mouse. The Laboratory's involvement in mouse genetics actually dates to the 1920s, when Drs. Clarence Little and Carlton MacDowell of the Carnegie Institution of Washington staff began to develop pure strains of inbred mice that have unusually high incidences of cancer. Little went on to found the Jackson Laboratory in Bar Harbor, Maine, now a major supplier of genetically pure strains of mice, while MacDowell continued work here, developing the important high leukemic mouse strain C58 black.

New Building Projects Continue Apace

Throughout this decade we have completed an average of one or more major construction projects per year: the Davenport restoration in 1980, Delbrück laboratory and Sammis Hall in 1981, the Harris Animal Facility in 1982, and the Demerec protein chemistry–nucleic acid addition in 1983. This trend has continued with the completion, in late fall, of the monoclonal antibody extension to James lab.

Construction costs for the extension were underwritten by grants of $425,000 from the National Cancer Institute, $400,000 from the Pew Memorial Trust, and $30,000 from the William Randolf Hearst Foundation. An additional grant of $30,000 from the Hearst Foundation, a $100,000 grant from the Fannie E. Rippel Foundation, $15,000 from the Samuel Freeman Charitable Trust, and $50,000 from various Long Island businesses went toward purchasing equipment.

The 6400-square-foot addition to the north end of James contains offices and two very spacious laboratories—one for Joe Sambrook's and Mary-Jane Gething's group and another for Ed Harlow—a cell fusion facility, and a large kitchen. Combined with the Harris animal house, we now have state-of-the-art facilities for the production of monoclonal antibodies needed to isolate cellular proteins and probe their structures. Already the James extension is fulfilling its role as a clearinghouse of monoclonal antibody technology for the entire campus; Ed Harlow provides expert technical advice, with Carol Schley available to perform cell fusions and screening procedures.

In May, we began the long-awaited construction of the Oliver and Lorraine Grace Auditorium. When completed in late fall 1985, this 360-seat facility will alleviate the long-standing problem of inadequate seating at our major meetings. With its massive dormers, richly textured brick and stucco exterior, and airy public spaces, the Grace Auditorium will be perhaps the finest meeting facility on Long Island.

Now planned as a multifunction building, the upper floor of the auditorium, besides containing the Lecture Hall, will house our Information Services Department, while on the lower floor will be our Meetings Office and a new computer center that will be the hub of an integrated network serving the entire scientific staff.

Delays in the Construction of Our Squash Court

Two years ago we thought we would soon be starting construction on both the Grace Auditorium and a squash court, to be located near the tennis court, which would give us a year-round facility in which our scientific staff, steadily growing in number, could benefit from short intervals of intense exercise. But as planning for the Grace Auditorium proceeded, we expanded its scope greatly through the decision to build a fully functional basement so as to give us the freedom to move within it the headquarters of our ever-expanding computer system. With this decision, the cost of the building rose to nearly $3.6 million, a sum far in excess of the earlier anticipated $2 million expenditure. In making the decision to start construction on the auditorium, we have had to commit virtually all the free funds in our treasury, leaving no monies to cover the estimated $200,000 cost of the squash court facility. We thus have had no choice but to postpone construction of the squash court until we find funds specifically donated for this effort. This may not be easy to do, since our neighbors know by now how much we also need new monies to expand our facilities for research and the holding of meetings and courses. Hopefully, we can persuade one of the now growing number of Cold Spring Harbor alumni who have helped form the biotechnology industry to donate his name along with the necessary number of stock certificates to bring this very much needed building into existence.

Revised Plans for New Facilities for Plant Molecular Biology

Initially, the most sensible way for us to expand our efforts in plant molecular biology appeared to be the construction of a joint laboratory-field station complex at Uplands Farm, the nearby former dairy farm that was owned by Mrs. George Nichols and whose land and buildings now belong, through her donation, to the Nature Conservancy. We thus entered into a contract with the Conservancy in the fall of 1984 to purchase from them some 10 acres of this land, on which are situated two houses and a 6000-square-foot garage/apartment building, for a price of $705,000. As our planning for this complex grew more precise, we realized that instead of saving money by using the existing garage for a new lab, the renovations required would actually cost more money and provide less suitable facilities than a new laboratory facility on our main Bungtown Road site. We thus now plan to use the Uplands Farm land and buildings exclusively for a Field Station on which we shall grow corn and erect a major new greenhouse specifically designed for corn. At the same time, the garage will be extensively renovated to provide areas for corn seed storage and examination, microscope facilities for chromosome analysis, plant growth chambers, and the housing of the necessary farm equipment. We are simultaneously making detailed plans for a 6000-square-foot addition to Delbrück laboratory, made possible by moving the nearby Firehouse (which now contains three apartments) to a site 80 feet to the north. Relocation of the Firehouse is now planned for late August 1985, with the construction of the Delbrück north addition scheduled to start in September and hopefully to be completed in the early summer of 1986. The projected cost of these various efforts now totals $2.3 million. Toward this sum, we have received a grant of some $700,000 from the National Science Foundation, $350,000 of foundation support, and have hope of another major donation being received before construction is to begin.

Already we have initiated work on the Uplands Farm garage, with occupancy scheduled by the end of summer 1985. Bids will soon be asked for the new greenhouse, with its completion date hopefully in time for a winter planting of the corn seeds that will result from the summer 1985 crop of Steve Dellaporta and his associates.

2-D Gel Technology Attracts Major Support

After years of hard work, Jim Garrel's computerized system for analyzing proteins separated by two-dimensional gel electrophoresis is attracting the attention it deserves. In June, the Laboratory granted an exclusive license to Protein Databases, Inc. (PDI), to develop this technology for commercial use. In return for the license, the Laboratory will receive $400,000 in research support from PDI, which has opened a facility on Oakwood Road in Huntington Station, N.Y.

Later in the year, we received a $2 million Biotechnology Resource grant from the National Institutes of Health to develop Cold Spring Harbor as a major center to train scientists in the uses of 2-D gel analysis in biomedical research. The Biotechnology Resource Center will be set up as a separate unit on the lower floor of the Grace Auditorium and will contain three MassComp computer workstations powerful enough to generate the complex graphics that are part of the analysis. Serving on the NIH advisory committee to the Center are Matthew Scharff, Barney Clarkson, Rich Roberts, Sidney Strickland, and James Schwartz.

Two-dimensional gel electrophoresis, first developed in 1975 by Patrick O'Farrell at the University of Colorado, can resolve more than 2000 different proteins in a single trial. Because of this exquisite sensitivity, 2-D gels contain far more information than can possibly be analyzed by visual observation alone. Jim Garrels, who came to the Laboratory in 1978 from the Salk Institute, was among the first to work out a practical, computerized method for analyzing the complex spot patterns generated by 2-D gels. One major goal of the Biotechnology Resource Center will be to establish complete protein databases for normal vs. transformed rat cell lines, yeast cell lines, and mouse embryo cell lines.

Our Summer Teaching Program Flourishes

Educational outreach—in the form of meetings and courses—has been a Cold Spring Harbor hallmark since the founding of the Biological Laboratory here in 1890. At that time, the harbor, mill ponds, and Long Island Sound were used as a natural laboratory in which to test Darwin's notions about how species evolve to exploit various environmental niches. Although our bucolic surroundings are no longer the object of experimentation, they do continue to inspire a serious, though informal, approach to science.

Our specialized courses in molecular genetics and neurobiology continue to attract three times as many applicants as there are available spaces. More than 400 applicants vied for 89 slots in six genetics courses, while 185 researchers applied for 91 positions in six neurobiology courses. Far and away the most popular courses, Molecular Cloning and Advanced Cloning drew a record 200 and 64 applicants, respectively.

The Neurobiology Teaching Program, which has functioned exceptionally smoothly under the direction of Sue Hockfield, was substantially boosted by the receipt in December of a $110,000 equipment grant from the McKnight Foundation. The grant will allow us to purchase centrifuges, incubators, and other equipment necessary to bring recombinant DNA techniques into our *Drosophila*, immunoglobin probes, and single channel methods courses. A Banbury meeting on Computational Neuroscience—sponsored by the Sloan Foundation—was so well received that in 1985 we are creating a new lecture course at Banbury to explore the topic.

A High-level Symposium on Genetic Recombination

The 49th Symposia on Quantitative Biology had as its major theme recombination at the DNA level, a topic that brought the return of many old friends to the Laboratory as well as first-time visits by many whom we hope will also come back many times. Serving as its major organizers were Amar Klar and Jeff Strathern who brought together a most distinguished and varied collection of speakers. Starting off the meeting on a high intellectual level was Bruce Alberts, who gave his view of how proteins work during DNA replication. Equally satisfying was Allen Campbell's final summary. Greatly assisting our standing-room-only audience was the use of a very large TV screen which often provided more than life size views of speakers faces to those watching the proceedings in the adjacent room of Bush Lecture Hall. As usual, our more than competent Meetings Office staff received much praise from our visitors for the kindly way in which they were introduced to the way of life here, a feature we must always maintain if we are to expect to keep our position as the leading meetings center for molecular biology.

The Robertson Research Fund Provides Our Main Source of Discretionary Research Funds

The gift from Mr. Charles S. Robertson in 1973 of an $8 million fund whose income should go exclusively to the support of research changed radically the nature of this Laboratory. In 1984, we used some $600,000 of this income to further help our newly created Mouse Embryology Group, to provide major items of equipment for the new north extension to James lab, to provide carryover funds needed for the temporary maintenance of the late Ahmad Bukhari's research efforts, to support our meeting programs, to help buy a cell sorter for James lab, and to support the stipends of several postdoctoral fellows and visiting scientists.

The Banbury Center Now Functions with a Balanced Budget

With each year we have made increasing use of our Banbury Center located on the site of Mr. Charles S. Robertson's former estate in Lloyd Harbor. During the five-month-long meeting and summer courses period its facilities are now in full-time use. Particularly important is its conference facility, which serves as the site of our summer neurobiology lecture courses. During the rest of the year, it serves as the perfect site for a series of small meetings arranged by Mike Shodell, the Director of Banbury Center. While Mr. Robertson provided a generous endowment fund for the upkeep of his marvelous house and the grounds of this beautiful 40-acre estate, each of the meetings we hold there demands additional outside funding. Toward this end, applications for grants are constantly being made to potentially interested governmental agencies and requests made to major industrial sources and private foundations for core-type support. The raising of this money has never been easy, and so it is with much satisfaction that I can report that all of last year's $550,000 in operating expenses were raised, leaving us, in fact, with a minor surplus. This coming year I fear may not be so satisfactory. Grant support for meetings is now very hard to get, even with high priority scores, due to the proposed reductions anticipated in the number of grants awarded. We shall thus have to work even harder to see that Banbury Center remains an important site of incisive small meetings, many devoted specifically to the interaction between the latest biological research and human society.

LIBA Continues to Provide Us with Critically Needed Building Funds

Our neighbors who work so effectively on our behalf under the auspices of the Long Island Biological Association (LIBA) came again to our rescue in a moment of great need by providing an additional $100,000 toward the costs of the Oliver and Lorraine Grace Auditorium. This project, initiated with the enthusiastic support of LIBA, will radically upgrade our facilities for the holding of our major meetings, allowing us to respond effectively to the enormous expansion in the number of molecular biologists that is accompanying the recombinant DNA revolution. So we remain deeply indebted to all of our LIBA members for their continued assistance which allows us to remain a major center of DNA research for the entire world.

Over the next year the officers and directors of LIBA will be engaged in discussions with our Trustees as to how we should prepare for the 100th anniversary of our founding in 1890. The five years still to pass before this major anniversary provide an obvious interval in which to reassess where we want to be on our

100th birthday and hopefully to plan out a major program for achieving these objectives.

Formation of an Ad Hoc Committee of Our Board of Trustees for Neurobiology

While we offer the most comprehensive series of summer courses in neurobiology in the world and are now generally recognized worldwide as a major asset for the promotion of neurobiology, we still do not possess a laboratory in which neurobiologists can work throughout the year. Jones laboratory, our sole facility specifically equipped and renovated to do neurobiology, must also function each year as the site of our summer courses. During the summer our neurobiologists must go away or crowd together in a modernized mini-lab in the old Mouse House, where effectively they must stop most of their research. This is clearly an intolerable situation in the long-term. This situation recalls the similar circumstances of our Yeast Group when their booming research on mating-type switching led us to build for them an addition to Davenport (now Delbrück) laboratory. There is no way, however, that a major addition can be added to the 1893-built Jones lab without destroying the intrinsically beautiful way our complex of old buildings appears when seen from both the Lab grounds and from across the inner harbor.

It was thus virtually inevitable that we would witness the premature breakup of the small Neurobiology Group that has worked in Jones lab over the past several years. This is inherently very distressing in view of their seminal role in showing the great potential of monoclonal antibodies not only for probing the anatomy of the nervous system, but also for potentially revealing the identity of the key molecules whose interactions lead to linking of nerve cells to their correct cellular partners during embryonic development. First to leave us for the prospect of a more stable long-term future was Ron McKay, who moved in the summer of 1984 to the Whittaker School of MIT. Soon afterwards, Birgit Zipser left to continue her research on the leech at the National Institutes of Health. And earlier this year we had to accept the fact that Sue Hockfield would be leaving in mid-1985 for the Neuroanatomy Department of Yale Medical School.

The prospective long-term absence of any year-round efforts in neurobiology is clearly incompatible with the running of a major summer program. We must thus design, and then find the funds for, a new laboratory specifically designed for neurobiology. Toward this end, a committee of our Board of Trustees has been created whose members are Charles Stevens, Eric Kandel, John Klingenstein, and William Robertson. Hopefully, semiprecise plans as to how we should proceed will be available by the end of the summer of 1985.

A Strong New Set of Additions to Our Board of Trustees

That our Board of Trustees combines science and the public sector at its best remains one of the strongest assets of the Laboratory. I consider myself most fortunate that from our trustees I can call on the collective wisdom of many very talented scientists as well as the financial and legal savvy necessary for quick, experienced reactions to a multiplicity of problems that need answers. It is thus with much regret that the statutory six-year terms of three very devoted trustees expired this past November. Roderick Cushman, Mary Lindsay, and Alexander Tomlinson have all served us with distinction. In particular, I wish to note that this

is the second time Mary Lindsay has completed a six-year term of office. Happily, all our departing trustees live near to us, and we are sure that their repeated help can be relied upon. Following his move to Princeton in the fall, Tom Shenk now becomes our trustee from Princeton University, with the Stony Brook slot now ably filled by Sidney Strickland. Newly elected as individual trustees are Mrs. Charles Dolan of Cove Neck, who, with her husband, has been a major imaginative force in the development of cable TV in the United States; Mrs. Sinclair Hatch of Oyster Bay, a longtime activist for a variety of medically important goals; and Mr. Harvey Sampson of Cold Spring Harbor, a distinguished leader of business and Chairman of the Harvey Electronic Corporation.

We Are Going Through a Major Transition in Leadership

That I have been the director for 17 years and still enjoy the many tasks that go with my position owes much to the fact that I have for almost all this time effectively shared most of my burdens with Joe Sambrook, who has headed James lab since his arrival here in 1969 and who for the last seven years has functioned also as our Assistant Director for Research. It was thus with both unease and regret that last August I learned of Joe's decision to resign from his position here in September of 1985 to join the University of Texas Medical School in Dallas as Head of its Department of Biochemistry. Filling Joe's shoes will be no easy task, since Joe has a forceful, innovative mind that consistently and wisely has worked for the good of this institution. In doing so, he has played a far more major role than generally perceived by the outside world in preserving Cold Spring Harbor as a major site for teaching and research in molecular biology.

To help me now with many of the tasks that Joe carried out, I have asked Terri Grodzicker to serve as Assistant Director with the particular charge of overseeing our summer teaching and meetings programs. Still to be filled is the position akin to that of the Scientific Director in many other research institutions, where one individual has the major role of overseeing the recruitment and subsequent promotions of the scientific staff. This is a task that Joe ably performed for many years, since as the Laboratory grew larger, I no longer had the time to effectively know the world of younger scientists. The recruitment of a high-level scientist versed in all aspects of DNA research, if not of biology itself, thus stands out as the most important goal for me to accomplish over the next year.

In this period when I have more tasks than one individual can handle, I take relief that in the recent appointment of G. Morgan Browne, we now have a new Administrative Director in whom we already have great confidence. A 1957 graduate of Yale, he is an experienced business executive, with a strong financial background, particularly in the high technology area. A member of LIBA for several years, and with a keen liking for and interest in science, Morgan knows many of the Lab's longtime supporters and needs no introduction either into the unique roles of the Laboratory or to our community that so long has been an indispensable asset.

The Grace Auditorium Will Transform the Way We Are Seen by Incoming Visitors

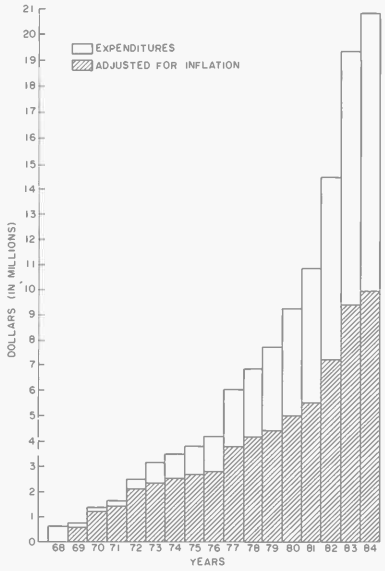
With the completion of the Grace Auditorium, our Laboratory will be ending a 23-year era that commenced in 1953 with the completion of Bush Auditorium and Demerec lab. With these new buildings in hand, Cold Spring Harbor had the re-

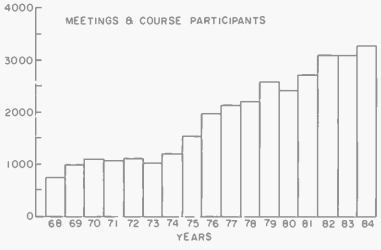
sources to participate in the biological revolution that took off with the discovery of the double helix. Though we have continued to make many important new additions to our physical plant, the Laboratory has looked much the same to uninitiated visitors. The hill overlooking the Blackford entrance hid much of the western afternoon sun and was never a site of beauty, remaining permanently scarred from the removal of the sand used in the 1906 building of Blackford itself. Thanks to the powers of modern earth-moving equipment, Blackford Hall now looks out onto a newly made open area created so that our auditorium would be graced with an adjacent area into which our meeting participants can move freely during coffee breaks and on the way to meals. Soon the now pushed-back hill will be landscaped and on its top will be placed an ancient eight-sided Gazebo given to us when a recently sold estate was broken up. Back of the gazebo we shall be creating a brand new parking area for some 70 cars to handle the increased number of visitors that shall come to the new lecture hall. In opening up this area, we shall be losing a few more trees than we would like, but to compensate for this, we shall plant even more to eventually allow a forestlike feeling to return. As we rework this upper space, we will face the facts that the 26-bed Page Motel built in 1953 functions too often like a 1930s New Hampshire motel and that guests chosen to live in our four 1930s-type cabins often remember the experience more than they wish. So we are already thinking about what their replacements should look like and tentatively have decided to have our architects come forth with the "Adirondack Style" revisited. And next to their potential site is an area that could be perfect for a new neurobiology building.

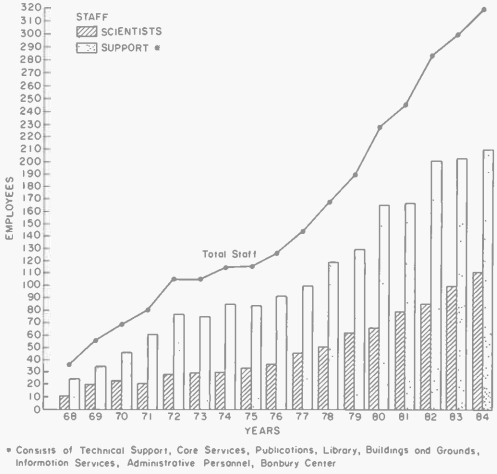
Hopefully, all of these dreams will be reality by our 100th birthday. If so, what a glorious next 100 years we can share with the world of biology.

June 26, 1985 **J.D. Watson**

DEPARTMENTAL REPORTS







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

Recto: Nichols Administration Building (foreground), originally a research laboratory, was built in 1928 as a memorial to George Lane Nichols, a great-nephew of Franklin Hooper, who helped found the Biological Laboratory. Extensive interior renovations were done in 1984. James laboratory is seen in the background.

ADMINISTRATION

The continued growth of the Laboratory over the past years has had a dramatic impact on the Administrative staff. Since 1968, the Laboratory's expenditures have increased from $580,000 to over $20 million, almost a 3500% increase. The number of research grants has grown from less than 10 to over 200 (not including subprojects within a grant), and the Laboratory staff has increased from approximately 35 to more than 325. Meetings and course participants went from approximately 700 in 1968 to 3300 in 1984. Although the challenges have been considerable throughout this period, they have been met through increased efficiency in virtually all areas.

From a small, in some ways "patchwork," operation, Administration has evolved into a modern, computerized organization built around a core of experienced professional managers and highly qualified clerical personnel. In streamlining our operations to provide greater support and assistance for the Laboratory's scientific endeavors, we have not, however, lost sight of the Laboratory's small-town, spontaneous, and scientifically intensive atmosphere that has served science so well for so long.

Installation of the Administrative Computer

Prior to the installation of the administrative computer, records and data were spread throughout the campus in separate offices located in separate buildings. The flow of paper from building to building and office to office precluded timely and coordinated information exchange. Now, with direct access to our MAI Basic Four computer, the various administrative segments can instantly and accurately reflect and report their daily activities to related departments.

In addition to greatly assisting clerical functions, the computer has eased the burden of processing purchase orders, maintaining personnel records, and managing finances. Up-to-date financial and grant reports are now readily available to senior management and scientific staff. It is difficult to imagine how the present volume and variety of transactions would have been managed without the accuracy, simplicity, and consolidation afforded by a computer.

Formation of the Commerical Relations Committee

The advance of biotechnology at the Laboratory has drawn the interest of several commercial organizations. To safeguard the Laboratory's basic research mission and to avoid potential conflicts of interest, the Commercial Relations Committee was established by the Board of Trustees to review such relationships and to generate policies and guidelines to govern staff interactions with commercial organizations. Townsend Knight, Chairman of the Committee, has contributed enormously toward reviewing the frequently complex and somewhat unprecedented new partnerships between commerce and basic research institutions.

Nichols Renovation

After years of neglect and overcrowding, the Nichols Administration building had become inefficient and no longer suited to the increased administrative tasks. It

had also become structurally unsound and could no longer support the number of people using the facility. Renovation plans were drawn up and Jack Richards turned the attention of his staff toward making Nichols into a more efficient and better place to work. The results are dramatic from both an aesthetic and functional point of view. Offices are now arranged on the basis of operating efficiency and functional considerations, and the staff has a much more productive and pleasant environment.

Tax Exempt Bond Issue

What began over 15 years ago as a critical program to restore the Laboratory's deteriorated physical plant has evolved into a substantial program of capital improvements and expansion which include large additions to Demerec and James labs, construction of the dramatic Grace Auditorium, modernization of Blackford Hall, and the addition of a much needed new parking area. To provide the funds necessary for these ambitious projects, we applied for and received an $8 million Nassau County Tax Exempt Industrial Bond Issue. With Morgan Guaranty Trust as bond purchaser, we can proceed with all of these capital projects without limiting the ongoing research program. Special recognition is due William R. Udry who, working together with our former Treasurer and Trustee, Clarence Galston, provided long hours of effort and invaluable financial savvy, which enabled this bond issue to succeed.

Consolidation of Core Services

Some of the most direct and important services provided in support of the scientific staff are the operations referred to as core services. These include the safety department, machine shop, electronics repair shop, equipment repair shop, Harris animal care facility, photography and technical illustrating, and the scientific computer staff. In 1984 these services were centralized under the direction of our highly capable Safety Director, Art Brings. With Art's well-established leadership, we expect that these vital services will prove even more useful to the Laboratory.

The Laboratory continues to be fortunate to have a high-quality administrative staff. We welcome to the staff Fred Lukas, who has already proven to be an able assistant to our Comptroller, Bill Keen. Roberta Salant continues to provide invaluable assistance to me and to the members of the Board of Trustees. Guy Cozza and Bob Pace deserve specific mention, for without them our computer installation could never have succeeded.

John Maroney

BUILDINGS AND GROUNDS

The overall expansion of the Laboratory since 1969 has resulted in an expanding workload for the Buildings and Grounds Department and an increase in personnel from 10 to 42 (including Banbury personnel). Maintenance of an ever-ex-

panding physical plant and the attendant landscaping has become increasingly more sophisticated and time consuming. In sharp contrast to the one part-time handyman of years gone by, who maintained five or six buildings using a hammer and screwdriver, our maintenance personnel today include men with a knowledge of air conditioning and heating, plumbers, and electricians. The advanced planning of future buildings, done in conjunction with architects, engineers, and contractors, and the supervision of ongoing construction and renovations also increase the demands on the department.

James Lab Extension for Monoclonal Antibody Research Completed

The major highlight of 1984 was the completion in mid-summer of the new James laboratory extension by the contracting firm of Robins and Cowan of Huntington, Long Island. The Buildings and Grounds staff soon after finished the offices, installed the kitchen, and coordinated the long awaited move of the scientists and their equipment from their previous quarters in James lab. In addition, the parking area behind James was extended, and the site around the building and lot was landscaped.

First Underground Conduits in Place for Utility Lines

A goal of the Laboratory is to bury all telephone, electrical, and computer lines for all facilities by late 1986 or early 1987. Through 1984, the lines from Harris to the James extension, including extra computer conduits for future use, have been buried. This leg was quite an undertaking and only with Owen Stewart's excellent planning were we able to proceed, despite the inevitable complications associated with dealings with major utility companies.

James Lab extension

Oliver and Lorraine
Grace Auditorium

Work Is Begun on Much Needed Auditorium

Construction of the new Grace Auditorium began early in 1984, and although the job is being done by an outside contractor, A.D. Herman, much time has been spent by members of the department in helping the contractor along. Hopes to complete construction in time for the 50th Symposium in June 1985 faded as labor strikes halted work during the summer. Completion is now expected in fall 1985.

Facilities Must Keep Pace as Needs Change

Interior renovations in 1984 were numerous. With a laboratory vacancy comes the planning for renovation of the facility, and it always seems that it was only "last year" that we were reworking the same rooms. The Slaughter and Tamanoi-Stillman labs in Demerec were completed and the entire first floor of Demerec A was reworked, including a new kitchen, halls, hall floors, and media room. In McClintock, the first floor south office and laboratory were redone as a full lab. Refurbishing of James Annex Library included the removal, refinishing, and reinstallation of all book shelves on foam-insulated walls. The James Annex coffee kitchen received a facelift with refinished cabinets and a ceramic tile countertop, while a laser printer room was constructed in lower James Annex. To correct a water problem caused by our hills and springs, it was necessary to install along the west wall of the James Annex a drainage grate and pipe leading to a sump pump pit. Now that the James (North) extension is completed and occupied, a complete remodeling of the original James (Middle) lab is planned for 1985.

Work was begun on the first floor of the Nichols administration building to modernize the facility and to correct problems that have developed over the years as the result of rushed expansions to meet critical needs. Duplicate equipment was installed in the wastewater treatment plant, increasing the capacity by at least one third. None of these renovations could have been accomplished without the advance sketching done by Charlie Schneider, as well as the talents of Diamond Scarduzio in carpentry and overall planning and coordination.

General Maintenance and Repairs Are Ever Present

Over and above the capital improvements and renovations, Buildings and Grounds works closely with our scientists and their families to assist them in both their work and personal lives. Rarely a day goes by without a maintenance or repair request from the scientific laboratories and tenants in our housing, both on and off grounds. With off-grounds rentals, the Banbury site, and Uplands Farm, the workload can become quite staggering. The summer courses and meetings increase the workload in all departments, and so too in Buildings and Grounds which must respond to the additional strain placed on all facilities through increased use.

Department Saddened by Three Losses

Our department was saddened early in the year by the deaths of Fred Pfeiffer in March, Mary Hill in April, and Jim Stanley (who retired in 1980) in June. We hope that in the future we will be fortunate to have people of their caliber on our team.

Jack Richards

INFORMATION SERVICES

Two Programs Aimed at Nonscientists

One major goal of the Information Services Department is to help explain the Laboratory and its research to nonscientists. Toward this end, "The Biological Revolution," a six-week series of public seminars, was held last spring in conjunction with Hutton House Lectures of Long Island University. Dave Micklos, Joe Sambrook, Mary-Jane Gething, Steve Dellaporta, Sue Hockfield, and Mike Shodell presented talks on recombinant DNA and its applications to biomedicine, agriculture, and industry.

Also in 1984, the Laboratory's outreach to local schools was formalized as the Cold Spring Harbor Curriculum Study. In conjunction with Superintendent Francis Roberts from Cold Spring Harbor High School, a cooperative program was developed to improve teacher competence and to bring state-of-the-art research concepts into school biology classes. Building upon an initial $10,000 grant from Citibank N.A., we were able to attract pledges of $10,000 apiece from eight neighboring school districts who are participating for the 1984–1985 school year: Cold Spring Harbor, East Williston, Great Neck, Herricks, Jericho, Northport-East Northport, Oyster Bay, and Syosset. It is hoped that the Curriculum Study will serve as a model for similar efforts throughout the United States.

Support from Foundations Increases

The department set out to match a $100,000 grant from the Fannie E. Rippel Foundation for the purchase of equipment for the new Monoclonal Antibody Extension to James lab. The first attempt to involve local companies in the Labora-

tory's research – the Long Island Business Cancer Campaign – yielded $50,000 in cash and donated equipment. Grants of $30,000 and $15,000 were secured from the William Randolf Hearst Foundation and the Samuel Freeman Charitable Trust.

The department also coordinated efforts to augment a $691,000 National Science Foundation grant to expand the Laboratory's research on the molecular basis of plant development. A reception held at the home of Ambassador John Humes led to grants from the William and Maude Pritchard Charitable Trust ($60,000) and the Griggs and Burke Foundation ($10,000) and numerous individual gifts. Two other major grants were secured: $75,000 from the Charles E. Culpeper Foundation and $50,000 from the Surdna Foundation.

Another highlight was the receipt of a $110,000 grant from the McKnight Foundation to purchase equipment to teach molecular genetic techniques in our summer neurobiology courses. Also heartening was news of a four-year, $74,000 grant in support of the Undergraduate Research Program from the Alfred P. Sloan Foundation.

Inauguration of the Corporate Sponsor Program

The 1984 Corporate Sponsor Program achieved its two goals: to provide a stable base of support for our meetings program and to increase dialog between academic and industrial researchers. Last year, 260 industrial scientists attended Cold Spring Harbor Laboratory and Banbury Center meetings. A new series of Banbury conferences dealing with technical aspects of genetic engineering was especially well received. So enthusiastic was the response to the inaugural year that 1985 membership has been expanded from 15 to 22 companies. Represented among the Laboratory's sponsors are prestigious Fortune-500 pharmaceutical and chemical companies and specialty biotechnology firms.

David Micklos

LIBRARY SERVICES

Reference Services Increase and Archives Expand

In 1984, patron/reference services became the focal point for the Library staff. Following the resignation of Audrey Powers as Librarian in July 1983, we were fortunate to have Genemary Falvey, a staff member for three years, step in as Acting Librarian while she completed her Master's Degree in Library and Information Science. In December 1984, Ms. Falvey graduated with honors and was promoted to Librarian. Genemary's expertise is in reference services, with emphasis on computer-based literature searches tailored to the needs of individual users. The impact of these new services on library activity is clear: Scientific searches increased from a total of 60 in 1983 to 105 in 1984 and reference and information questions increased by 41% (from 5000 in 1983 to 8500 in 1984).

Several major objectives were accomplished in the reference and archival areas this year. The reference collection has been weeded and updated, and the

foundation has been laid for upgrading the textbook collection. A DEC personal computer was purchased so that extensive on-line searches would no longer sap the campus-wide system. The preservation and cataloging of 32 maps related to the history of Cold Spring Harbor Laboratory and its environs were completed. Organization of the historical records of the Laboratory, including completion of the registry of the Carnegie correspondence from 1930 to 1950 and the streamlining of the portrait files, continued. On the recommendation of Trustee Ed Pulling, the library also established a Long Island Cultural Arts/Activities File for the Laboratory community. This service provides announcements from all entertainment facilities on Long Island.

Permanent Collection and Patron Services Grow Amid Staff Economies

The growth of library resources included the addition of 14 periodical titles, while 14 others were withdrawn, keeping our journal subscription count stable at 433. The book collection grew by a net total of 1,404 bound volumes, bringing the number of bound volumes to 27,299. Patron services increased an overall 35% from 1983 to 1984.

I would like to publicly acknowledge the contribution made by the entire Library staff in 1984. All individuals contributed beyond their usually high performance, allowing a smooth operation and reducing salary expenditures by the equivalent of one full-time staff member.

Susan Gensel

PUBLICATIONS

New Titles Published and a New Series Launched

The Publications Department continued to grow in 1984, with a total of 11 new titles published in addition to 18 reprintings. A new series, *Cancer Cells,* was inaugurated with the publication of "The Transformed Phenotype" and "Oncogenes and Viral Genes." This series supersedes *Cold Spring Harbor Conferences on Cell Proliferation,* and its journal format provides a forum for scientists from diverse disciplines. Two new monographs were published this year: *Microbial Development,* issued simultaneously in cloth and paperback, and *Gene Function in Prokaryotes,* which was later released in paperback in early 1985.

The practice of paperbacking selected titles to extend readership particularly to the student market was continued with the publication of *RNA Tumor Viruses/1, Text.* This volume and *RNA Tumor Viruses/2, Supplements and Appendixes* (due in 1985), with a combined total of more than 2500 pages, constitute an updated and expanded edition of the *RNA Tumor Viruses* volume originally published in 1982.

Experiments with Gene Fusions, a manual based on the Bacterial Genetics courses held at Cold Spring Harbor in 1981 and 1982 was well received by reviewers and by our teaching and research colleagues. A Strain Kit for use with this manual will be available in 1985.

Other titles produced include *Modern Approaches to Vaccines*, which was an outgrowth of a 1983 Cold Spring Harbor meeting. So successful was the meeting that its organizers, Robert Chanock and Richard Lerner, were able to contract for five more annual meetings. From these meetings will be published a five-year series, beginning with *Vaccines 85*. Another timely publication was *Human T-Cell Leukemia/Lymphoma Virus*, which studies the role of this virus family in malignancies and in AIDS. A meeting in April 1984 gave impetus to the publication in December of *Molecular Biology of the Cytoskeleton*.

In 1984, we assumed publication of a biennial series entitled *Genetic Maps: A Compilation of Linkage and Restriction Maps of Genetically Studied Organisms*. Originally published and distributed as a service in 1980 and 1982 by Steve O'Brien of the National Cancer Institute, the growing demand for, and complexity of, the volume required the expertise of a publisher and we were happy to add this series to our list.

In recent years, we have strived to publish the results of our meetings as rapidly as possible, even though most of our books are multi-authored and inevitably turn out to be at least 100 pages longer than initially planned. In spite of this, it has been our goal to publish our annual *Symposium* volume in the same year in which the meeting takes place. Although the proceedings of the 1983 Symposium on Molecular Neurobiology was at the printers in 1983, bound books were not available until 1984. For the first time, the *Symposium* was published simultaneously in cloth and paperback editions. When the subject matter warrants the publication of a paperback edition for the student market, this practice will be carried out in the future. In addition to these new titles, we published abstracts of the 11 meetings held here in 1984 and the Annual Report.

Use of In-house Computer Saves Time and Money

In-house computer typesetting was utilized in producing seven of the 1984 titles. This technology saves time and cuts composition costs by approximately 30%. We achieved even greater efficiency and savings by initiating the practice of sending authors letter-quality computer printouts instead of typeset galleys. This allows all author corrections to be inserted before the manuscript is transmitted to the typesetter.

Move to Paperbacks Increases Volume

Although unit sales increased from approximately 35,000 units in 1983 to more than 37,000 in 1984, net sales declined to slightly more than $1,500,000. This decline in the wake of increasing volume is directly related to our major move into offering lower-priced paperback editions of many titles. To continue this program of providing paperbacks priced within reach of the economically hard-pressed student market, every means of increasing sales volume while controlling costs must be explored.

Marketing Pushes for Greater Foreign Sales

With an eye toward increasing volume, the Marketing Department has been vigorously pursuing new channels for foreign distribution, including finalizing an agreement with a new distributor for India, Panima Educational Book Agency, and opening negotiations for a new Japanese distributor with the prospect of in-

creasing sales to Japan over the next three years from $100,000 to $250,000 annually.

A consistent program of follow-up by Marketing on all books sent to journals for review has yielded an impressive 40–43% response with a review and/or listing within 18–24 months, well above the industry norm of a 25–30% response within the same time frame.

Fulfillment Moves Toward Consolidating All Warehousing and Shipping

Equally responsive to the need for greater volume, the Fulfillment Department increased the ease of placing orders by offering customers the convenience of an 800 toll-free number. To speed delivery, 48% of 1984 orders were shipped directly from the storage facility at Cold Spring Harbor. With this increase in direct shipments it became imperative that sufficient warehousing space be acquired locally not only to solve the on-grounds storage problem, but also to consolidate all warehousing and shipping under our direct control. Having completed negotiations with the Board of Education to lease a portion of the no-longer-needed East Side School in Cold Spring Harbor, it is expected that all inventory will be transferred to this new warehouse by mid-summer of 1985.

Publications Completes First Full Year in Urey Cottage

The start of 1984 saw the Publications group happily installed in our attractive new quarters. Specifically renovated to meet our growing needs, the function-oriented planning has more than lived up to expectations in providing the quietude and ease of communication needed for efficiently handling the myriad details involved in editing and producing high-quality scientific books. This combination of an environment conducive to productivity and a superb staff, both knowledgeable and dedicated, should enable us to continue to increase the number of titles published over the next few years while maintaining present staff levels.

Nancy Ford

RESEARCH

TUMOR VIRUSES

Thirteen years ago, the National Cancer Institute funded a major Program Project Grant to support work on tumor viruses at Cold Spring Harbor Laboratory. This grant is now in its third cycle and has allowed us to focus the efforts of a multidisciplinary team toward the study of adenoviruses, SV40, and papilloma viruses. Because this work has been funded by the Program Project mechanism, it has been characterized by extensive collaboration both within and between the various sections whose activities are described in the following pages.

In addition to the inherent challenge of understanding how they work, these viruses have provided exciting model systems and tools for exploring the molecular biology of eukaryotic organisms in general. Thus, viral vectors have been designed that enable the overproduction of both viral and host proteins. Studies of viral transformation are already leading into the more general phenomena of gene regulation and cellular growth control. The adenovirus E1A region and its gene products are the subjects of intensive research efforts. Studies of RNA splicing and viral enhancers continue while the role of the adenovirus VA RNAs finally seems to be unraveling. In vitro systems that replicate adenovirus and SV40 DNAs have led to the identification of essential viral and host proteins.

Our long-time colleagues, Tom Broker and Louise Chow, have left Cold Spring Harbor Laboratory to take up appointments at the University of Rochester. They have played key roles in many of our past projects and will be sorely missed. Likewise, Earl Ruley, who has moved to the Massachusetts Institute of Technology, has been a valued and productive colleague.

MOLECULAR BIOLOGY OF TUMOR VIRUSES

Y. Gluzman
T. Grodzicker
E. Harlow
W. Herr
H.E. Ruley

T. Adams
R. Gerard
J. Harper
M. Manos
K. Maruyama
M. Quinlan
K. Van Doren
P. Whyte
M. Yamada
B. Ahrens

N. Beck
N. Caplin
D. Chao
R. Chisum
J. Clarke
J. Cosetti
M. DeLuca
M. Goodwin
R. McGuirk

M. McKeever
M. Merle
J. Moomaw
S. Rook
L. Sansome
C. Schley
L. van der Wal
J. Wiggins
N. Williamson

The major emphasis of our research continues to be the analysis of DNA tumor viruses, especially SV40 and adenoviruses. A major effort is directed toward the analysis of the transforming genes of these viruses. A wide variety of mutants in these genes, viral vectors that overproduce transforming proteins as well as a large set of monoclonal antibodies directed against transforming proteins, provide reagents used in a variety of studies. Thus, many projects are concerned with the role of viral transforming genes in the regulation of cellular and viral gene expression, in the establishment and transformation of primary cells, in the interaction of viral transforming genes with each other and with other oncogenes, and with the interaction of virus-en-

coded transforming proteins with other cellular proteins. Experiments on the cellular localization and modification of transforming proteins are also being carried out. We are continuing studies of controlling elements of viral early genes that are involved in the regulation of transcription, mRNA processing and translation, and the interaction of viral and cellular proteins with these controlling elements. Work has continued on the construction and analysis of viral vectors that has allowed us to produce, in mammalian cells, large amounts of eukaryotic proteins, including transforming proteins that can be used for purification and biochemical studies.

Analysis of Wild-type and Mutant Ad2 E1A cDNAs

H.E. Ruley, T. Grodzicker [in collaboration with E. Moran, B. Zerler, and M.B. Mathews]

The adenovirus early region 1A (E1A) gene is required for adenovirus-induced cell transformation and is involved in the regulation of expression of other adenovirus genes. To distinguish the individual roles of the 13S, 12S, and 9S E1A gene products, we have isolated the corresponding cDNA clones and have recombined them into both plasmids and viruses (see Protein Synthesis in this section).

The 13S virus plaques equally well in human 293 cells, HeLa cells, and A549 cells, a human lung-oat cell carcinoma line that does not contain adenovirus DNA. Plaque titers of the 12S virus are much reduced in HeLa and A549 cells as compared with that in 293 cells. The 12S virus is multiplicity-dependent leaky in both HeLa and A549 cells. At low multiplicities of infection, the yield of 12S virus in HeLa cells is low; however, in A549 cells, even at low multiplicities of infection, the yield of 12S virus increases with time to approach the maximum yield from 293 cells. These results suggest that the A549 tumor cell line, even though it contains no adenovirus DNA, can partially complement the E1A defect of the 12S virus so that transcription of other early adenovirus genes is activated. Immunofluorescence studies indicate that the adenovirus DNA-binding protein is made during infection of HeLa cells with the 13S virus but is not detectable during infection with the 12S virus. Primary baby rat kidney (BRK) cells are immortalized by the 12S virus at very high efficiency, whereas infection of these cells with 13S virus, as with wild-type E1A virus, results mainly in cell death. Transfection with 12S or 13S plasmids shows that both the 12S and 13S plasmids can, like genomic E1A, establish BRK cells or cooperate with plasmids carrying the *ras* gene to transform these cells. In a transient expression assay in HeLa cells, the 13S plasmid stimulates expression of the chloramphenicol acetyltransferase (*cat*) gene from the adenovirus E3 promoter, but the 12S plasmid does not. The 12S plasmid appears to interfere with the stimulation of E3-*cat* expression by the 13S plasmid. To differentiate the functions of the E1A products further, we have used oligonucleotide-directed mutagenesis to obtain several point mutations in the genomic E1A or cDNA clones. A point mutation at position 961, which converts a glutamic acid residue to lysine, appears to impair the transformation function seen in the transfection assay while not interfering with the gene-regulating functions studied in the E3-*cat* transient expression assay. We are presently transferring the mutant E1A 12S and/or 13S regions into adenovirus so that their lytic and transforming properties can be assessed. Several other point mutations at nearby sites have also been made and are being analyzed.

Analysis of the Transforming Functions of Adenovirus E1A

P. Whyte, K. Maruyama, T. Grodzicker, H.E. Ruley [in collaboration with E. Moran and B. Zerler]

The polyoma virus middle T antigen, T24 Ha-*ras*-1, and adenovirus E1B genes are all individually unable to transform cultured primary BRK cells. Adenovirus E1A expresses functions that enable these genes to transform. We have speculated that the oncogene cooperativity in transformation is linked to the E1A functions which promote the in vitro establishment of cultured primary cells. To probe the function of E1A in transformation, plasmids containing the E1A regions from Ad5*hr*1 and Ad5*hr*440, as well as

cDNAs for the 12S and 13S E1A mRNAs, were transfected into primary BRK cells alone and together with the polyoma middle T antigen and T24 Ha-*ras*-1 genes. The results obtained indicate that the establishment functions of E1A are located in the aminoterminal segment of the protein.

To determine the minimal aminoterminal sequences required for oncogene cooperation, a series of deletion mutants have been constructed. A plasmid containing the E1A region was linearized at the *Sma*I site, located within the 13S unique coding region, and deletions were generated by digesting with the exonuclease BAL-31. A linker encoding a termination codon was inserted and the plasmids were recircularized, resulting in plasmids coding for truncated E1A proteins. The extent of the deletions was determined by sequencing using the dideoxy method.

The ability of these E1A mutants to cooperate in transformation was assayed by cotransfecting plasmids containing the E1A mutants and the T24 Ha-*ras*-1 gene into primary BRK cells and scoring for transformed foci.

We have determined the minimal aminoterminal sequence of E1A necessary for oncogene cooperation. This region is common to both 12S and 13S mRNAs and is different from the E1A functions necessary for transcriptional activation of the viral early-region genes. These mutants are currently being used to investigate the relationship between functions required for oncogene cooperation and in vitro establishment of primary cells.

Adenovirus E1A Blocks In Vitro Differentiation of Rat PC12 Cells

K. Maruyama, H.E. Ruley

Adenovirus E1A and genes related to the *myc* oncogene of avian myelocytomatosis virus express establishment functions that circumvent the commitment of cultured primary cells to growth arrest and senescence. To investigate the role of establishment functions in the regulation of cell proliferation further, we have introduced E1A and *myc* genes into cell lines that can be induced to differentiate in vitro and that experience growth arrest in the process.

PC12 is a rat adrenal pheochromocytoma cell line that differentiates in response to both nerve-growth factor (NGF) and dibutyryl cyclic AMP (dcAMP) by extending neuronal processes and by cell growth arrest. Plasmids, containing E1A and *myc* genes, linked to the bacterial neomycin phosphotransferase (*neo*) gene, were introduced into PC12 cells, and G418-resistant clones were isolated. Clones were characterized for E1A and *myc* expression and for the ability to differentiate in response to NGF and dcAMP. In the case of E1A, clones expressing moderate-to-high levels of E1A-related RNA failed to differentiate in response to even high levels of NGF and dcAMP, whereas clones expressing lower levels of E1A-related RNA responded only weakly to NGF. Variation in the ability of *myc-neo* transfectants to differentiate was also observed, and we are presently measuring the levels of *myc*-related RNA to correlate *myc* expression with the inability to differentiate in vitro.

Cellular Immortality Is Not Sufficient for Oncogenic Transformation by the T24 Ha-*ras*-1 Gene

H.E. Ruley, R. Franza, J. Garrels

Although the functions required by the Ha-*ras*-1 gene to transform primary cells have not been separated from functions that promote in vitro establishment, we have recently demonstrated that the REF52 cell line behaves like primary cells with regard to transformation by the T24 Ha-*ras*-1 oncogene. Thus, the T24 Ha-*ras*-1 gene alone is unable to transform REF52 cells, but the adenovirus E1A gene enables the T24 oncogene to transform. These results indicate that in vitro establishment is not a sufficient prerequisite for transformation of cultured cells by the T24 Ha-*ras*-1 gene and suggest that the role of E1A in primary cell transformation extends beyond in vitro establishment.

Initial evidence that the T24 Ha-*ras* gene is unable to transform REF52 cells came from microinjection studies. Microinjection of the T24 oncogene plasmid induced neither transient nor

permanent morphological transformants. In contrast, 1–2% of the cells microinjected by a combination of the T24 Ha-*ras*-1 and adenovirus E1A genes transiently acquired the rounded morphology of highly transformed cells, and approximately 0.1% of the microinjected cells grew progressively as morphological transformants.

To probe the requirements for transformation of REF52 further, the T24 Ha-*ras*-1 and adenovirus E1A genes were linked to the bacterial aminoglycoside transferase gene (*apt*) from the Tn*5* transposon expressed from the SV40 early promoter (SV*apt*). Plasmids were transfected into REF52 cells by the calcium phosphate coprecipitation method. Phenotypic transformation was scored either by focus formation on cell monolayers or following selection in G418. The following results were obtained: (1) The T24 Ha-*ras* and Ad5E1A genes individually are unable to transform REF52 cells. Indeed, REF cells expressing the T24 Ha-*ras* p21 protein (T24REF) have been isolated. They are indistinguishable from REF52 cells expressing the SV*apt* gene (REFneo) with regard to growth rate, saturation density, failure to grow in 1% serum, and failure to form tumors in syngeneic rats. Cell lines expressing the E1A gene (1AREF) have also been isolated. These cells are morphologically altered but remain flat and are similar to REFneo cells with regard to growth rate and saturation densities. (2) Transformation of REF52 cells, like that of primary cells, can require at least two collaborating oncogenes. Thus, transformants arose only from cultures cotransfected with E1A and T24 Ha-*ras*-1 genes. The transformants grew to high saturation density (10^6/cm^2) and formed tumors in syngeneic rats; however, the tumors regressed within 5 weeks. (3) E1A does not collaborate with the T24 Ha-*ras* gene in transformation by enabling the cell to overcome some toxic consequence of introducing the T24 Ha-*ras*-1 gene. Thus, neither E1A nor T24 Ha-*ras*-1 was toxic to REF52 cells, as judged by the fact that pKOneo (the SV*apt* gene alone), p1Aneo, and pT24neo plasmids induced G418-resistant colonies with similar frequencies.

T24REF cells differ from REFneo cells in at least two respects. First, T24REF cells can be transformed by plasmids containing the Ad5E1A gene linked to the bacterial guanine phosphoribosyl transferase gene expressed from the SV40 early promoter (SV*gpt*), whereas REFneo cells cannot be transformed by E1A. This result indicates that the T24 Ha-*ras*-1 gene and Ad5E1A can transform REF52 cells in a stepwise manner. Second, T24REF cells require much lower serum concentrations to escape growth arrest induced by 1% serum. Over three times more serum was required to induce 50% of the REFneo cells to enter S phase (within the labeling period) as compared with T24REF cells (9–2.5%); 1AREF cells were intermediate in their responsiveness, requiring 5% serum. Experiments are in progress to determine the physiological mechanisms underlying this responsiveness. It is possible that constitutive expression of the E1A and T24 Ha-*ras*-1 genes complements specific serum growth factors in the mitogenic response of serum-arrested cells. Alternatively, the oncogene proteins may play some role in growth-factor signal transduction and thereby enhance the responsiveness to certain serum growth factors.

Monoclonal Antibodies to the Adenovirus E1A Proteins

E. Harlow, C. Schley

Following infection of mammalian cells with adenovirus, the first virus-coded proteins that are synthesized are the products of the E1A region. To help in the studies of the E1A proteins, we have constructed a number of monoclonal antibodies specific for these polypeptides. A bacterial expression plasmid (kindly supplied by K. Spindler and A. Berk, University of California, Los Angeles), which 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-PAGE, 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. Eighty-three hybridomas secreting antibodies specific for the fusion protein have been isolated and single-cell-cloned. As demonstrated by indirect immunofluorescence, 29 of these hy-

bridomas secrete antibodies that will bind to E1A proteins synthesized in HeLa cells infected with Ad5 virus. However, of these 29 antibodies, only 12 will efficiently immunoprecipitate the E1A proteins from lytically infected or transformed human cells. Analysis of the E1A polypeptides by immunoprecipitation and two-dimensional IEF/PAGE has shown that the E1A products can be resolved into approximately 60 polypeptide species (Fig. 1). We have used mutant viruses that can only synthesize portions of the E1A region to localize the binding sites of these antibodies. All of the monoclonal antibodies will precipitate the products of the 13S mRNA, but two of the hybridomas secrete antibodies that will not bind to the 12S polypeptides. In addition to the authentic E1A proteins present in the immunoprecipitates prepared from an adenovirus-transformed human cell line (293 cells), there are several coprecipitating proteins. These proteins are not immunochemically related to the E1A polypeptides and are precipitated from these cells because they are stably bound to the E1A proteins. We are currently investigating the possible roles of these virus-host complexes.

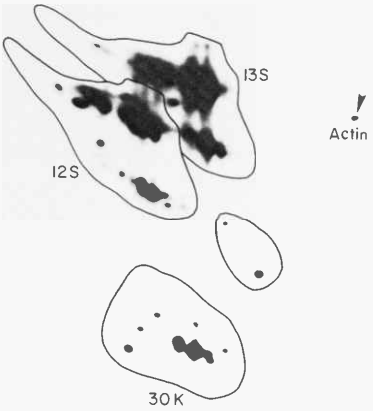


FIGURE 1 Two-dimensional analysis of the adenovirus E1A proteins. The adenovirus E1A proteins from Ad5-infected HeLa cells were immunoprecipitated with M73 hybridoma tissue-culture supernatant and separated by two-dimensional IEF/PAGE. The region of the autoradiogram that contains the E1A polypeptides is shown. The autoradiogram has been annotated to show the polypeptides that are encoded by the 12S and 13S mRNAs. The mRNAs that direct the synthesis of the 30K and 35K–40K polypeptides have not been identified.

Mutations in the Nuclear-envelope-associated Adenovirus E1B 19K Tumor Antigen Cause the Degradation of Chromosomal DNA

T. Grodzicker [in collaboration with B. Stillman and E. White]

We reported last year that the adenovirus mutant Ad2*ts*111 contains a mutation in the early region 2A (E2A) gene that encodes the single-stranded DNA-binding protein (which results in thermolabile replication of virus DNA) and a mutation in early-region 1 (E1) that causes the degradation of intracellular DNA. A recombinant virus, Ad2-*cyt*106 has been constructed that contains the Ad2*ts*111 E1 mutation and the wild-type E2A gene from Ad5. This virus, like its parent Ad2*ts*111, has two phenotypes; first, it has the ability to cause enhanced and unusual cytopathic effects on the host cell (cytocidal phenotype, Cyt) and, second, it induces degradation of cellular DNA (DNA degradation phenotype, Deg). We have mapped the mutation responsible for these phenotypes into the early region 1B (E1B) by marker rescue, transferring the left-end adenovirus DNA fragments from *ts*111 to wild-type DNA and sequencing the relevant portion of *ts*111 E1B DNA.

The mutation responsible for these phenotypes is a single point mutation in the gene encoding the adenovirus E1B 19K tumor antigen. This mutation causes a change in the twentieth amino acid from the amino terminus of the protein from a serine to an asparagine. Three other mutants that affect the E1B 19K protein function have also been examined. The mutants Ad2*lp5* and Ad5-*dl*337 (from G. Chinnadurai [St. Louis University Medical Center] and T. Shenk [SUNY, Stony Brook]) have both the cytocidal and DNA degradation phenotypes (Cyt and Deg), whereas Ad2*lp3* only has the cytocidal phenotype and does not induce degradation of cellular DNA (Cyt and

Deg⁻). Thus, the DNA degradation is not caused by the altered cell morphology. Furthermore, the mutant Ad5*dl*337 does not make any detectable E1B 19K protein product, suggesting that the absence of E1B 19K protein function is responsible for the mutant phenotypes. A fully functional E1B 19K protein is not absolutely required for lytic growth of Ad2 in HeLa cells. Although these E1B mutants clearly play a role in affecting cell morphology, the *ts*111 mutant, which has reduced cytotoxicity because of the mutation in the DNA-binding protein gene, can transform BRK cells at the nonpermissive temperature.

Molecular Cloning and Expression of the Human p53 Tumor Antigen

E. Harlow, N. Williamson, T. Adams

To study the biochemistry of the cellular tumor antigen p53, we have begun experiments designed to purify p53. Analysis of a number of cell lines and animal tissues that might serve as good sources for purification has shown that these cells do not express high enough levels of p53 to allow easy purification. Therefore, we have cloned a cDNA copy of the p53 gene and plan to overexpress the gene for p53 in mammalian expression vectors. A cDNA copy of the mRNA for the human p53 from A431 cells was cloned in a bacterial plasmid vector, and the sequence of the largest insert was determined by the dideoxy-sequencing method. Comparison of the predicted amino acid sequence of this clone and the amino acid sequence predicted from similar clones prepared from mouse mRNA confirms the authenticity of this clone. This comparison has also shown that these two proteins have long regions of amino acid homology, particularly in the aminoterminal 20 amino acids and in the carboxyterminal 200 amino acids. Preliminary results have shown that this cDNA sequence may be difficult to express in mammalian cells in its present form. Therefore, we have isolated a number of subclones that have deletions in both the 5′- and 3′-untranslated regions, and we are studying the ability of these sequences to direct the correct synthesis of human p53.

Expression of p53-specific Transcripts during Mouse Development

T.E. Adams

We have established the pattern of expression of p53 during mouse embryogenesis and in adult tissues. Polyadenylated mRNA was prepared from total RNA isolated from a variety of adult mouse tissues and from embryos at various stages of development. p53-specific transcripts were identified by Northern blot hybridization using a cDNA probe derived from 3′-coding sequences of the mouse gene. The pattern of expression obtained was in keeping with the concept of a role for p53 in cellular proliferation; highest levels of p53 transcripts were observed in embryos taken early in development and postnatally in hematopoietic and lymphopoietic tissues and in skin. Additional studies on chemically transformed cell lines, using both Northern hybridization and "sandwich" radioimmunoassays, have indicated post-transcriptional regulation of the level of p53 in these cell lines.

Transcriptional Modulation of Gene Expression by the SV40 Enhancer: Duplications in a Mutated Enhancer Restore Its Activity

W. Herr, Y. Gluzman, J. Clarke

The SV40 enhancer contains two 8-bp stretches of alternating purines and pyrimidines that have been implicated in transcriptional activation (Nordheim and Rich, *Nature 303*: 674 [1983]). We investigated the role of these sequences in the function of the enhancer by mutating two nucleotides in each 8-bp segment such that the alternation of purines and pyrimidines was destroyed. The relative potential of the wild-type and mutant enhancers to activate transcription was measured by placing them upstream of the human β-globin gene, followed by measurement of the levels of properly initiated β-globin mRNA after transient expression in HeLa cells. In this assay, the mutated enhancer is four- to sixfold less active than the wild-type enhancer. Furthermore,

SV40 carrying these four point mutations grows very poorly in CV-1 cells.

Revertants with improved growth potential could be obtained after passage of mutant virus stocks in CV-1 cells. Analysis of the DNA from 18 plaque-purified revertants showed that the enhancer regions had rearranged. Each of these

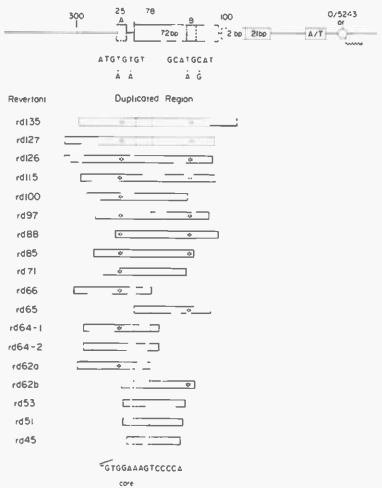


FIGURE 2 Structure of revertants of an SV40 enhancer mutant. At the top of the figure is a diagram of an SV40 control region that contains only one copy of the 72-bp element. Shown from right to left are the early transcriptional start site (zigzag arrow), the origin of replication (*ori*), the AT-rich TATA-like element (A/T), the two GC-rich 21-bp repeats, and the single 72-bp element. Hatched boxes indicate the location of the two 8-bp stretches of alternating purines and pyrimidines; directly below these boxes are the sequences of each of these stretches and the base substitutions found in each of these segments in the *dpm*12 mutant. The series of rectangular boxes represents the extents of the tandemly duplicated region in each of the 18 independent revertants of the enhancer mutant. They are ordered from top to bottom by size. The two series of double Xs identify the positions of the original point mutations where duplicated. The stippled area within each rectangular box corresponds to the 15-bp region that is common to all of the duplications; the sequence of this region is shown at the bottom of the figure with a bracket outlining the "core" element (Weiher et al., *Science 219:* 626 [1983]).

rearranged enhancer elements was studied in the enhancer assay and exhibited increased transcriptional activation of the β-globin promoter in HeLa cells. The nucleotide sequence of these revertants showed that each rearrangement was due to tandem duplication of the enhancer region in which the mutations were preserved (see Fig. 2). Although the duplicated regions range from 45 bp to 135 bp in length and the sequences contained in each duplication vary, there is a 15-bp sequence, TGTGGAAAGTCCCCA, that is consistently duplicated in each reactivated enhancer. This region contains the "core" sequence, GTGGAAAG, that was initially suggested (Laimins et al., *Proc. Natl. Acad. Sci. 79:* 6453 [1982]) to be functionally significant, since related sequences were identified in a variety of viral enhancers. Our results suggest that the core and surrounding sequences are a *cis*-acting element that can function independently of and compensate for the alternating purine and pyrimidine sequences.

We have tested the hypothesis that the core element is important for enhancer function by making point mutations within this sequence and have found that indeed these mutations are deleterious to both SV40 growth and enhancer function. We are presently analyzing the structure of revertants of these core mutations to determine what alterations of the SV40 genome can compensate for these new defects. Preliminary results indicate that duplication of either one or the other of the regions containing the alternating purine and pyrimidine sequences is able to compensate for the mutations in the core element. These results suggest that the SV40 enhancer is composed of multiple independent *cis*-acting elements that are able to compensate for one another. At present, we do not know whether these independent *cis*-acting elements are interacting with the same or different complementary *trans*-acting factors.

Diethylpyrocarbonate as a Chemical Probe for DNA Secondary Structure

W. Herr

Last year, Y. Gluzman and I reported a study in which we analyzed the significance of two 8-bp

alternating purine and pyrimidine sequences that lie within the SV40 enhancer. These two sequences had been suggested by Nordheim and Rich (*Nature 303:* 674 [1983]) to have the potential to form left-handed Z-DNA, and these authors proposed that Z-DNA may be functionally important for enhancer activity. We found that transition mutations, which maintain the alternating patterns of purines and pyrimidines, had little effect on the potential of the SV40 enhancer to activate transcription, whereas transversion mutations, which destroy the alternation of purines and pyrimidines, had much greater deleterious effects. These results were consistent with the possibility that Z-DNA is involved in enhancer function, because alternating purine and pyrimidine sequences favor the formation of Z-DNA. However, analysis (with A. Nordheim and A. Rich) of the ability of the mutant DNAs to form Z-DNA using anti-Z-DNA antibodies has been inconclusive because of the difficulty in identifying the precise DNA-binding site of the antibodies.

To identify more precisely sites of Z-DNA formation, I searched for a chemical probe that would discriminate between the B-DNA and Z-DNA secondary structures. As a test substrate, I used the plasmid pLP32 (Peck et al., *Proc. Natl. Acad. Sci. 79:* 4560 [1982]) which carries a 32-bp stretch of alternating GC residues. This 32-bp sequence is in the B conformation when the plasmid DNA is relaxed, but upon negative supercoiling, it will flip to the Z conformation. I found that diethylpyrocarbonate (DEPC), which modifies the N7 position of adenines and guanines, is hyperreactive to the 32-bp alternating GC sequence in the Z conformation, compared with the same sequence in the B structure. The comparative reactivity of DEPC to specific nucleotides was displayed as follows: Linear and negatively supercoiled pLP32 DNAs were partially modified with DEPC, digested with a restriction enzyme, and end-labeled, and the strands of singly end-labeled molecules were cleaved as described by Maxam and Gilbert (*Proc. Natl. Acad. Sci. 74:* 560 [1977]).

Reactivity of DEPC to natural sequences was analyzed by examining the modification patterns of a region of the plasmid pBR322 that has been shown to bind anti-Z-DNA antibodies when the plasmid is negatively supercoiled and contains a 9-bp stretch of alternating purines and pyrimidines. pBR322 plasmid DNAs carrying increasing densities of negative supercoils were modified with DEPC. As the supercoil density increased, DEPC exhibited hyperreactivity to the purines within the 9-bp alternating purine and pyrimidine sequence. Purines flanking the alternating purine and pyrimidine sequence became hyperreactive at higher supercoil densities. These results suggest that Z-DNA is favored within the alternating purine and pyrimidine sequence and then spreads to the adjacent nonalternating purine and pyrimidine sequences as the supercoil density increases.

I am currently applying this technique to study the Z-DNA potential of the SV40 enhancer region. In preliminary experiments, I have not detected any differences in the reactivity of DEPC to the enhancer and early promoter regions of relaxed and negatively supercoiled SV40 DNAs. These results indicate a potential lack of correlation between the binding to DNA of anti-Z-DNA antibodies and the hyperreactivity of DEPC. This enigma will hopefully be explained by further investigation.

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

M. Manos, Y. Gluzman

We have continued the analysis of DNA replication-defective, but transformation-competent, SV40 mutants such as C2, C6, and C11 and have isolated and analyzed two more such mutants (C8/SV40 and T22/SV40). These mutants were isolated from the C8 and T22 simian cell lines that were transformed with UV-irradiated SV40. Viral DNA was rescued by fusion of these lines with COS-1 cells, which provide a functional T antigen in *trans* and therefore allow 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 pro-

tein defective for viral DNA replication but efficient in transforming cells in vitro. The mutations responsible for the defects in viral DNA replication are His→Glu at residue 203 (T22) and Lys→Glu at residue 224 (C8). There is an additional mutation in the C8 mutant that converts glutamine (amino acid 660) to a stop codon; therefore, C8 T antigen lacks the 49 carboxyterminal amino acids. These two mutations were separated to form mutants pC8A and pC8B. All mutants, pC8, pC8A, pC8B, and pT22, are comparable to the recombinant wild-type SV40 DNA in transforming primary mouse embryo fibroblasts or primary BRK cells. Whereas pC8, pC8A, and pT22 are completely defective in viral DNA replication, pC8B is able to replicate viral DNA but only at 10% wild-type efficiency. Although C8B DNA replication occurs with the same efficiency in both CV-1 and BSC-1 cells, virus only grows on BSC-1 cells. Thus, truncation of 49 amino acids from the carboxyl terminus of T antigen results in a host-range mutant phenotype. Analysis of the origin-binding activity of mutant T antigen reveals that C8, C8A and C8B T antigens bind to SV40 origin as efficiently as wild-type T antigen, but T22 T antigen is completely defective in the origin-binding assay. All mutants are positive in ATPase assay.

All of the replication-defective T antigen mutants we have so far analyzed have been cloned into an adenovirus expression vector, and large quantities of mutant T antigen have been produced. In collaboration with C. Prives (Columbia University), the biochemical properties of these mutants are being further analyzed with greater scrutiny.

Permissive Cell Lines That Inducibly Express SV40 Antigen

R.D. Gerard, Y. Gluzman

We wanted to generate cell lines in which the replication of transfected SV40 origin sequences could be controlled. One way to accomplish this is to replace the SV40 early promoter with the mouse methallothionein promoter to render expression of T antigen inducible by heavy metals. Transformation of permissive monkey cells with this chimeric gene has yielded cell lines (CVMT and BSCMT) that synthesize three- to tenfold more T antigen upon induction. Uninduced levels of T antigen are comparable to those present in COS cells. Withdrawal of these cell lines from inducer results in a rapid decay in the rate of T-antigen synthesis, with estimates of the half-time ranging from 3 hours to 5 hours, depending on the clone. In addition, pulse-chase experiments indicate that T antigen is stable, with a half-life (20–30 hr) comparable to that in COS cells.

The rate of replication of transfected SV40 *ori* sequences rapidly slows after heavy metals are withdrawn even though T antigen is stable. The rate of DNA replication decays with a half-time of 5–8 hours and parallels the decay of the rate of synthesis of T antigen. One explanation of this phenomenon is that continual synthesis of T antigen is required for DNA synthesis because only newly synthesized T antigen will support DNA replication. Further analysis of SV40 *ori* replication in CVMT and BSCMT cell lines indicates that induction of T-antigen synthesis by heavy metals results in a 20–100-fold greater accumulation of replicated DNA than obtained under uninduced conditions after 48 hours. COS cells replicate DNA to levels comparable to those present in uninduced CVMT and BSCMT cells.

To examine the ability of these cell lines to maintain SV40 *ori* sequences in a episomal state, we have transformed them with the *neo* gene carried on an SV40 *ori* plasmid (pON3). Low-molecular-weight DNA was isolated from G418-resistant cultures and analyzed by both Southern blotting and plasmid rescue. The results demonstrate the persistence of pON3 DNA as an episome and an increase of 20–100-fold in the copy number of these sequences upon induction of T-antigen synthesis by heavy metals. Although significant rearrangement of the DNA sequences sometimes occurs, the majority of these alterations occur within plasmid sequences presumably because alterations in the SV40 *ori* and *neo* structural genes are selected against.

Transformation of CVMT and BSCMT cells with a similar DNA construct containing the *neo* gene under SV40 early promoter control, the SV40 *ori*, and the influenza virus hemagglutinin (HA) gene under SV40 late promoter control (provided by M.-J. Gething, Molecular Genetics

of Eukaryotic Cells Section) (neoHA DNA) has also given rise to G418-resistant clones. Analysis of low-molecular-weight DNA by Southern blotting demonstrates the persistence of intact neoHA DNA and a 10–100-fold amplification of these sequences after induction of T-antigen synthesis by heavy metals for 48 hours. Synthesis of the HA protein is easily assayed by immunoprecipitation and gel electrophoresis and shows a 3–15-fold increase above control levels upon heavy metal induction.

Although we do not know if chromosomal integration of these sequences has occurred, these observations suggest that CVMT and BSCMT cell lines may be a useful system for maintaining cloned genes in an amplifiable episomal state in mammalian cells.

Monoclonal Antibodies Specific for SV40 Nucleoprotein Complexes

R. 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. Among these were antibodies specific for the SV40-coded large T antigen and viral capsid proteins. Another hybridoma in this series produces antibodies that precipitate NPCs that have been pulse-labeled with [^{3}H]thymidine. This antibody will immunoprecipitate a 70K phosphoprotein from infected or mock-infected cells, and we are currently attempting to identify this antigen.

An Adenovirus Vector That Expresses the Protein Product of a Nonselected Foreign Gene at a High Level: Synthesis of HSV-1 Thymidine Kinase and Human α-Chorionic Gonadotropin

M. Yamada, T. Grodzicker

We have been developing an adenovirus vector system that can express foreign gene products to a high level in mammalian cells. We have shown previously that SV40 T antigen is produced at the highest levels when its coding sequences are placed adjacent to the third segment of the adenovirus tripartite leader. We have also shown that defective adenovirus-SV40 recombinants expressing SV40 large T antigen can be selected by virtue of the large T helper function that allows adenovirus to grow on normally nonpermissive monkey cells. To insert other foreign genes into the third leader of the adenovirus genome, we used a combination of in vivo and in vitro recombination, followed by the selection of hybrid viruses that also express the helper function of SV40 T antigen (Thummel et al., *Cell 33:* 455 [1983]). We inserted the thymidine kinase (*tk*) gene of herpes simplex virus I (HSV), the genomic sequences coding for human α-chorionic gonadotropin (α-HCG), or its cDNA form at the third segment of the tripartite leader. These viruses also contain the SV40 early promoter and T-antigen-coding region downstream from the *tk* or α-HCG genes.

Human or monkey cells infected by one of the adeno-*tk* hybrid viruses (AdTkSVR591) produced in the late stage of infection (1) an abundant Tk mRNA with the length expected for a molecule containing both the tripartite leader sequence and *tk* sequences, (2) a 45K protein that reacted with anti-Tk antisera and with the same molecular weight as that found in HSV-infected cells (see Fig. 3), (3) a Tk protein that was synthesized to high levels and represented the third most heavily labeled protein in the cells, after hexon and fiber, and (4) severalfold higher Tk activity than is found in HSV-infected cells.

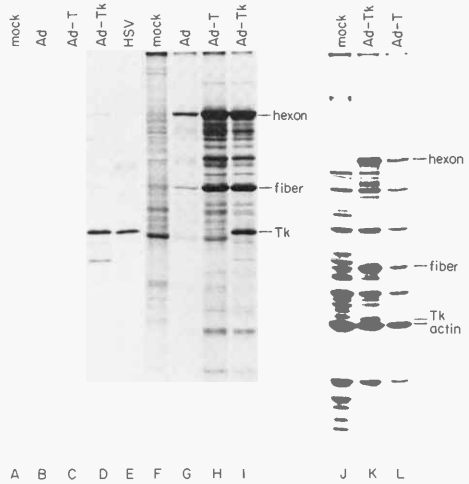


FIGURE 3 Analysis of hybrid-virus-encoded Tk protein by gel electrophoresis. CV-1 cells were infected with the virus indicated. After incubation at 37°C for either 8 hr for HSV or 20 hr for adenovirus, cells were labeled with 100 μCi [^{35}S]methionine for 1 hr and lysed with NP-40. An amount of supernatant fraction, equivalent to 2×10^5 cells, was incubated with 2 μl of anti-Tk antiserum at 4°C, and immunocomplexes were precipitated with an excess of protein A–Sepharose precoated with rabbit IgG directed against goat IgG. The precipitate was boiled in sample buffer and analyzed by electrophoresis on a 10% acrylamide gel *(A-E)*. An amount of the same extract, equivalent to 2×10^4 cells, was also analyzed without immunoprecipitation by electrophoresis on the same gel *(F-I)*. Extracts were also prepared from nonlabeled cells at 26 hr postinfection. An amount equivalent to 2×10^5 cells (containing about 70 μg protein) was analyzed on an 8% acrylamide gel *(J-L)*. *(A,F,J)* Mock; *(B,G,)* wild-type adenovirus 1 × 51 (moi = 50); *(C,H,L)* AdSVR599 (contains SV40 T antigen sequences but no *tk* gene) (moi = 200, hyb = 10%); *(D,I,K)* AdTkSVR591 (contains SV40 T antigen sequences and HSV-1 *tk* gene) (moi = 200, hyb = 30%); *(E)* HSV (moi = 10).

We also have stocks of virus that contain the α-HCG gene. Populations are a mixture of hybrid (30%) and helper virus. In CV-1 and HeLa cells infected with these viruses, α-HCG can be detected by immunoprecipitation of [^{35}S]methionine-labeled proteins from cell extracts and from the culture medium. Time course experiments show that α-HCG is synthesized as a late viral protein. CV-1 cells do not synthesize α-HCG, and HeLa cells have low endogenous levels that are turned off late in adenovirus infection. In both cell types, large amounts of α-HCG are made, equivalent in amount to late viral proteins; thus, α-HCG can be detected among [^{35}S]methionine-labeled infected cell proteins without immunoprecipitation. The α-HCG made is glycosylated and secreted as is cellular α-HCG, and several forms are seen in both the cytoplasm and the medium. The molecular-weight species (20K and 16K in the cytoplasm and 22K in the medium) seen in α-HCG virus-infected HeLa cells are the same as those of endogenous α-HCG. When infected cells are treated with tunicamycin and labeled proteins are immunoprecipitated with α-HCG antiserum, the predominant forms are absent, but one lower-molecular-weight α-HCG protein is detected in the cytoplasm. The secreted α-HCG is insensitive to treatment with endoglycosidase H, although some of the cytoplasmic forms are cleaved.

Construction of Defective Adenovirus Recombinants That Express Polyoma T Antigens

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

We wanted to produce large amounts of polyoma T antigens so that these proteins can be more easily purified and their biochemical properties studied and compared to SV40 tumor antigens. We have been able to overproduce SV40 T antigen using adenovirus vectors, and we have now constructed two defective adenovirus recombinants that carry polyoma virus early coding sequences fused to the adenovirus third leader segment. These viruses also contain the SV40 early

promoter and T-antigen-coding region to allow helper function selection for recombinants.

One virus, RAdPySVR498, carries the entire polyoma early coding region. This virus expresses proteins that are immunoprecipitable with anti-polyoma tumor serum and that comigrate with the authentic polyoma T antigens on SDS-polyacrylamide gels. The hybrid virus, AdPySVR498, overproduces both middle T antigen (5-fold) and small T antigen (20-fold) in infected cells. Small T antigen can be detected among total [^{35}S]methionine-labeled infected cell proteins without immunoprecipitation.

S1-nuclease analysis of the hybrid-virus-encoded mRNAs suggests that the polyoma splicing signals are being correctly utilized and that the mRNA levels reflect the levels of the three T-antigen proteins. In addition, at least the third adenovirus leader segment is present at the 5′ end of the mRNAs. It should be noted that, although all three polyoma T antigens are synthesized in AdPySVR498-infected human or monkey cells, the ratios between the proteins are different from those found in polyoma-infected mouse cells. However, these lower levels of large T antigen relative to levels of middle and small T antigens correspond to the amounts of the respective mRNAs produced by the hybrid virus. This suggests that the donor splice sites for large T antigen versus middle- and small-T-antigen mRNAs are used with different efficiencies in adenovirus-infected cells. A similar situation is found with adenovirus-SV40 hybrid virus-infected monkey cells, where the donor splice sites for SV40 large and small T antigens are differentially utilized (Thummel et al., *Cell 23:* 825 [1981]).

The second virus, RAdLTSVR545, was constructed to carry a cDNA coding for the large T antigen of polyoma virus rather than the entire early coding region. Immunoprecipitation of proteins late in an RAdLTSVP infection shows a fivefold overproduction of 100K polyoma large T antigen. S1-nuclease analysis of RAdLTSVR545-encoded mRNA shows that the large T message contains the adenovirus third leader at its 5′ end. Using a fragment immunoprecipitation assay, we showed that the RAdLTSVP large T antigen binds specifically to the polyoma noncoding region, i.e., to the region around the origin of viral DNA replication and the early and late promoters. Independent binding to fragments on both sides of the *BglI* site was observed.

We are currently attempting to purify sufficient amounts of polyoma large T antigen from RAdLTSVR545-infected HeLa spinner cells to carry out detailed DNA-binding studies and to determine whether this protein will repress transcription from the polyoma early promoter in vitro. The hybrid virus, AdLTSVR545, contains pBR322 sequences downstream from the polyoma insert and represents less than 5% of the viral population. Thus, it is difficult to assess the maximum amount of polyoma large T antigen that could be produced. We do know that recombinant viruses containing the genes encoding HSV-1 Tk or α-HCG that constitute 10–20% of the viral population make very high levels of these proteins (Yamada and Grodzicker, see above).

Helper-free Ad5 Vectors: Expression of Complete or Truncated SV40 T Antigens by Recombinant Viruses during Lytic Infection

J. Harper, Y. Gluzman

As we reported last year, large amounts of SV40 T antigen are synthesized late after infection of human 293 cells with recombinant viruses carrying SV40-T-coding sequences. The largest amount is produced in cells infected with the virus containing the Ad2 MLP with 2-1/2 leader segments. Threefold less T antigen is produced in cells infected by viruses containing the Ad2 MLP with only half of the first late leader segment. Analysis of T-antigen-specific mRNAs extracted late after infection of 293 cells by the recombinant viruses revealed that the amount of these messages correlated with the amount of protein produced in these cells. These data demonstrate that under certain (as yet unknown) conditions, the mRNA with 2-1/2 leaders is not translated more efficiently than mRNA with only half of the first late leader segment. In addition to authentic T antigen, smaller polypeptides recognized by the large-T-specific monoclonal antibody PAb416 were seen in cells infected by viruses carrying the SV40 insert in the left-to-right ori-

entation. The truncated T antigens produced by viruses containing the Ad2 MLP + 2-1/2 leader segments appear to be 26K, and T antigens produced by viruses containing the Ad2 MLP + 1/2 leader segment appear to be 20K. The correlation between the orientation of the SV40 insert in the vector and synthesis of truncated T-antigen polypeptides suggested that the mRNA for these proteins might be produced by aberrant splicing of the SV40 RNA. Several examples of aberrant splicing of the RNA encoded by genes inserted into adenovirus have been reported (Thummel et al., *J. Mol. Appl. Genet. 1:* 435 [1982]; Berkner and Sharp, *Nucleic Acids Res. 10:* 1925 [1984]; Westphal, *J. Virol. 40:* 526 [1981]). We have analyzed the T-antigen-related polypeptides synthesized in rabbit reticulocyte lysates primed with RNA from cells infected by various recombinant viruses. The occurrence, size, and relative amounts of truncated T antigens synthesized in vitro were similar to those of the in vivo products. These data suggest that these proteins are primary translation products of RNA molecules specific to viruses that carry the SV40 insert in a left-to-right orientation. Both the 20K and 26K truncated T antigens contain the aminoterminal region of T antigen, since they are immunoprecipitated by monoclonal antibodies PAb430 (which recognizes both small T and large T antigens) and PAb416 (which is specific for a large-T-antigen determinant located within the aminoterminal 20% of the molecule), but are not immunoprecipitated by a mixture of carboxyterminal-specific T-antigen monoclonal antibodies (PAb414, PAb431, and PAb405).

S1-nuclease analysis of corresponding mRNAs indicates that truncated RNA is produced by at least two splicing reactions: removal of the normal large T intron and an additional splice using a donor in the second exon approximately 150 nucleotides downstream from the normal splice acceptor. A good splice donor consensus sequence appears in the SV40 DNA sequence at approximately this position (nucleotide 4424). Use of a splice donor in this region has been reported for one of the Ad2+ND_4 RNA species (Westphal, *J. Virol. 40:* 526 [1981]).

Our data show no obvious differences between RNAs encoding the 26K and 20K T antigens in the SV40 *Hin*dIIIB region. Differences in the protein-coding capacity of these RNAs may be due to small differences in splice sites that are not detected by this method, the use of different splice sites in the SV40 sequences 3′ to the region probed, or the use of different splice acceptors in the adenovirus E1B sequences. S1-nuclease experiments are in progress to examine the latter possibilities.

Integration and Stable Expression of Foreign Genes Introduced by Helper Independent Adenovirus Vectors in a Variety of Mammalian Cells

K. Van Doren

Last year, we reported that recombinant adenoviruses containing a selectable marker for neomycin resistance in E1 will integrate into the host-cell genome. The data obtained from analysis of the integrated viral DNA indicate that, in general, the viral DNA is present as almost full-length linear genomes. These observations suggest either that the viral DNA is preferentially integrated from the ends of the genome or that this occurs due to a positive selective pressure created by having the selectable gene at one end of the viral chromosome. Recombinant viruses in which the selectable marker for neomycin resistance replaces early region 3 (E3) of the adenovirus vector were used to address this point. These recombinant viruses contain the selectable marker far from the ends of the viral genome. Because the selectable marker is not located at the end of the viral chromosome, there should be no positive selective pressure exerted for the integration of the ends of the viral genome into the host-cell chromosomes. Recombinant viruses containing the neomycin resistance gene in E3 transformed CV-1 cells to G418 resistance as efficiently as recombinant viruses containing the neomycin resistance gene in E1. Preliminary analysis of the viral DNA contained in cell lines derived from these viruses suggests that the pattern of integration is very similar to that found for E1 replacement viruses. This indicates that integration occurs preferentially from the ends of the adenovirus genome.

Previously, we reported that a recombinant vi-

rus containing the SV40 early region with a defective origin of replication, located in E1, would efficiently transform human fibroblasts and that the pattern of integrated DNA was identical to that reported for the neomycin recombinant viruses. Because human fibroblasts are semipermissive for SV40 DNA replication, we were unable to assess the state of the integrated DNA in cells transformed by recombinant viruses containing an SV40 functional origin of replication due to the presence of large amounts of freely replicating DNA. Upon subcloning of the parental transformed cell lines, we observed that two of the three subcloned cell lines had lost the ability to produce freely replicating SV40 DNA molecules. The subclones contained T antigen as detected by immunofluorescence, indicating that they had not lost the SV40 DNA. It was possible that a mutation occurred in a cellular permissivity factor or in the viral T antigen so that it could no longer function in DNA replication. We fused the subclones to either COS-1 or CV-1 cells and found that the T antigen in the human cells had mutated so that it no longer supported SV40 replication, which suggests that there is a strong selective pressure against having a functional SV40 replication system in human cells. The pattern of integrated viral DNA in these cells was found to be identical to that observed for human cells transformed by recombinant viruses containing a defective SV40 origin of replication.

We are currently characterizing the use of a double-replacement vector in which foreign genes can be cloned into both E1 and E3. This vector will make it possible to introduce nonselectable genes into mammalian cells by using a selectable marker in the second replacement site. Double recombinant viruses containing the human β-globin gene with a selectable marker for either neomycin resistance or methotrexate resistance (DHFR) are being constructed and analyzed for the ability of the viruses to introduce the globin gene into a variety of cells. The feasibility of using the DHFR gene as a method to amplify the integrated viral DNA will also be explored.

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DNA SYNTHESIS

B.W. Stillman P. Cotton, J. Diffley, E. White, G. Prelich, B. Faha, S. Penzi

Over the past 5 years, we have concentrated our studies on the biochemical analysis of eukaryotic DNA replication using adenovirus as a model system. This work is now at a stage where it is possible to replicate completely adenovirus DNA in vitro with purified proteins. Three of these proteins are encoded by the virus and two are produced by the host cell. Thus, despite the atypical mechanism of adenovirus DNA replication, two cellular proteins required for adenovirus DNA replication have been identified, and it is possible that these proteins function in important cellular processes such as DNA replication. However, because adenovirus replicates its DNA differently from the way a cell replicates its chromosomal DNA, we have turned to the biochemical analysis of DNA replication of another DNA tumor virus, SV40, which provides an excellent model for chromosomal replication in cells. During this year, we have developed a cell-free system for the replication of SV40 DNA. Since the replication of SV40 DNA and that of cellular DNA appear to be similar, we expect that characterization of this system will provide valuable insight into the mechanism of initiation, elongation, and termination of eukaryotic DNA replication and ultimately enable a molecular analysis of the control of these processes.

In addition to our studies of DNA replication, we have continued to probe the biochemical functions of the adenovirus tumor antigens. One of these proteins has been shown to be localized to the nuclear envelope of infected and transformed cells, and mutations in the protein induce the degradation of cellular DNA. We have now found that this DNA degradation is specific for the type of human cell that is infected by the mutants.

Adenovirus DNA Replication

J. Diffley, G. Prelich, B. Stillman

Two cellular proteins are required for the complete replication of adenovirus DNA in vitro. One of these proteins, nuclear factor II, contains a type I topoisomerase activity and is required for complete elongation of nascent DNA strands. The other protein, nuclear factor I, is a site-specific DNA-binding protein that binds to sequences within the origin of adenovirus DNA replication, and this binding is required for efficient initiation of replication. It is known that the protein can bind specifically to cellular DNA sequences that are present at approximately 1 in every 100,000 base pairs in the human cell genome (Gronostajski et al., *Proc. Natl. Acad. Sci. 81:* 4013 [1980]), but the function of the protein in uninfected cells is not known. At present, the only assay for this protein for purification purposes is its ability to stimulate adenovirus DNA replication in the presence of the three virus-encoded proteins, the single-stranded DNA-binding protein (DBP), the adenovirus DNA polymerase (Adpol), and the adenovirus precursor terminal protein (pTP). However, this assay requires that these three proteins be substantially pure prior to purification of nuclear factor I. We have therefore developed a simple, more rapid assay for nuclear factor I that can detect the functional protein in crude extracts of human cells and is independent of its role in initiation of adenovirus DNA synthesis.

The assay for nuclear factor I utilizes its specific DNA-binding properties. Briefly, DNA containing the binding site for nuclear factor I is labeled at each terminus with ^{32}P and mixed with crude extracts derived from the nuclei of HeLa cells and a mixture of synthetic homopolymers as competitor DNA. The binding of nuclear factor I to the ^{32}P-labeled DNA is assayed by a simple nitrocellulose-filter-binding method, and the retention of labeled-DNA–protein complexes on the nitrocellulose is detected by autoradiography or scintillation counting. The assay is dependent on time and temperature, the presence of Mg^{++}, and the concentration of NaCl in the reaction mixture. The competitor DNA is an equal mixture of the synthetic homopolymers poly(dA):

oligo(dT) and poly(dC):oligo(dG) and must be added to the nuclear extract prior to the addition of labeled DNA. It is preferable that the labeled DNA be as short as possible, and we routinely use fragments in the 35–200-bp range. As a control for nonspecific binding to DNA of proteins present in the crude extract, a labeled DNA fragment that does not contain the binding site for nuclear factor I is used, usually a DNA fragment containing a mutated binding site. The presence of nuclear factor I in the extract is observed when DNA containing the binding site is preferentially retained on nitrocellulose, but the control DNA fragment fails to bind. Using this assay, we have purified nuclear factor I from HeLa cells, and the activity coelutes at every purification step with the reconstitution of adenovirus DNA replication activity of the protein. We are currently concentrating our efforts on determining the normal cellular function of the nuclear-factor-I protein.

This assay for site-specific DNA-binding proteins in extracts prepared from eukaryotic cells is generally applicable to other proteins. Site-specific DNA-binding proteins have been shown to regulate transcription of certain genes in eukaryotes (Dynan and Tjian, *Cell 35:* 79 [1983]; Parker and Topol, *Cell 36:* 357 [1984]) and may be important in other cellular processes such as DNA replication and cellular differentiation. Using the rapid filter-binding assay, we have detected in crude extracts from HeLa cells a protein that binds specifically to the 21-bp repeat region of the SV40 early promoter, and it is most likely that this protein corresponds to the transcription factor SP1 required for transcription of the SV40 early promoter in vitro (Dynan and Tjian, *Cell 35:* 79 [1983]). This will provide a rapid assay for the purification of this protein and identification of its molecular weight and any associated enzymatic activities.

In addition to biochemical analysis of these cellular encoded proteins, structural and functional studies on the adenovirus DBP are continuing. This protein is required for elongation of adenovirus DNA replication, and, in addition, it is involved in the control of early mRNA synthesis and late gene expression. We have now sequenced the mutation in the DBP from the mutant Ad2*ts*111 and have found that it lies in the middle of the protein sequence, away from the other well-characterized DBP temperature-sensitive mutation in Ad5*ts*125. The Ad2*ts*111 mutation lies adjacent to another mutation present in Ad5*ts*23 (J. Williams, pers. comm.); we have obtained this virus and are currently comparing the in vitro biochemical properties of the mutant and wild-type proteins. These studies should complement the structural studies on DBP by Tsernoglou et al. (*J. Mol. Biol. 172:* 237 [1984]), who have crystallized a fragment of the DBP and are currently determining the three-dimensional structure of the protein.

SV40 DNA Replication

B. Stillman

The tumor virus SV40 contains a double-stranded, circular DNA genome of 5243 bp and replicates efficiently in monkey cells. DNA replication can also be detected in some, but not all, human cell lines but does not occur in mouse cells. The viral DNA is part of a nucleoprotein complex that is very similar in structure to the cell's chromosome, and replication of SV40 DNA in vivo is followed by duplication of the nucleosome structure. Replication proceeds from a single origin of replication and is dependent on a single virus-encoded protein, the SV40 tumor (T) antigen. Because of these properties, it is most likely that SV40 replicates its DNA in a similar way to cell chromosomal DNA replication. We therefore set out to prepare cell-free extracts that would support complete SV40 DNA replication and eventually study the mechanism and control of eukaryote DNA synthesis in some detail.

Recently, Li and Kelly *(Proc. Natl. Acad. Sci. 81:* 6973 [1984]) have developed a cell-free system for the replication of SV40 DNA, using extracts from monkey cells, and we have modified this system for the use of human cells. We decided to use human cells for SV40 DNA replication studies, since monkey cells proved to contain high levels of protease activities and were difficult to maintain in suspension cultures, which would be necessary for obtaining large amounts of cellular protein for purification purposes. It was shown previously that human 293 cells could support efficient SV40 DNA replication in vivo but that replication in human HeLa cells was very ineffi-

cient (J. Manley and M. Calos, pers. comm.). Human 293 cells, which are adenovirus-transformed human embryo kidney cells that express adenovirus tumor antigens encoded by the E1A and E1B genes, were adapted for growth in suspension cultures.

Because cell extracts were prepared from uninfected cells, it was necessary to supply the SV40 T antigen to reconstitute DNA replication. For this purpose, a modified adenovirus that produces large amounts of SV40 T antigen was constructed by Y. Gluzman and his colleagues (this section). The T antigen produced from this vector was purified to homogeneity by immunoaffinity chromatography on a protein A–Sepharose column to which the anti-T monoclonal antibody PAB419 had been covalently bound (Simanis and Lane, *Virology* [1985] in press). With this purification procedure and the adenovirus vector system, milligram quantities of highly active T antigen can be purified with great ease.

Extracts prepared from both the nucleus and cytoplasm of human 293 cells were used in combination with the purified SV40 T antigen. In the presence of the cytoplasmic extracts and SV40 T antigen, replication of plasmids containing the origin of DNA replication was achieved. Replication was shown to be very efficient; up to 70% of the input DNA molecules could be replicated (calculated on the basis of nucleotides incorporated per nucleotides added), and DNA synthesis occurred in both directions from the origin region. Multiple rounds of SV40 DNA synthesis were also observed, and the available data suggest that replication proceeds in a manner similar to that of SV40 DNA replication in vivo. Furthermore, initial studies with SV40 origin mutants have demonstrated similar DNA sequence requirements in vivo and in vitro.

The products of the replication reaction made in the presence of SV40 T antigen and the cytoplasmic extract were shown to be covalently closed, circular, but relaxed DNA. Addition of an extract prepared from the nuclei of human 293 cells converted these relaxed circular DNA molecules to negatively supercoiled DNAs (see Fig. 1). The introduction of negative supercoils into replicated DNA required factors from the cytoplasmic extracts because the nuclear extract alone did not alter the topological state of the DNA. Indeed, the process of negative supercoiling of the

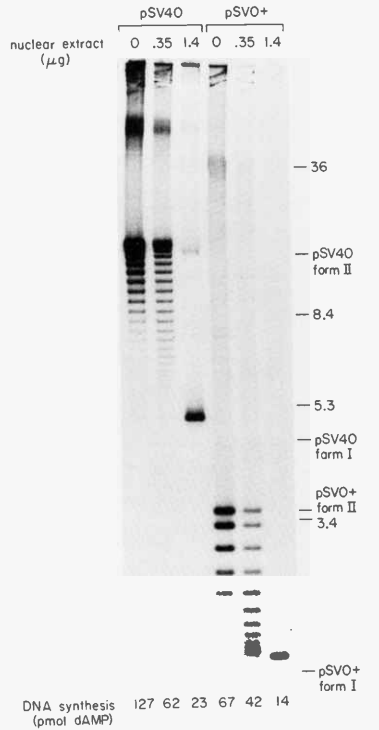

FIGURE 1 Replication and supercoiling of SV40 DNA in vitro. Plasmid DNAs containing the entire SV40 genome (pSV40) or a 200-bp fragment from the SV40 origin region (pSV0+) were incubated for 2 hr at 37°C with an extract derived from the cytoplasm of human 293 cells and purified SV40 T antigen. In addition, the reactions contained either 0, 0.35, or 1.4 μg of an extract prepared from the nuclei of 293 cells as indicated. The reactions were stopped, and the DNA was isolated and subjected to agarose gel electrophoresis. The marker DNAs were pSV40 DNA (forms I and II) and pSV0+ DNA (forms I and II) and adenovirus DNA or *Hin*dIII restriction fragments of adenovirus type-2 DNA. The gel shows an autoradiogram of the ^{32}P-labeled replication products.

DNA only occurs during the process of replication itself. It is thus likely that negative supercoiling of the DNA in this in vitro system occurs

concomitantly with DNA synthesis. This result was of some interest because the negatively supercoiled DNA was stable, even in the presence of high levels of the type I topoisomerase in the extracts. This latter activity normally relaxes negatively supercoiled DNA. It therefore seems that a novel regulation of the higher order of structure of DNA is occurring in these extracts. It will be of interest to determine whether the DNA is supercoiled by a DNA gyrase activity that is present in the extracts or whether the DNA is associated with protein that causes the DNA to alter its topological structure.

The SV40 replication system should prove useful for the elucidation of the mechanism of DNA synthesis in eukaryotic cells and eventually lead to the identification of replicative proteins. It is expected that a better understanding of the control of DNA replication and cell proliferation will be obtained.

Adenovirus Tumor Antigens

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

In addition to our studies on DNA replication, we have continued with the biochemical analysis of the adenovirus tumor antigens that are produced from the E1B genes. Two tumor antigens are synthesized from this early gene region and both proteins appear to be required for complete transformation of cells. In last year's Annual Report, we described our studies on the E1B 19,000-dalton (19K) tumor antigen. Single point mutations in, or a deletion within, the coding region of the E1B 19K gene in the adenovirus genome caused a drastic phenotype in infected cells. The cellular morphology was severely altered, and cellular DNA was degraded into low-molecular-weight fragments during the course of infection of human HeLa cells with these viral mutants. We have characterized these phenotypes, which are the result of two separable functions of the E1B 19K protein: The *cyt* mutations caused an altered cytopathic effect in mutant infected cells and the *deg* mutations caused the degradation of the cell's chromosomal DNA. All of the *deg* mutants demonstrated the Cyt phenotype, but the *cyt* mutant *lp3* did not induce degradation of cellular DNA. The phenotype of these E1B 19K mutants is summarized in Table 1.

We had previously demonstrated by indirect immunofluorescence at the light microscopic level, and by biochemical fractionation, that the E1B 19K protein in wild-type adenovirus-infected HeLa cells was localized to the nuclear envelope; 50% of the protein was tightly associated with the nuclear envelope lamina and the remaining fraction was less tightly associated with the lamina and was probably localized in (or on) the nuclear envelope membranes. In adenovirus-transformed cells, the E1B 19K protein localized to the nuclear envelope and also to a cytoplasmic membrane structure. Nuclear envelope localization of the tumor antigen in infected cells was confirmed by immunoelectron microscopy, which was done in collaboration with S. Blose (Cell Biology Section). The protein localized to both the inner and outer nuclear envelope membranes but did not localize in the interior of the nucleus (see Fig. 2).

TABLE 1 Phenotypes of E1B 19K Gene Mutants

Virus	Cyt[a]	Deg[b]	19K protein	Transformation[c]
Ad2 wild type	Cyt⁻	Deg⁻	+	Tra⁺
Ad2*cyt*106	Cyt	Deg	+	Tra[d]
Ad5*lp3*	Cyt	Deg⁻	+	Tra
Ad5*lp5*	Cyt	Deg	+	Tra
Ad5*d*337	Cyt	Deg	–	Tra

[a]The Cyt phenotype is an enhanced and abnormal cytopathic effect in infected cells. Wild-type phenotype is Cyt⁻.

[b]The Deg phenotype is the production of degraded cellular DNA during lytic infection. Wild-type phenotype is Deg⁻.

[c]Tra⁺ indicates that the virus has transforming potential.

[d]Only when the mutation is in a temperature-sensitive background.

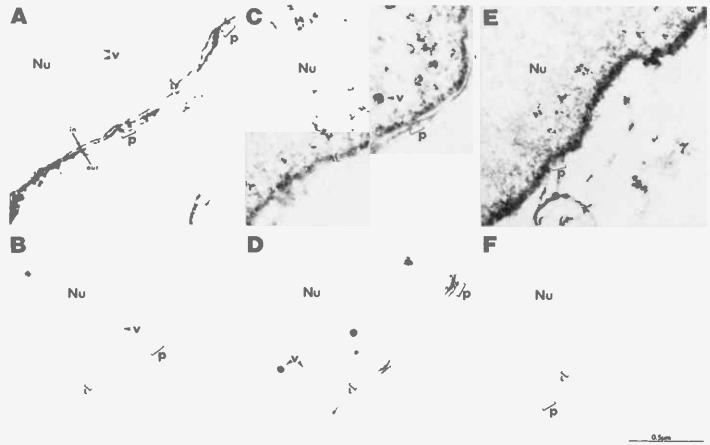

FIGURE 2 Immunoperoxidase electron microscopic staining of the nuclear-envelope-associated E1B 19K tumor antigen. HeLa cells were infected with wild-type adenovirus for 41 hr or mock-infected, and the cells were then fixed and stained with antibodies against either the E1B 19K protein or the nuclear lamina. Electron micrographs of thin sections through nuclei of Ad2 (*A–D*) or uninfected HeLa cells (*E,F*) stained with anti-19K protein (*A*) or anti-lamin (*C,E*) antibodies. For comparison, the control panels represent reactions with goat anti-rabbit (*B*) or rabbit anti-human (*D,F*) horseradish-peroxidase-conjugated second antibodies alone. The nucleus (Nu), pore complex (p), virion (V), inner (in) and outer (out) nuclear membranes, and lamina (L) are indicated. Magnification, 83,915×.

These results confirmed our previous data and combined suggest that the E1B 19K protein is associated with the nuclear lamina on the membrane side of this structure, as well as with the nuclear envelope membranes.

In addition to the E1B 19K tumor antigen, we observed that late in infection, several adenovirus structural proteins were also found associated with the lamina. The precursor (but not the processed forms of virion proteins VI and VII) and protein IX were selectively enriched in the nuclear lamina preparations. Furthermore, the major nonstructural protein produced late in infection, the 100K phosphoprotein, was shown to be enriched in nuclear envelope membrane preparations but not in the nuclear lamina preparations. It is therefore likely that this protein is associated with the nuclear envelope membranes. These observations suggest that certain aspects of virion morphogenesis occur on either the nuclear envelope or an internal nuclear structure that is part of a nuclear matrix.

Of particular interest was the association of the E1B 19K tumor antigen with the nuclear envelope and the fact that mutations in the E1B 19K gene caused degradation of cellular DNA. This correlation was observed in virus-infected HeLa cells, and in these cells, the yield of virus was only reduced about tenfold compared with the wild-type virus. As part of a search to find a human cell line in which the E1B 19K mutants were more defective than wild type, it was noticed that in some cells, the mutants did not induce degradation of cellular DNA. When primary human embryo kidney (HEK) cells, or WI38 human diploid fibroblast cells, were infected with the E1B 19K mutants, the mutant viruses did not induce degradation of cellular DNA and surprisingly grew to significantly higher titers than the wild-type virus. On the other hand, the E1B 19K mutants

were extremely defective for virus production in human KB cells and caused enhanced DNA degradation, whereas wild-type virus grew efficiently in these cells.

We have thus identified three classes of human cells that respond in different ways to the E1B 19K mutants and that clearly demonstrate the host-range phenotype of these mutants. The first class, represented by HEK and WI38 cells, allow the E1B 19K mutants to grow efficiently and escape the events that lead to the degradation of DNA seen in the other cells. In the second class, represented by HeLa cells and 293 cells, the mutants cause degradation of cellular DNA, but the virus is able to grow at nearly wild-type levels. In the third class of cells, represented by KB cells, the mutant viruses are severely defective for growth and cause enhanced destruction of the cellular DNA. We are currently examining at which stage of the lytic cycle the mutant viruses are blocked in infected KB cells.

The identification of a cell line in which the virus is defective should enable revertants of the E1B 19K mutants to be isolated and characterized. The E1B 19K mutant, *dl*337, which does not produce any 19K protein due to a large deletion of coding sequences for this protein, can still induce DNA degradation upon infection into HeLa and KB cells. Thus, since the 19K protein is absent in *dl*337-infected cells, another virus protein (or proteins) must be responsible for causing the Cyt and Deg phenotypes. It is hoped that the revertants will identify this gene product. We are also creating double mutants in vitro, in which selected early gene products are mutated in the *dl*337 or *cyt*106 viral genomes.

The other protein produced from the E1B region is the 57,000-dalton (57K) tumor antigen. We have previously demonstrated that mutations in this protein cause a delay in the onset of DNA synthesis during lytic infection but that the protein is not required for DNA replication directly. The E1B 57K protein also has been shown to associate with the p53 cellular tumor antigen, as does the SV40 T antigen, so that it is possible that SV40 T antigen and the E1B 57K protein share a common biochemical activity. In an attempt to identify a role for this protein in lytic infection and cell transformation, we are currently examining methods of overexpressing the protein and eventually purifying the protein. To this end, a number of monoclonal antibodies directed against this protein have been isolated and are currently being characterized.

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NUCLEIC ACID CHEMISTRY

R.J. Roberts A. Bhagwat, J. Dellaporta, G.A. Freyer, C. Keller, A. Kiss, P.-M. Lin, P.A. Myers, K. O'Neill, K. Tohill, M. Wallace, B. Zerler

The *Bsu*RI Restriction-Modification System

A. Kiss

We have continued the characterization of the GGCC-specific *Bsu*RI restriction-modification system of *Bacillus subtilis* that we have cloned and expressed in *Escherichia coli*. The *Bsu*RI genes, cloned originally on a 9.5-kb *Sph*I-*Sal*I fragment, were localized to a 4.3-kb *Hpa*I-*Sal*I fragment. Since the sequencing of the methylase gene, using a clone that codes only for methylase (Kiss and Baldauf, *Gene 21:* 111 [1983]), was already in progress in P. Venetianer's laboratory in Hungary, we concentrated our efforts on the endonuclease gene.

A detailed restriction map of the region has been established, and specific fragments have been cloned in the M13 phage vectors mp18 and mp19 and sequenced by the chain-termination method. The region we have sequenced is 2904 bp in length and codes for the endonuclease and the aminoterminal 32 amino acids of the methylase. The endonuclease is coded by a 1728-bp open reading frame specifying 576 amino acids. The two genes are oriented in the same direction, with the methylase gene being downstream from the endonuclease gene. The coding regions of the two genes are separated by 780 bp, which is longer than any other intergenic region found so far in restriction-modification systems. This region contains an open reading frame coding for 146 amino acids. A Shine-Dalgarno sequence preceding this reading frame suggests that it may direct the synthesis of a small protein, but experiments with derivatives in which this reading frame has been disrupted indicate that even if this protein is produced, it probably has no role in the functioning of the *Bsu*RI system. The open reading frame coding for the methylase is preceded by a strong Shine-Dalgarno sequence, whereas the sequence preceding the start codon of the endonuclease gene allows for only weak Shine-Dalgarno interactions. This difference may be important in regulating the expression of the *Bsu*RI system.

We have not been able to clone the endonuclease in the absence of methylase, indicating that the expression of the restriction enzyme is not dependent on the presence of active methylase. The two genes are expressed when cloned on two different plasmids in the same cell, suggesting that they do not constitute an operon. Transcription of the *Bsu*RI genes is currently being studied by analysis of the cDNA products synthesized on in vivo RNA using specific oligonucleotide primers. These experiments confirm that the two genes are transcribed separately and indicate that the methylase-specific mRNA is more abundant in the *B. subtilis* cell than the endonuclease-specific transcript.

Our main purpose in studying restriction and modification enzymes is to find out how these proteins recognize specific DNA sequences. Comparison of different proteins interacting with the same DNA sequence may be helpful in this respect. A search of the amino acid sequences derived from the DNA sequence revealed no homology between the *Bsu*RI endonuclease and methylase. However, regions of homology were found between the *Bsu*RI methylase and two other GGCC-specific DNA methylases, the *Bsp*RI and SPR methylases. We wish to use mutagenesis of the cloned enzymes to determine which parts of the proteins are responsible for sequence-specific DNA recognition.

The *Eco*RII Restriction-Modification System and the *dcm* Gene

A. Bhagwat

The *Eco*RII endonuclease, the *Eco*RII methylase, and the product of the *Escherichia coli dcm* gene

all recognize the sequence 5′-CC(A/T)GG-3′. The *Eco*RII genes were originally found to reside on a low-copy-number *E. coli* plasmid N3, and were subsequently cloned onto multicopy plasmids. The *dcm* gene, which resides at 43 minutes on the *E. coli* chromosome, codes for a methylase with the same specificity as *Eco*RII methylase. We have found that despite this known identity in their specificities, in vivo, the *dcm* gene is unable to complement endonuclease$^+$ methylase$^-$ (R^+M^-) mutants of *Eco*RII.

To understand better the relationship between these genes, we have cloned the *dcm* locus of wild-type *E. coli* on to pBR322. From a *Sau*3A partial library of *E. coli* DNA in pBR322, the clone carrying the *dcm* locus was selected by treating the DNA from a mixture of clones with the *Eco*RII endonuclease, followed by transformation into a *dcm* deletion (Ddcm) strain. Of the 18 transformants thus obtained, 1 carried a plasmid that was found to be resistant to the *Eco*RII endonuclease. Restriction analysis showed that this plasmid, designated pDCM1, has an 11.3-kbp insert at the *Bam*HI site.

When used as a probe in Southern blots, this plasmid hybridizes to several *Eco*RV fragments from the chromosomal DNA of a dcm$^+$ strain but to only one *Eco*RV fragment from the DNA of the Ddcm strain. We thus conclude that the insert in pDCM1 is derived from the same region in which the Ddcm strain has a deletion. On the basis of genetic evidence, *E. coli* codes for only one cytosine methylase, *dcm*; hence, we conclude that pDCM1 must carry the *dcm* locus.

A probe containing the *Eco*RII methylase gene does not hybridize to the insert within pDCM1, nor does it hybridize to any *Eco*RV fragments of *E. coli* chromosomal DNA. However, pDCM1 is able to complement an R^+M^- mutant of *Eco*RII. Thus, *dcm* is able to complement the *Eco*RII methylase from a multicopy plasmid location but not from the single-copy chromosomal location. This is probably due to the greater production of the enzyme from the plasmid. Finally, a host carrying pDCM1 does not restrict incoming phage, which indicates the lack of any endonuclease. In summary, the products of the *dcm* gene and the *Eco*RII methylase gene share the same sequence specificity, but the genes are not homologous. From its chromosomal location, the *dcm* gene product is not able to methylate all of its substrate sites, but it is able to do so from the plasmid location. There may be no endonuclease gene linked with *dcm*.

We have constructed protein fusions between the *Eco*RII endonuclease and β-galactosidase of *E. coli*. The endonuclease gene was cloned, in both orientations, in front of the β-galactosidase gene lacking its promoter, initiator methionine codon, and ribosome-binding site. The resulting phenotype was R^+Lac^-. *E. coli* strains (Dlac) carrying one of these two plasmids and a compatible plasmid carrying the methylase gene were streaked out on MacConkey-lactose plates. Several red (Lac^+) colonies spontaneously grew up for one of the orientations of the endonuclease gene but not for the other. These Lac$^-$ colonies were picked and analyzed. Seven independent colonies contained plasmids with deletions at the endonuclease–β-galactosidase junction ranging in size from 50 bp to 1.3 kb. Strains carrying these plasmids gave rise to white colonies on tetrazolium plates, suggesting the equivalent of 400 or more units of β-galactosidase activity as analyzed by the Miller assay. The two smallest deletions, which may have up to 30 amino acids missing from the carboxyl terminus of the endonuclease, restrict incoming phage as well as the wild-type gene. A deletion removing up to 66 amino acids reduces the ability of the cell to restrict incoming phage by a factor of 2×10^2 to 5×10^2. The plating efficiency of the phage is still 20–50-fold lower on this strain compared with that on an R^+ strain. Larger deletions completely destroy the ability of the cell to restrict phage. These protein fusions define the direction of transcription of the endonuclease gene. They also suggest that the carboxyl terminus of the endonuclease may not be essential for its action and argue that a hybrid protein carrying both the endonucleolytic activity and lactose metabolizing activity can be constructed.

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 original clone contains a 15-kb insert. A series of subclones have been prepared, and one of these, containing a 3-kb insert, still expresses both activities. The entire insert of this clone has been sequenced. Three open reading frames are present, of which one, encoding a polypeptide of 418 amino acids (M_r = 47,664), has been shown to encode *Msp* methylase. A second open reading frame encodes a polypeptide of 262 amino acids (M_r = 29,833) and is the restriction endonuclease gene. A third open reading frame, of unknown function, partially overlaps with the carboxyl terminus of the restriction endonuclease gene and continues uninterrupted into the flanking pBR322 sequences.

The genes for the restriction enzyme and the modification methylase are transcribed in opposite directions, and the first methionines present in each reading frame are separated by 110 nucleotides. Upstream of both AUGs are sequences that closely resemble the Pribnow box found in *Escherichia coli* promoters. The two genes are extremely AT-rich and show no obvious homology with each other at either the nucleic acid level or protein sequence level. Furthermore, they show no clear homologies with any other restriction enzyme or modification enzyme genes that have been sequenced to date.

Restriction Endonucleases

P.A. Myers, B. Zerler, R.J. Roberts

The collection of restriction enzymes continues to grow and more than 500 such enzymes are now known; 116 different specificities have now been characterized, including eight in the last year. Among these new specificities, the recognition sequences for *Bsm*I (GAATGC), *Nhe*I (G↑CTAGC), *Ssp*I (AAT↑ATT), and *Spe*I (A↑CTAGT) have been characterized, as part of a collaborative program with I. Schildkraut and D. Comb (New England BioLabs). In addition, we have shown that *Mja*I is an isoschizomer of *Mae*I (CTAG), *Mja*II is an isoschizomer of *Asu*I (GGNCC), and *Nfl*aI is an isoschizomer of *Eco*RV (GATATC). As shown in Table 1, 44 of the possible 64 enzymes that could recognize hexanucleotide palindromes have now been discovered.

TABLE 1 Simple Hexanucleotide Palindromes

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

In Vitro Splicing of Ad2 Late RNA

G.A. Freyer, K. Tohill

Extracts made from HeLa cell nuclei have been used to study RNA splicing in vitro. The substrate, which contains the first and second exon and the first intron of the tripartite leader of Ad2 major late RNA, is synthesized in vitro using a HeLa whole-cell extract. Additionally, we have constructed recombinant plasmids that can produce similar precursor RNAs when driven by the bacteriophage λ promoter P_L or the *Salmonella* phage promoter SP6. All of these transcripts contain a modified version of the first intron, in which 934 of the 1020 nucleotides have been deleted. These substrates have all been shown to splice efficiently and correctly.

When a ^{32}P-labeled runoff transcript of 168 nucleotides is incubated in nuclear extracts, the correct joining of the first and second exons takes place to generate the final splice product; in addition, three potential intermediates are detected. On a 10% polyacrylamide-urea gel, these inter-

mediates migrate with apparent lengths of 41, 68, and 118 bases. Fingerprint analysis has shown that the 41 species is the free first leader. The 68 species has not yet been completely characterized, although it appears to come from the 3′ end of the RNA. On the basis of electrophoretic mobility and fingerprint analysis of the 118 band, it appears to be a circular molecule, 63 bases in length, which contains sequences from the 5′ end of the intron. It is almost certainly the circular portion of the lariat molecules described by other investigators (Krainer et al., *Cell 36:* 993 [1985]; Grabowski et al. *Cell 37:* 415 [1985]). These lariats contain sequences from the complete intron and the adjacent exon but have an unusual structure in that the 5′ end of the intron has formed a 2′-5′ linkage to an A residue close to the 3′ end of the intron; thus, they contain a circular RNA structure with a tail. It is the tail that appears to be missing in the 118 band. The lariat is believed to be the intermediate that combines with the first exon to form the final splice product. Its absence in our system suggests either that it turns over extremely rapidly or that some other intermediate may be involved. The 68-nucleotide-long RNA, which contains the 3′ end of the precursor, is the most likely candidate for this role. Experiments are under way to test this possibility.

To investigate the necessity of lariat formation, we have synthesized a mutant template, in which both A residues at and close to the lariat attachment site were changed to G residues. When the ^{32}P-labeled transcript, made from this plasmid, was incubated in our splicing extract, the only visible product was the 41-nucleotide band that results from cleavage at the donor splice site for the first leader RNA. Although this accumulated to high levels, the remaining RNA was degraded extensively. This result suggests that at least one function of the lariat may be to protect the RNA from degradation. It also argues that lariat formation is essential for splicing.

Fate of Ad2 Introns In Vivo

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

Our initial interest in examining introns from in vivo RNA was to test a hypothesis concerning the mechanism of splicing. This model proposed a simple reciprocal cleavage and ligation event occurring at the intron/exon boundary, leading to a linear final splice product and a circular intron. The system that we chose to examine was the tripartite leader from the major late RNA of Ad2. Two oligonucleotides were synthesized that would hybridize across the predicted junctions of circular introns corresponding to either the intron between leaders 1 and 2 or the intron between leaders 2 and 3. Nuclear and cytoplasmic RNAs were prepared separately from Ad2-infected HeLa cells. The RNA was electrophoresed on denaturing agarose gels, blotted, and probed with these ^{32}P-labeled oligonucleotides. The oligonucleotide, designed to probe the first intron, failed to hybridize to any RNA species. This result is consistent with the circularization of this intron at an internal position as indicated above. However, the oligonucleotide, designed to probe the second intron, did hybridize to several RNA species. In control experiments, it was shown that some of these species were present in uninfected HeLa cells. The structures of the Ad2-specific species are under investigation.

To investigate the fate of the first intron sequences, two oligomers were synthesized, with sequences complementary to linear portions of the first intron. These oligomers gave an interesting and surprising result. They detected RNA species that were present in abundance in the cytoplasm of Ad2-infected cells but were undetectable in the nucleus. Two species of 1000 and 1100 nucleotides were found, which is the approximate size of the linear intron. Although the precise structures of these RNAs are still being examined, one behaves like a linear molecule, and the other migrates anomalously on gels and may well be a circular molecule.

Both of these oligomers have been used as primers in dideoxynucleotide sequence analysis of these RNAs. A unique sequence was obtained that begins near the 3′ end of the oligomers and extends up to the 5′ penultimate nucleotide of the intron. These results show that the oligomers are hybridizing specifically and also indicate that the structure of the intron at its 5′ boundary is such that it prevents further extension with reverse transcriptase. Such a block could be a free 5′ end as might be found in a linear molecule cleaved at the donor splice site or could be an unusual linkage, such as the 2′-5′ linkage that characterizes

lariat attachment sites. Additionally, it is clear from these results that the oligomer is priming on a processed intron, rather than merely detecting the linear sequences from the precursor RNA.

Several other oligomers have been synthesized and used to probe specific regions of these RNAs. First, an oligomer complementary to the 23 nucleotides between the lariat attachment site and the 3′ end of the RNA was used as a probe. Although this oligomer hybridized to a high-molecular-weight RNA in the nucleus, there was no detectable hybridization to the cytoplasmic RNA. An oligonucleotide complementary to the 3′ region of the intron that spans the lariat attachment site was used as a probe at low stringency and was found to hybridize to both species, suggesting that the boundary of these RNAs is at or near the lariat attachment site. Finally, a third oligomer was made that spans the lariat attachment site by 13 nucleotides on either side. Under low stringency, this oligomer was shown to hybridize to only one RNA species.

From these results, we conclude that the two intron species contain similar, if not identical, sequences and that the difference in mobilities is likely due to a difference in structure, i.e., a linear and possibly circular RNA. Neither contains the 3′-terminal fragment comprising the "handle" of the lariat. However, both hybridize to a probe near the 2′-5′ junction. This is the first report of the presence of free intron sequences in the cytoplasm of cells.

Ad2 E1A Gene Products

B. Zerler

The adenovirus E1A gene is required for adenovirus-induced cell transformation and is involved in the regulation of other adenovirus gene products. Early in infection, two mRNAs are produced from E1A, the 12S and 13S products. Late in infection, a 9S mRNA product is made. The nucleotide sequence of the 12S and 13S products indicate that they code for proteins 243 and 289 amino acids long, respectively. These proteins differ only by the presence of an additional 46 amino acids unique to the 13S product. The 9S product is predicted to encode a protein 55 amino acids long, which as a result of splicing enters a different reading frame from that of the 12S and 13S products. Therefore, only the aminoterminal 26 amino acids of the 9S protein are common to the 12S and 13S proteins. Our aim is to distinguish the roles of the individual E1A products. Last year, we separated the 12S and 13S products by isolating the corresponding cDNAs. We also constructed a 9S cDNA clone by using synthetic oligomers that span the splice junction. These cDNAs were subcloned into plasmids and also rebuilt into the entire viral genome in place of the genomic E1A region. For a detailed description of our results on the biological roles of the individual E1A gene products using these cDNA plasmids and viruses, see Mathews et al. (this section).

We are presently concentrating on expressing E1A in other eukaryotic systems. We have recently expressed the 12S and 13S E1A cDNA products in yeast in order to take advantage of the extensive potential for genetic analysis of wild-type and mutant E1A functions in this organism. The E1A cDNAs were cloned into a yeast expression vector under transcriptional control of the inducible *GAL10* promoter. Expression of both E1A gene products has been detected in yeast colonies by an immunological method described by Lyons and Nelson (*Proc. Natl. Acad. Sci. 81:* 7426 [1984]). In addition, immunoprecipitation and Western blot analysis show the presence of two 13S-specific proteins with molecular weights similar to those of the two major 13S proteins present in HeLa cells during Ad2 infection. Similar studies with the 12S-E1A-encoded product in *Saccharomyces cerevisiae* are in progress.

When grown under inducing conditions, *S. cerevisiae* containing either of the E1A gene products does not exhibit any observable phenotype. Generation time and morphology appear similar to those of controls. We are currently investigating the ability of the E1A 13S and 12S products to complement various yeast temperature-sensitive *cdc* mutants.

In addition to the above studies, we are cloning E1A into mammalian high-expression vectors to enable purification of the proteins. A BPV vector containing genomic E1A has been constructed and transfected into NIH-3T3 cells. Transformants have been isolated and are currently being screened by immunofluorescence and immunoprecipitation for the presence of E1A proteins. In

addition, we have completed construction of a plasmid containing E1A and the amplifiable dihydrofolate reductase (DHFR) gene. This plasmid will be transfected into DHFR⁻ Chinese hamster ovary cells.

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PROTEIN SYNTHESIS

M.B. Mathews G.P. Thomas, T.M. Harrison, C.V. Dery, J.M. Langstaff, E. Moran, M.R. Sadaie, C.C. Bunn, R. O'Malley, M. Freedman, C.H. Herrmann, D.P. Pascucci, P.A. Reichel

We have continued to work on the regulation of adenovirus infection and on the heat-shock (stress) response of human cells. In addition, our studies of autoantibodies in rheumatic disease have expanded in depth and scope during the past year.

Functional Analysis of the E1A Gene

E. Moran, C.V. Dery, T.M. Harrison, M.B. Mathews

The adenovirus E1A gene is required for virus-induced cell transformation and for the transcription of other adenovirus genes. Early in infection, two mRNAs are made from E1A, the 12S and 13S RNAs (see Fig. 1). Their nucleotide sequences indicate that they encode proteins 243 and 289 amino acids long, respectively, differing only in that the larger protein contains an additional 46 amino acids between positions 140 and 185. Our aim is to examine the roles of the separate E1A products. This goal is being pursued in collaboration with B. Zerler, R. Roberts, and T. Grodzicker (this section). Last year, we took the first step, the isolation of cDNA clones corresponding to the 12S and 13S cDNAs and the construction of a cDNA clone corresponding to the

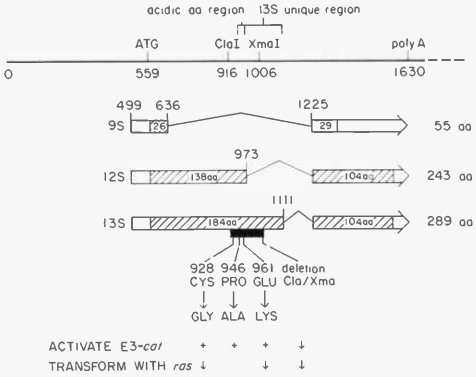

FIGURE 1 The adenovirus E1A gene. The E1A gene is located at the left end of the adenovirus genome, and some landmarks are indicated on the top line. The structures of the three spliced E1A mRNAs are depicted by arrows and their coding regions are shaded. The coding regions of the 12S and 13S mRNAs (hatched) are identical except that the first exon of the 12S RNA is shorter; in both cases, the codon for one amino acid is split between the first and second exons. The amino terminus of the 9S protein is identical with that of the 12S and 13S products, but after the splice junction, the 9S product enters a different reading frame (stippled). Mutations of individual nucleotides (numbered) and a deletion of the *ClaI/Xma*I fragment (shown as a bar) have been used to study the functions of the 12S and 13S products (see text).

9S mRNA synthesized by the E1A gene later in infection. To allow further analysis, the cDNAs were inserted into the viral genome in place of the genomic E1A region, giving so-called 9S, 12S, and 13S viruses.

The simplest test of E1A gene function available at present is the transient expression assay, which measures the ability of the E1A products to regulate transcription from other adenovirus promoters. In one version of this assay, HeLa (human) cells are transfected with an E1A derivative, together with the E3-*cat* plasmid in which the adenovirus E3 promoter governs the expression of the readily assayed bacterial enzyme chloramphenicol acetyltransferase (*cat*). As described last year, plasmids carrying either the entire E1A gene or the 13S cDNA stimulate E3-*cat* expression, whereas the 12S and 9S cDNA plasmids do not. These experiments have now been repeated with the E2-*cat* plasmid in which enzyme production is governed by a different adenovirus early promoter. Again the results indicate that transcriptional activation is a property of the 13S product only. We are presently constructing a set of additional promoter-*cat* fusions in order to assess the generality of this conclusion and to measure the sensitivity of other promoters to E1A stimulation.

The functions of the E1A gene were also studied during infection of human cells with the cDNA viruses. The 13S virus is as competent as wild-type virus for lytic growth in HeLa cells, whereas the 12S and 9S viruses are defective. Indirect immunofluorescence studies indicate that the 72K protein made by the adenovirus E2A gene is present at comparable levels in cells infected with 13S or wild-type virus, but the protein is not detected after infection with 12S virus. As expected, corresponding differences are seen when viral mRNA levels are compared. These results confirm that the stimulation of expression of other adenovirus early gene products is a function of the E1A 13S product but not of the 12S or 9S products.

The transforming activities of the cDNA plasmids and viruses were investigated in primary baby rat kidney (BRK) cells. Both the 12S and 13S plasmids can establish BRK cells in culture in a manner similar to that of the intact E1A gene, and all three can cooperate with the *ras* oncogene to generate stable transformants. Similar results are obtained when the E1A promoter is replaced by the mouse metallothionein promoter, implying that the genetic element responsible for the transforming function lies within the 12S and 13S coding sequences. Remarkably, the 12S virus transforms BRK cells at an efficiency 50–100-fold higher than wild-type virus. However, the establishment activity observed with the 13S plasmid is not seen after infection with the 13S virus, probably because it is obscured by the cytopathic effect of this virus. No establishment activity has been detected in the 9S cDNA plasmid or virus constructs.

From these results, we conclude that the establishment function, exhibited by both the 12S and 13S products, is separate from the function that stimulates expression of other adenovirus early genes and resides exclusively in the 13S product. Presumably, the 13S unique region is required for efficient stimulation of adenovirus early genes. On the other hand, plasmids carrying the E1A region of an existing E1A mutant virus, *hr*-A, are able to establish BRK cells and to cooperate with *ras* to transform these cells stably. The *hr*-A mutant fails to produce the 12S mRNA, and its 13S mRNA encodes a truncated E1A product comprising the first 140 amino acids of the 289-amino-acid protein. Thus, it appears that the first 140 amino acids of the 13S product (139 of which are shared by the 12S product) are sufficient for this activity and that the activity persists in the truncated protein.

To dissect further the functions of E1A, we have begun a mutational analysis of the gene. We first excised the *Cla*I to *Xma*I fragment of E1A, producing an in-frame deletion mutant that lacks the 12S splice donor site and amino acids 121–150 of the 289-amino-acid protein (Fig. 1). The mutant plasmid fails to stimulate transient expression of E3-*cat* in HeLa cells even though it retains coding information for 35 of the 46 amino acids of the 13S unique region, including the five cysteine residues that are a distinctive feature of this region. It also fails to cooperate with *ras* in transformation of BRK cells. These results suggest that amino acids 121–150 are required for both functions and in particular that the section from residue 121 to residue 140 is required for the establishment activity.

The most striking feature of this sequence is a string of six acidic amino acids from residue 133 to residue 138. The unusual concentration of negative charges might form part of an active site. Interestingly, the *myc* oncogene, which like E1A can cooperate with *ras* to transform BRK cells, has a similar run of acidic amino acids. We have used oligonucleotide-directed, site-specific mutagenesis to substitute lysine for glutamic acid at position 135 (nucleotide 961) to discover whether introduction of a positive charge in this negatively charged region will disrupt activity. The mutation was constructed in both E1A genomic and 12S cDNA plasmids. The E1A-961 mutation retains the ability to stimulate E3-*cat* activity, but both the E1A-961 plasmid and the 12S-961 plasmid are defective in the ability to cooperate with *ras* to transform BRK cells. In addition, when rebuilt into virus, the 12S-961 construct is also defective in the ability to establish foci of immortalized BRK cells, compared with the wild-type 12S virus, although it does appear to give these cells some extended growth potential. Immunoprecipitation from infected HeLa and BRK cells demonstrates that the mutant protein is produced and is reasonably stable. Thus, the acidic-to-basic amino acid change at position 135 has a marked effect on the establishment function but little or no effect on stimulation of E3-*cat* expression. The activity that stimulates adenovirus early gene expression can be dissociated from the establishment function, just as the establishment function can be uncoupled from the early gene stimulating activity. We are currently rebuilding the E1A-961 construct into whole virus to discover whether the mutation affects the growth properties of the virus.

We have also used oligonucleotides to change the cysteine residue at position 124 (nucleotide 928) to glycine and the proline residue at position 130 (nucleotide 946) to alanine. These amino acids are conserved in the adenovirus serotypes, Ad2, Ad7, and Ad12, suggesting that they may have functional significance. The E1A-928 plasmid construct has a phenotype similar to that of E1A-961: It stimulates expression of E3-*cat* but is defective in cooperating with *ras* to transform BRK cells, again indicating that the ability to stimulate early gene expression is not dependent on the normal establishment activity. The 13S-946 plasmid is able to stimulate expression of E3-*cat*, but its establishment potential has not yet been determined. Both mutations are currently being incorporated into viruses to assess their lytic and transforming functions.

We are continuing to use oligonucleotide-directed mutagenesis as well as random mutagenesis procedures to isolate additional changes in the region between the *Cla*I and *Xma*I sites of E1A. These mutants will be assayed for their lytic and transforming functions and for their ability to regulate expression of adenovirus genes, cotransfected genes, and host-cell products. Furthermore, we have recently expressed the E1A cDNA products in yeast in order to exploit the potential for genetic analysis of wild-type and mutant E1A

functions in this organism. We also hope to obtain a sufficient amount of E1A proteins for biochemical analysis both from yeast and from mammalian expression vectors. This will permit analysis of the effects of E1A mutations on the chemical modification and function of the E1A proteins.

VA RNA and Translational Control

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

The "virus-associated" RNAs, VA RNA_I and VA RNA_{II}, are small, abundant adenovirus transcripts that do not code for proteins. They are transcribed by RNA polymerase III from two closely spaced genes located between map units 29 and 31 on the viral chromosome. Both species are about 160 nucleotides in length, exhibit extensive secondary structure, and terminate with a run of U residues that interacts with a cellular protein known as the La antigen. Until recently, the role of the VA RNAs was a matter for speculation, but, as described in last year's report, work with the Ad5 mutant *dl*331 (Thimmappaya et al., *Cell 31:* 543 [1982]) pointed to a role in protein synthesis. Cells infected with the mutant virus lack VA RNA_I, the major VA RNA species, and at late times of infection, they are defective in their ability to translate mRNAs of either viral or cellular origin.

We have now pinpointed the level at which VA RNA_I functions in translation. Cell-free protein-synthesizing extracts of *dl*331-infected HeLa cells, like the intact cells from which they are derived, suffer from a greatly reduced ability to initiate translation of mRNA. Surprisingly, the defect cannot be overcome by addition of VA RNA or of VA RNA together with the La antigen, implying that the small RNA acts only indirectly. Furthermore, mixing experiments indicated that *dl*331 extracts contain an inhibitor capable of blocking initiation in other cell extracts. The mixing experiments also disclosed that protein synthesis can be effectively restored by various fractions from a rabbit reticulocyte extract, leading us to examine several highly purified reticulocyte initiation factors for their ability to rescue protein synthesis in the *dl*331 extracts. These factors were kindly provided by W. Merrick (Case Western Reserve University) and J. Siekierka (Roche Institute). The activity proved to reside solely in eukaryotic initiation factor 2 (eIF-2) and in an ancillary enzyme, the guanosine nucleotide exchange factor (GEF), which enables "used" eIF-2 to function catalytically in initiation.

Figure 2 summarizes the relationships between

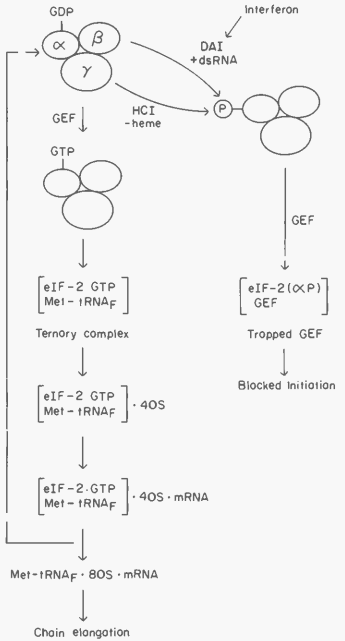

FIGURE 2 Function and control of initiation factor eIF-2. During the initiation process, this factor, composed of three polypeptide subunits, forms a ternary complex with GTP and the initiating tRNA (methionyl-$tRNA_F$). The complex binds mRNA, the 40S ribosomal subunit, and the 60S ribosomal subunit to complete the initiation sequence. In the last step, eIF-2 is released bound to GDP, which must be replaced by GTP before eIF-2 can recycle. The replacement reaction is catalyzed by GEF. If eIF-2 is phosphorylated on its α-subunit by the double-stranded RNA-activated inhibitor (DAI) or the heme-controlled inhibitor (HCI), it sequesters GEF. The GTP/GDP exchange reaction is then blocked, preventing other molecules of eIF-2 from recycling. VA RNA_I prevents phosphorylation of eIF-2 in adenovirus-infected cells. (Diagram from Reichel et al., *Nature 313:* 196 [1985].)

these two factors in the initiation process and illustrates a mechanism whereby their activity can be modulated through phosphorylation of eIF-2. Two protein kinases are known to catalyze this reaction and thereby block protein synthesis: DAI is activated by double-stranded RNA, whereas HCI is triggered by a multiplicity of effectors. Working with T. Mariano and J. Siekierka (Roche Institute), we have demonstrated the induction of such an activity in *dl*331-infected cells and have shown that it is able to block initiation. Although the nature of the kinase is not yet certain, in view of the extensively base-paired structure of VA RNA and its ability to interact with viral mRNA, our working hypothesis is that VA RNA prevents activation of DAI during adenovirus infection. We have also tested the possibility that VA RNA_I interacts directly with eIF-2 or GEF but have failed to detect significant binding.

Studies begun last year of the interaction of VA RNAs with specific viral mRNAs in vivo have continued. A transient expression system was developed that allows us to detect changes in the amount of the E2A-72K DNA-binding protein (DBP) synthesized in response to VA RNA. A plasmid containing the complete gene for the DBP is cotransfected into human 293 cells together with plasmids encoding VA RNA, and the synthesis of DBP is assayed by immunoblotting or immunoprecipitation. In this assay, VA RNA_{II} has little or no effect on DBP synthesis, whereas VA RNA_I increases the amount of DBP significantly. Deletion mutants have allowed preliminary mapping of the VA RNA_I sequences required for enhancement of DBP synthesis. The region between nucleotides 79 and 157 in the 3′ half of the molecule appears to be especially important. More detailed analysis with new mutants is projected for the near future, and rapid screening and assay methods are currently under development for this purpose.

Ribonucleoprotein Particles and Autoimmunity

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

Because of our long-standing interest in VA RNA and the La antigen that binds to it, the study of autoantibodies has developed into a research project in its own right. Our work in this area benefits from a close association with R. Bernstein and G.R.V. Hughes (Rheumatology Unit, Hammersmith Hospital, London). Autoantibodies circulate in the blood of patients suffering from certain rheumatic and connective-tissue diseases, including systemic lupus erythematosus (SLE) and myositis. In these disorders, the built-in safeguards of the immune system are breached, permitting the production of normally "forbidden" antibodies that react with the body's own cellular components. Examination of these antibodies not only sheds light on the nature of autoimmune disease, but also affords valuable reagents for molecular and cytological research. Current work is focused on the proliferating cell nuclear antigen (PCNA) and antibodies characteristic of the muscle-wasting diseases polymyositis and dermatomyositis.

Last year, we reported that PCNA is a protein with a molecular weight of 35,000, identical with the protein "cyclin" that accumulates in dividing and transformed cells. PCNA reacts with autoantibodies from a group of patients with SLE. Its level and localization within the nucleus of cultured Wil-2 cells and mitogen-stimulated human B lymphocytes vary with the phases of the cell cycle (Takasaki et al., *J. Exp. Med. 154:* 1899 [1981]). This protein is therefore of potential significance in the regulation of cell division as well as in autoimmunity.

We have begun to study the metabolism of PCNA in HeLa cells. It is mainly, if not exclusively, nuclear and can be released in large measure by digestion of DNA, which may point to an association with chromatin. Indirect immunofluorescence using anti-PCNA serum shows that PCNA is distributed heterogeneously in asynchronous cells, but in synchronized cells, several distinct patterns are seen (Fig. 3). Particularly intriguing is its apparent migration into and out of the nucleoli. This peripatetic behavior remains to be explained, and the interpretation is further clouded by the discovery that PCNA antibodies are not entirely monospecific. A group of nine nuclear proteins with molecular weights of 12,000–110,000 are detected by immunoblotting, whereas PCNA alone is immunoprecipitated from our cell extracts. Immunoprecipitation of proteins synthesized in vitro gives a single protein with the same electrophoretic mobility as PCNA.

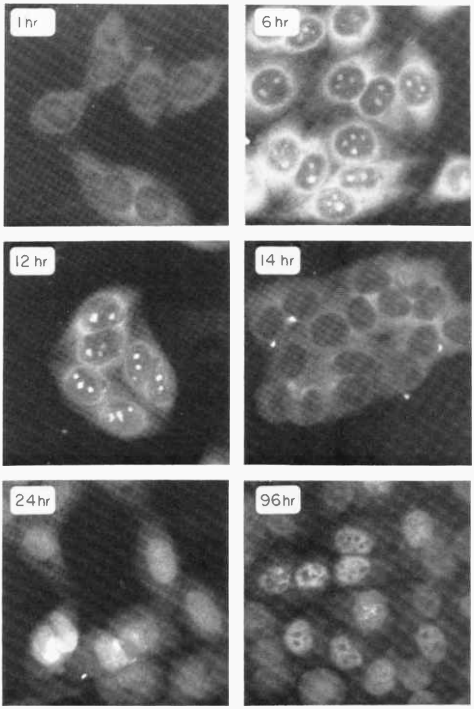

FIGURE 3 Changes in PCNA distribution through the cell cycle. HeLa cells synchronized by mitotic shake-off were reseeded and allowed to grow for various periods of time before staining with human anti-PCNA antibody and a fluorescent second antibody. The patterns illustrated correspond roughly to the following phases of the cell cycle: 1 hr, early G_1; 6 hr, late G_1; 12 hr, late G_1 early S; 14 hr, late S; 24 hr, G_2; 96 hr, confluency (G_0). Note the intense nucleolar fluorescence beginning in G_1, the homogeneous nuclear staining in G_2, and nonnucleolar staining at confluency.

This finding suggests that PCNA is neither made from a larger precursor protein nor subject to major posttranslational modifications. Our present intention is to isolate a cDNA clone to determine the structure of the protein and to permit more sophisticated analysis of its regulation.

In our work on myositis, we have now characterized three autoantibody systems associated with this disease (see Table 1). It is striking that in each case the antibody reacts with a specific aminoacyl-tRNA synthetase, the enzyme responsible for charging a tRNA with its cognate amino acid. In two cases, Jo-1 and PL-7, the corresponding tRNA can immunoprecipitate with the protein antigen but is not itself recognized by the

TABLE 1 Common Myositis-related Autoantibody Systems

System	Frequency (%)	Antigens
Jo-1	~30	histidyl-tRNA synthetase
PL-7	<5	threonyl-tRNA synthetase
PL-12	<5	alanyl-tRNA synthetase and $tRNA^{Ala}$

The frequency of occurrence of the antibodies in polymyositis and dermatomyositis is tabulated together with their cellular antigenic targets

antibody. PL-12 sera, on the other hand, recognize alanine-accepting tRNA independently of the synthetase protein. In these patients, the two separate antibodies to tRNA and protein coexist, suggesting that the autoimmune response was originally mounted against the enzyme-tRNA complex. An alternative theory proposing that the initial immunogen is some unidentified cross-reacting macromolecule seems much less likely. With PL-12 as a possible exception, our data also argue against the anti-idiotype model of autoimmunity and favor the notion that the antigens are seen as foreign (rather than "self") constituents because they become associated with some foreign molecule. In the case of the myositis antigens, the likeliest candidate for this role would seem to be viral RNA, although firm evidence is lacking.

The Mammalian Stress Response

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

As in past years, our interests in the mammalian stress (or heat-shock) response have centered on the mechanisms of translational control operating during stress and the analysis of genes encoding the human stress-response proteins. Because of the relative intractability of the former topic, more emphasis has been placed this year on the latter.

Probes for the gene encoding the 90,000-dalton (90K) human stress-response protein have been isolated through a "plus-minus" approach and have been used to isolate bacteriophage clones from a library of genomic DNA, as indicated in previous Annual Reports. One such clone, containing what appears to be the entire protein-coding sequence for the inducible 90K protein, in addition to about 10 kb of upstream sequence, has been studied intensively. The protein-coding sequences have been identified through S1-nuclease protection experiments coupled with nucleotide sequencing. The sequence of a stretch of about 4.5 kb, including the coding regions and an approximately 1-kb region upstream of the likely initiator methionine, has been determined and is largely complete.

Comparing this sequence with the recently published sequences of its counterparts from *Drosophila* (fruitfly) and *Saccharomyces* (yeast), we have identified the putative initiator methionine (Fig. 4). The derived protein sequences are very similar, and in some stretches more than 90% conservation is observed. In *Drosophila*, the 90K gene equivalent (HSP83K) is unique in that it is the only heat-shock gene with an intervening sequence. Its mRNA initiates some thousand bases upstream of the initiating ATG, but most of this sequence is removed through splicing during mRNA formation. In yeast, the transcript is not spliced, and the protein-coding region follows about 50 bases from the start of the mRNA. Our upstream sequence does not contain likely matches with the so-called "consensus" (CT-GAA-TTC-AG) sequence implicated in the induction of this type of gene, suggesting that the 90K gene may be activated by a different mechanism. The alternative possibility, that our sequence may not extend far enough upstream to contain this region, remains to be excluded, although S1-nuclease protection has failed to reveal its existence. We hope to resolve this issue in the near future by primer extension and protection experiments using fragments located even further upstream than those used so far.

The 90K stress-response gene differs from many other stress-response genes in that it is expressed at fairly high levels under normal circumstances. The 72K/73K family of stress proteins is subject to subtle and varied controls: Some members are expressed normally and repressed under stress, others are expressed normally and seemingly unaffected by stress, and yet others are greatly induced during stress. The latter class has drawn attention for two reasons. First, it is a favorable model gene to investigate the mechanisms of induction since the magnitude of induction exceeds 50-fold. Second, its protein product is the major stress protein of many organisms, has been conserved throughout evolution, and is required for growth at elevated temperatures. The "noninducible" members of the family have yet to enjoy such detailed study, yet are of no less interest, also being well conserved and expressed at substantial levels under normal circumstances. We have isolated cDNA probes for both types of genes, the induced form (72K) from HeLa cells treated with the amino acid analog and, more recently, the uninduced form (73K) from normal rat fibroblasts.

Using the 72K cDNA clone, we have isolated genomic clones, and these are currently being

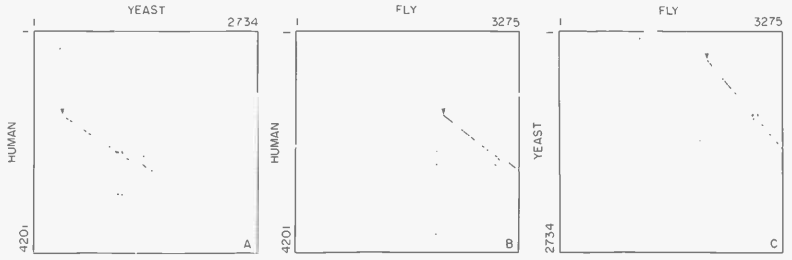

FIGURE 4 Nucleotide sequence comparison of the human 90K stress protein gene and its counterparts from *Drosophila* and yeast. We have compared the nucleotide sequences of the 90K genes of human, *Drosophila*, and yeast using the DIAGON program of Roger Staden. In essence, two sequences are laid at right angles and where they share a required degree of homology, a dot is deposited; comparison of a given sequence with itself therefore generates a diagonal lying 45° from the perpendicular and if the sequences are of different lengths the angle of the diagonal is dictated by their ratio. For the comparisons here, we have required that the two sequences be homologous to 75% or greater over a span of 17 nucleotides. (*A*) Comparison of human and yeast; (*B*) comparison of human and fly *(Drosophila)*; (*C*) comparison of yeast and fly. The yeast sequence (Farrelly and Finkelstein, *J. Biol. Chem. 259:* 5745) is 2734 nucleotides long and includes roughly 300 bases of noncoding sequence on either side of the protein coding region. The *Drosophila* sequence (Hackett and Lis, *Nucleic Acids Res. 11:* 7011) is 3275 nucleotides long, and the protein coding region starts at base 2151. The presumptive initiator ATG and the beginning of open reading frame in the human sequence are at position 1557, and the presumptive initiator ATGs of the other two sequences are indicated by the arrows. From the diagonal plots, the three sequences are seen to be closely related, but not identical with extensive but interrupted regions of homology extending over the protein-coding sequences. The predicted amino acid sequences display less fragmented homology, indicating better conservation of the protein sequence than the nucleotide sequence. This may prove to be a general feature of the stress proteins as a whole. No obvious homologies upstream of the coding sequences were detected either by this analysis or by direct comparison of the sequences in regions where, for the fly sequence at least, a so-called "Pelham" box has been identified. Each of the proteins has the unusual (acidic_{3-4})-(basic_2) motif repeated in the region between amino acids 220 and 280.

mapped with a view to isolating their upstream regulatory sequences. They are of particular interest because the gene is induced both by stress and as a result of infection with certain viruses. Moreover, in the case of adenovirus, evidence points to the involvement of E1A proteins in the induction. Limited sequencing of the 72K cDNA has identified protein-coding sequences that are highly homologous to the corresponding genes of *Drosophila* and yeast and has indicated that the cDNA in hand contains all but the aminoterminal 100 amino acids. In primer-extension experiments aimed at creating a full-length copy, the cDNA is extended by approximately 700 bp when induced cell (not uninduced cell) RNA is used as template, implying that the cDNA is indeed the major induced form of the 72K/73K family. Similarly, as demonstrated by Northern blotting of RNA from normal cells, very little 72K mRNA is detected with this cDNA clone, whereas vast quantities are found in RNA from stressed cells. Under conditions of similar stringency, Southern blotting of human genomic DNA reveals a large number of bands. This may indicate the presence of intervening sequences in the 72K protein gene (unexpected if the *Drosophila* genes are an accurate paradigm) or of multiple genes closely related to the inducible 72K protein gene. To resolve this issue and to characterize the 72K gene, we are currently reexamining genomic digests as well as the bacteriophage clones that we have already isolated, using shorter, more specific probes from amino- and carboxyterminal regions of the induced cDNA.

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PROTEIN CHEMISTRY

C. Slaughter T. Fischer
C. Moomaw

A Protein Chemistry laboratory has been established for the isolation and chemical characterization of the protein products of normal and transformed cells. The facility has been equipped both with conventional chromatography systems to isolate proteins available in quantities large enough for X-ray crystallography (100 mg or more) and with high-performance liquid chromatography (HPLC) and electrophoresis systems to isolate minor cell proteins in microgram quantities for chemical analysis. Two automated amino acid sequencers have been brought into operation. The first, a Beckman model 890C, which was on site previously, has been upgraded to model 890M by the addition of a microprocessor controller and an autoconverter system. This instrument is now being used to perform amino-terminal amino acid sequence analyses on protein and peptide samples of between 500 pmoles and 5 nmoles. The second sequencer, an Applied Biosystems model 470A, has been newly installed and has been used for protein and peptide samples of between 10 pmoles and 20 nmoles. It has been possible to identify up to 45 amino acids in aminoterminal runs on unknown samples using both instruments.

Phenylthiohydantoin (PTH)–amino acids generated by the sequencers are being identified on a newly installed Waters automated HPLC system by reverse-phase chromatography on a Waters Nova Pak C18 column. The procedure has proved to be capable of quantitating 10 pmoles of each PTH amino acid on a routine basis and detects considerably less. Since the turnaround time for each analysis is 23 minutes, and the cycle time on each sequencer is approximately 1 hour, it is

possible for the PTH-amino acid identification system to keep pace with the simultaneous output of both amino acid sequencers indefinitely. Amino acid sequencing and PTH-amino acid identification are being conducted by T. Fischer.

Estimation of amino acid composition of peptides and proteins is being carried out on a newly installed Waters automated HPLC system employing the PICO.TAG procedure. In this procedure, phenylthiocarbamate (PTC)-amino acid derivatives are synthesized in a one-step manual operation, and the derivatives are separated by reverse-phase chromatography on a Waters Nova Pak C18 column. The PTC derivatives can be quantitated in the low picomole range by their intrinsic absorption at 254 nm. The turnaround time for the separation is 20 minutes.

The synthesis of peptides for the production of sequence-specific antibodies has been initiated using a Beckman model 990 solid-phase peptide synthesizer. The peptides are subject to verification prior to uncoupling from their solid support by sequence analysis on the Applied Biosystems model 470A. After hydrogen fluoride cleavage, purity of the uncoupled peptides is subject to further checking by reverse-phase HPLC and by amino acid analysis. Amino acid analysis and peptide synthesis are being conducted by C. Moomaw.

We are currently exploring procedures for extended carboxyterminal amino acid sequencing of both proteins and peptides using reverse-phase HPLC to identify dipeptides cleaved from the carboxyl terminus by dipeptidyl carboxypeptidase digestion. We hope to be able to carry out high-sensitivity sequence analyses at both amino and carboxyl termini of all protein and peptide samples studied in the laboratory.

Peptide-mapping procedures employing both gel permeation and reverse-phase HPLC separations in volatile buffer systems have been established. HPLC peptide mapping has the advantage over traditional gel electrophoresis procedures that the peptides are readily collected in a form suitable for further chemical analysis. The coupling of high-resolution peptide mapping with high-sensitivity analytical procedures will provide us with a powerful means of investigating the structural alterations involved in the processing of the protein products of oncogenes and tumor viruses.

Our laboratory has performed 2566 cycles of Edman degradation during its first 10 months of operation and has been involved with nine projects, seven of which were performed in collaboration with scientists on the grounds of Cold Spring Harbor Laboratory. Work on the proteins of interest in the field of cell growth has been equally divided between the human alkaline phosphatases, a family of cell-surface enzymes that are used extensively as tumor markers in clinical practice, and rat brain protein kinase C, an intracellular enzyme of the plasma membrane that is a receptor for phorbol ester tumor promoters. The latter project is being undertaken in collaboration with J. Feramisco's group (Cell Biology Section).

Structural Diversity of Human Alkaline Phosphatases

C. Slaughter, S. Cheley, T. Fischer, C. Moomaw

The research on alkaline phosphatases (APs) represents the principal focus of the laboratory's interest. APs are a family of membrane-associated enzyme glycoproteins that hydrolyze phosphate esters with a high pH optimum. APs from various tissues differ in biochemical, immunochemical, and genetic characteristics, suggesting that they are encoded by a family of related structural genes. Some of these genes are expressed in a highly tissue-specific manner, whereas others have a more general pattern of expression. In addition, some members of the gene family are expressed ectopically in tumors and in cell lines derived from tumors. In many respects, the APs provide a useful model system for exploring the general characteristics of multigene families. The overall aim of the work is to determine the extent of structural and genetic diversity in both normal and ectopically expressed APs. This problem is being approached at both the nucleic acid and protein levels.

Aminoterminal amino acid sequences have been obtained for the products of the principal genes expressed in human placenta, intestine, and liver. The results confirm the conclusion drawn previously from genetic and biochemical evi-

dence that different genes are involved in coding for these tissue-specific forms of AP. The aminoterminal amino acid sequences of adult and fetal intestinal APs have also been compared, and although these proteins display distinct antigenic properties and peptide-mapping patterns, their sequences thus far appear identical at the amino terminus. Placental AP has been subjected to digestion with cyanogen bromide, and peptides have been isolated by reverse-phase HPLC. In this way, a variety of amino acid sequences from regions of the molecule other than the amino terminus have been deduced. This information has been used by M. Zoller (Molecular Genetics Section) to synthesize oligonucleotides with which S. Cheley, in collaboration with D. Helfman (Cell Biology Section), is screening suitable cDNA libraries. The clones identified in this way will provide the means of deducing the complete amino acid sequences of human placental AP and of investigating the genetic and structural complexity of a major part of the AP family.

MOLECULAR GENETICS OF EUKARYOTIC CELLS

Included in this section are a diverse range of systems, spanning from yeast to man, and topics, spanning from the control of protein localization to developmental biology. The common thread is the application of new methods of molecular genetics to long standing biological problems. J. Sambrook and M.-J. Gething (Protein Traffic) describe investigations of protein modification, localization, secretion, and protein assembly in animal cells. Using an influenza hemagglutinin gene expression system and mutants constructed in vitro, they are outlining the pathways for these processes. J. Lewis (Cell-cycle-specific Expression) has cloned the hamster thymidine kinase gene and is examining the mechanisms by which expression of this gene is regulated during the cell cycle. D. Kurtz (Hormonal and Developmental Control of Gene Expression) describes the study of hormonal and tissue-specific expression of a family of genes encoding closely related proteins, the rat α_{2u} serum globulins. M. Wigler (Genetics of Cell Proliferation) details studies on human oncogenes, their isolation, structure, and function. The *RAS* oncogenes are highly conserved in evolution, and the function of homologs in yeast are also being explored. This work has been carried out in collaboration with F. Tamanoi (Biochemistry of Yeast *RAS* Proteins). L. Silver (Mouse Genetics) has been investigating the molecular basis of a group of closely linked developmental mutants of mice, which together comprise the T locus. These mutants are characterized by early developmental death in homozygotes, segregation distortion, and locus-specific inhibition of meiotic recombination. Finally, D. Hanahan (Transgenic Mice) and his group are redesigning the mouse by placing genes under new controls into the mouse germ line. This experimental system promises to be one of the most powerful approaches to understanding gene expression in the developing animal.

PROTEIN TRAFFIC

J. Sambrook
M.-J. Gething
E. Hunter
P. Bird
P. Gallagher
J. Hearing
M. Roth
C. Doyle
J. Henneberry
L. Rodgers
S. Sharma
D. York

The general aims of our laboratory are to map the major routes of protein traffic within mammalian cells and to understand how proteins are directed to specific destinations.

THE EXOCYTIC PATHWAY

Most of our work on the exocytic pathway involves the hemagglutinin (HA) of influenza virus, which is now the best studied of a large class of integral membrane proteins. Until a few years ago, work on HA depended almost entirely on the techniques of protein chemistry (Waterfield et al., *Br. Med. Bull. 35:* 57 [1979]), but since the advent of recombinant DNA techniques, the genes encoding the HAs of many strains of influenza virus have been cloned and sequenced, and this has allowed the complete amino acid sequence of many serotypes of the protein to be deduced (for review, see Lamb, in *Genetics of Influenza Vi-*

ruses, Academic Press, New York [1983]). At the same time, techniques to produce monoclonal antibodies became available, and such antibodies raised against HA have led to a detailed analysis of the antigenic structure of HA. In 1977, the glycoprotein spike of HA was crystallized, and a three-dimensional structure has since been determined to a resolution of 3 Å using X-ray crystallography (Wiley et al., *Nature 289:* 373 [1981]; Wilson et al., *Nature 289:* 366 [1981]). The culmination of all this work has been a detailed description of the physical domains of the molecule, 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.

The mature protein consists of three structural domains (see Fig. 1). One of these is a large hydrophilic carbohydrate-containing domain located on the external surface of the lipid bilayer and is itself divided into three regions: a globular portion that consists largely of an eight-stranded antiparallel β-pleated sheet, and a switch or hinge region formed from two antiparallel chains that connect the distal globular portion to the third region—a fibrous stem. The upper part of this stem is made from two antiparallel α-helices, and the lower part consists of a compact globular fold that includes a five-stranded, β-pleated sheet. The structure of the fibrous stem is buttressed by extensive contacts among the three monomers that comprise the trimer. Without these contacts (which stabilize the fibrous stem into a triple-stranded coiled coil), it seems unlikely that an individual monomer would form a stable stem. Consequently, the protein molecule may raise itself from the surface of the membrane only when it is assembled into a trimer.

The second structure is a small hydrophobic domain of approximately 30 amino acids located near the carboxyl terminus of the molecule and spans the membrane. The third structure is a small carboxyterminal hydrophilic domain of approximately 10 amino acids located on the internal side of the membrane.

Characteristically of transmembrane proteins, HA is synthesized on membrane-bound ribosomes as a precursor that includes an aminoterminal signal peptide; this peptide is subsequently removed by a protease that is found only on the luminal side of the endoplasmic reticulum (ER).

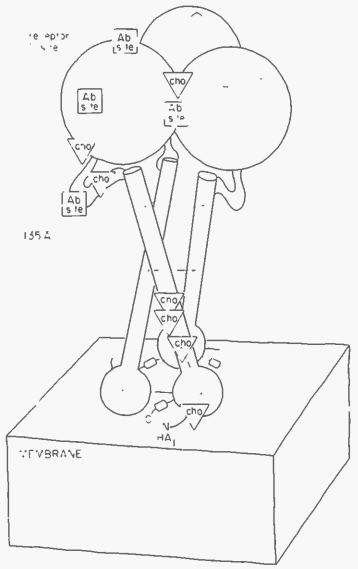

FIGURE 1 Structure of A/Aichi/68 HA. A schematic diagram of the HA trimer showing carbohydrate-addition sites and the arrangement of the molecule with respect to the membrane. (Modified from Wilson et al., *Nature 289*: 366 [1981]).

Each monomeric peptide chain is cotranslationally glycosylated at several sites, all of which are eventually located on the surface of the completed trimer (see Wiley et al., *Nature 289:* 366 [1981]).

In recent years, HA has been used increasingly as a model to study the biosynthesis, modification, and transport of integral membrane proteins in mammalian cells (for review, see Gething and Sambrook, in *Genetics of Influenza Viruses,* Springer-Verlag, Berlin [1983]). The groundwork for this approach was laid when it was shown that the cloned gene for HA could be expressed from eukaryotic vectors with very high efficiency. The newly synthesized molecule was translocated through the rough ER, modified, assembled into trimers, and transported *via* the Golgi apparatus to the cell surface along a pathway indistinguish-

able from that taken by a large number of endogenous cellular proteins.

These initial findings were extended to include a number of mutant forms of HA, in which DNA sequences coding for the putative hydrophobic signal and anchor regions of the molecule were deleted. Analysis of the expression of the resulting constructs led to the conclusions (1) that the hydrophobic aminoterminal sequences are necessary to cause translocation of the newly synthesized molecule across the membrane of the rough ER and (2) that the hydrophobic carboxyterminal sequences are sufficient to anchor the completed molecule in the plasma membrane. Deletion of these sequences results in secretion of a truncated HA from the cell (Gething and Sambrook, *Nature 300:* 598 [1982]).

ALTERATIONS TO THE AMINO TERMINUS AND CYTOPLASMIC TAIL OF HA CAN AFFECT TRANSPORT OF THE PROTEIN ALONG THE EXOCYTIC PATHWAY

During the last year, we have constructed and analyzed a number of additional mutant and chimeric forms of HA in which the DNA sequences coding for the signal sequence, the transmembrane region, and the cytoplasmic domain were mutated at specific sites or replaced by sequences encoding analogous regions from other proteins. The results of these experiments are summarized in Table 1 and discussed briefly below:

1. Substitution of the signal sequence of HA with those from other glycoproteins (the *env* glycoprotein of Rous sarcoma virus [RSV] or bovine preprochymosin) yields chimeric proteins that are translocated into the lumen of the ER, where they undergo core glycosylation. In both cases, the signal sequences are cleaved from the precursor, leaving an HA in which the first two amino acids have been replaced by either 9 (in the case of RSV *env)* or 12 (preprochymosin) novel residues. Neither of these chimeric proteins is folded correctly (see below) and neither is transported further along the exocytic pathway to the Golgi apparatus. However, by deleting the DNA sequences coding for the additional aminoterminal amino acids, we have been able to restore the ability of the *env*-HA protein to fold into a form that is efficiently transported to the cell surface. We conclude that a signal sequence obtained from a different eukaryotic protein is sufficient to guarantee translocation of HA into the lumen of the rough ER. Further movement of HA along the exocytic pathway is highly dependent on the configuration of the amino terminus of the protein.
2. Replacement of the transmembrane region and cytoplasmic tail of HA with analogous sequences from the G glycoprotein of vesicular stomatitis virus (VSV) or the C glycoprotein of herpes simplex virus yields chimeric glycoproteins that are transported to the cell surface, where they are displayed in an antigenically and biologically active state. The HA-G chimera is transported to the surface as efficiently as wild-type HA, whereas HA-gC traverses the early part of the pathway (from the ER to the Golgi apparatus) more slowly and asynchronously. We conclude that the anchoring function of the transmembrane regions of HA can be provided by hydrophobic sequences derived from different viral glycoproteins, without crippling the ability of the chimeric molecule to move through the exocytic pathway.
3. Certain alterations to the cytoplasmic tail of HA drastically lower the rate at which the molecule travels from its site of synthesis to the Golgi apparatus; other alterations affect transport of HA from the Golgi apparatus to the cell surface (see Table 1). These results can be interpreted two ways: Either the cytoplasmic tail of HA carries signals that determine the rate or efficiency with which the entire molecule progresses along the exocytic pathway or the tail is important for the structural integrity of the molecule, and changes in rates of transport are a consequence of an inability to fold into or maintain a stable structure.

To distinguish between these possibilities, we have used a number of approaches to compare the three-dimensional structure and folding of wild-type and mutant proteins. An antiserum raised against denatured HA that recognizes an epitope(s) cryptic in the external region of mature wild-type HA reacts strongly with mutant proteins that are blocked in the ER (*env*-HA and HAxpBR). Second, these mutants, unlike wild-

TABLE 1 Properties of HA Mutants

Mutant	Description	Phenotype	Final location
HA-S	aminoterminal hydrophobic signal sequence deleted	protein not translocated to ER and not glycosylated	cytoplasm
env$_s$HA12	aminoterminal signal and first two amino acids of mature HA replaced by signal sequence and six amino acids of RSV *env* glycoprotein	protein translocated and core glycosylated; signal cleaved; protein not transported to Golgi	ER
env$_s$-HA16	aminoterminal signal of HA replaced by that from RSV *env*; processed polypeptide identical to wild-type HA	chimeric protein translocated and core glycosylated; signal cleaved; protein transported to cell surface with wild-type kinetics	cell surface
HA-A$^-$	transmembrane anchor and cytoplasmic tail deleted and replaced by 11 heterologous amino acids	transport of protein between ER and Golgi slowed; truncated protein efficiently secreted from cell	medium
Ha-G	transmembrane anchor and cytoplasmic tail of HA replaced by the analogous regions of VSV G	chimeric protein anchored in lipid bilayer and transported to the cell surface with kinetics identical to those of wild-type HA; at cell surface, chimeric protein antigenically and functionally active	cell surface
HA-gC	transmembrane anchor and cytoplasmic tail of HA replaced by the analogous regions of HSV-1 glycoprotein C	chimeric protein anchored in the lipid bilayer, but transport between ER and Golgi slowed; at cell surface, chimeric protein antigenically and functionally active	cell surface
HA-Cg	transmembrane anchor and cytoplasmic tail of HA replaced by 67 heterologous amino acids	transport of protein between ER and Golgi slowed; protein efficiently secreted from the cell	medium
HA152*env*	cytoplasmic tail of HA replaced by analogous region from the *env* glycoprotein of RSV	chimeric protein wild type in all respects	cell surface
HA71	three of the four final residues of cytoplasmic tail altered	altered protein wild type in all respects	cell surface
HA11	cytoplasmic tail of HA deleted; three heterologous amino acids added	transport of protein between ER and Golgi slowed	cell surface
HA477*env*	cytoplasmic tail of RSV *env* added after the penultimate residue of the HA cytoplasmic domain	transport of protein between ER and Golgi slowed	cell surface
HA164	cytoplasmic domain of HA deleted; replaced by 16 heterologous amino acids	transport of protein between ER and Golgi slowed; terminally glycosylated molecules severely retarded in a post-Golgi compartment	post-Golgi and vesicles and cell surface
HAxpBR	16 heterologous amino acids added to the HA cytoplasmic domain	transport of protein from ER blocked	ER

type HA and normal amphipathic proteins, do not partition into the detergent phase of Triton X-114, perhaps because the transmembrane region of the malfolded proteins cannot form the stacked triple helices that stabilize the protein in the lipid bilayer (or the detergent). Finally, a complex between the mutant molecules and a cellular "binding protein" (BiP) found solely in the lumen of the ER can be immunoprecipitated from cell extracts using either anti-HA sera or an anti-BiP monoclonal antibody (kindly provided by L. Hendershot and J. Kearney, University of Alabama). BiP (M_r = 77,000) is thought to bind to newly synthesized proteins in the ER prior to folding. These and other data show that there is a relationship between the altered structure of

mutant forms of HA and their ability to be transported to the cell surface. Apparently, HA needs to be correctly folded in order to move efficiently along the exocytic pathway.

FOLDING PATHWAY OF HA

How a protein that is synthesized on one side of a membrane folds into a specific three-dimensional shape on the other is not known. The following is our current working model (see Fig. 2): The monomeric polypeptide is translocated essentially as a linear string of amino acids. It remains attached to the luminal face of the membrane not only by the carboxyterminal anchor, but also by the aminoterminal hydrophobic signal. After primary glycosylation is completed, but before trimerization has occurred, the distal region of the molecule folds into its globular shape and disulfide bonds form. The carboxyterminal hydrophobic domains of three monomers then associate to form a triple-stranded helical coil within the membrane. The three stem regions are then able to twist around each other to form the coiled coil that comprises the bulk of the external fibrous spike. After cleavage of the signal peptide, the completed trimer is free to move in the plane of the membrane of the ER toward the Golgi apparatus. Trimerization is therefore a prerequisite for movement. This model is consistent with the structural analysis of wild-type HA (Wilson et al., *Nature 289:* 366 [1981]; Wiley et al., *Nature 289:* 373 [1981]) and provides a plausible explanation for the phenotype of the mutants. Thus, alterations to the amino and carboxyl termini, although in regions of the protein that reside on opposite sides of the rough ER, might be expected to prevent or delay folding and oligomerization of the molecule. In the most severely affected mutants (HAxpBR and *env*-HA), the molecule may be completely unable to form trimers; in other cases (see Table 1) a disordered trimeric structure may retard movement of the protein in the plane of the membrane or obstruct its incorporation into transport vesicles that ferry it from the ER to the Golgi apparatus.

These ideas raise many questions: How does the cellular transport machinery distinguish between molecules that are correctly folded and those that are not? Does malfolding of HA affect its interaction with hypothetical sets of cellular receptors that may be involved in movement of proteins

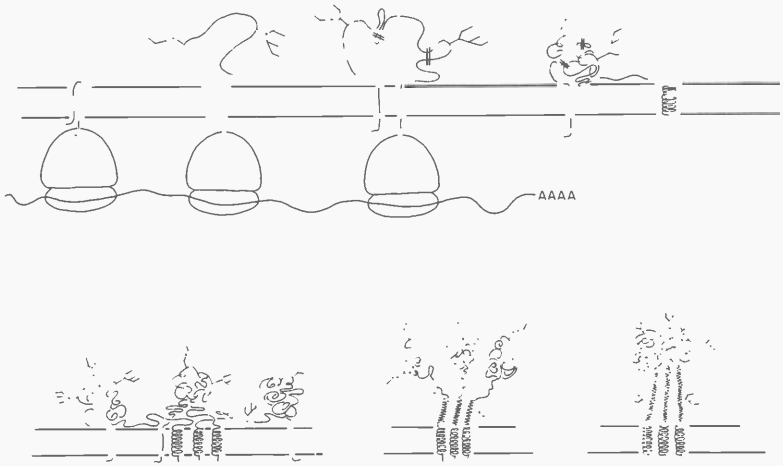

FIGURE 2 A model for the folding of HA and assembly into monomers.

along the exocytic pathway? If so, are these receptors located in the lumen of the ER, within the lipid bilayer, or on the cytoplasmic face of the membrane? Are there distinct sets of receptors that transport proteins through different stages of the exocytic pathway? We hope that these questions can be answered at least in part by further mutational analysis of HA and the elucidation of the mechanism by which the protein folds and trimerizes.

ROLE OF CARBOHYDRATE IN THE STRUCTURE AND BIOLOGICAL FUNCTION OF HA

Glycosylation of proteins is universal in eukaryotic organisms. The fact that such a complex and expensive process has been conserved over such a long period of evolutionary time suggests strongly that glycosylation must be of considerable advantage to higher organisms. However, there is no evidence for a single function for glycosylation. Instead, it appears (for review, see Pollack and Atkinson, *J. Cell Biol. 97:* 293 [1983]) that N-linked oligosaccharides may play a variety of roles including (1) the maintenance of the structure and stability of glycoproteins, (2) the direction of glycoproteins to various intracellular organelles, and (3) the specific interaction between cells that occurs during tissue organization.

The oligosaccharide groups attached to HA are thought to serve two functions—to modulate antigenicity by shielding potential antigenic sites (Knossow et al., *Nature 311:* 678 [1984]) and to stabilize the protein structure and thus facilitate the transport of the protein along the exocytic pathway. The magnitude of this latter effect varies widely between HAs of different strains. Thus, the nonglycosylated protein from the A/Aichi/68 strain is stable but does not reach the cell surface, whereas that from the A/Japan/57 strain is transported to the cell membrane, albeit at a reduced level. Most likely, lack of glycosylation prevents Aichi HA from folding into its correct configuration for transport; in the case of Japan HA, the molecule is transported to the cell surface, but it is more susceptible than wild-type HA to proteolysis.

To study these problems further, we have used site-directed mutagenesis to alter the canonical sequences specifying each of the seven carbohydrate attachment signal sites on Aichi HA. The altered genes have been inserted into SV40 vectors and expressed in CV-1 cells. The rate of biosynthesis, transport, cellular localization, and biological activity of these altered gene products is being analyzed in terms of the contribution of the individual side chains. We are also using site-directed mutagenesis to create sites for oligosaccharide addition in areas of the HA surface that do not normally carry carbohydrate groups. We hope to use the novel glycan side chains to shield particular surfaces of the molecule, e.g., those involved in transport signals or in the recognition of HA on the membrane of histocompatible target cells by cytotoxic lymphocytes.

EXPRESSION OF HA IN POLARIZED EPITHELIAL CELLS

The problem of protein traffic is particularly acute in polarized epithelial cells, in which secreted and membrane-bound proteins are directed to either of two distinct domains of the plasmalemma. These two domains, the apical and the basolateral, are separated by the *zonula occludans,* a circumferential belt of tight junctions that serves as a barrier to lateral diffusion of proteins and lipids in the plane of the plasma membrane. Polarized epithelial cells direct both their own endogenous surface proteins and the glycoproteins of the viruses that infect them to specific domains of the plasma membrane (Rodriguez-Boulan, in *Modern Cell Biology,* A.R. Liss, New York [1983]). Thus, during the early stages of infection of kidney epithelial cells by influenza virus, HA is expressed almost exclusively at the apical surface of the cell, through which the nascent viral particles will later bud. The infected cell recognizes HA as belonging to the "apical" set of proteins, and this recognition has been shown to be independent of either glycosylation of HA or of the presence of other viral proteins (Roth et al., *Cell 33:* 435 [1983]). The features of the HA polypeptide recognized by the cellular sorting machinery could reside either in the large ectodomain exposed to the luminal environment of the exocytic pathway, in the transmembrane region, or in the small carboxyterminal cytoplasmic domain. We have carried out two types of experiments to investigate which regions of HA are important for sorting. First, we have determined the

pattern of secretion in polarized cells of a form of HA lacking the transmembrane and cytoplasmic domains. This form of HA (HA^{sec}) has previously been shown to form trimers and to display all of the biological activities that reside exclusively in the ectodomain (Gething and Sambrook, *Nature 300:* 598 [1982]). Second, in collaboration with E. Rodriguez-Boulan (Cornell Medical School), we are investigating the distribution on the surface of polarized cells of forms of HA in which the transmembrane or cytoplasmic regions have been replaced with those of another glycoprotein. During the early phase of infection of MA104 rhesus monkey kidney cells with a recombinant SV40 vector expressing HA^{sec}, secretion of HA^{sec} is almost entirely from the apical surface. Thus, the transmembrane and cytoplasmic domains of HA are not needed for the directional transport of HA in this system. Preliminary results indicate that chimeric glycoproteins that contain transmembrane and cytoplasmic domains from viral glycoproteins normally expressed on the basolateral surface are concentrated on the apical surface of infected MA104 cells. A simple interpretation of these observations is that the large ectodomain of HA contains dominant features recognized by the cellular sorting machinery. We conclude that discrimination between apical and basolateral proteins probably occurs within the lumen of the organelle(s) of the exocytic pathway.

While analyzing the biosynthesis of these chimeric glycoproteins, we realized that they differed from wild-type HA in certain interesting respects. In particular, a chimera consisting of the ectodomain of HA and the transmembrane and cytoplasmic regions of VSV G protein became cleaved by protease, generating a protein consisting of two subunits (HA_1 and HA_2G) joined by a disulfide bond. The transport of HA-G must therefore at some stage diverge from that of wild-type HA, causing the chimeric protein to enter a cellular compartment in which the relevant protease(s) is active. The kinetics of cleavage are consistent with the idea that the chimeric protein enters this compartment at about the same rate as it reaches the cell surface, raising the possibility that HA-G is transported along the conventional exocytic pathway and is then removed rapidly from the cell surface by endocytosis: Proteolytic cleavage would therefore occur in endosomes. We are currently trying to test aspects of this hypothesis and to determine whether the signals that divert HA-G into this novel compartment are carried on the transmembrane region or the cytoplasmic tail of the protein.

SV40 T ANTIGEN AND THE EXOCYTIC PATHWAY

Proteins tend to have singular locations in cells. Thus, proteins present in one site (e.g., the cell surface) are by and large not detected in significant quantities elsewhere. Because specific locations are served by specific transport pathways, proteins generally need to carry only one set of transport signals. For example, proteins that are destined for the cell surface almost always contain hydrophobic regions that are readily identifiable as signal sequences. The large T antigen of SV40 does not fit easily into this simple pattern. Newly synthesized T antigen is rapidly transported from the cytoplasm into the nucleus of infected or transformed cells where it is believed to associate with cellular p53 and with specific sets of DNA sequences. However, a small proportion of T antigen is also displayed on the surfaces of transformed cells, where it can be detected in a specific configuration by a variety of immunological and biochemical techniques (for review, see Rigby and Lane, *Adv. Viral Oncol. 3:* 31 [1983]). However, in contrast to typical transmembrane proteins, T antigen carries no hydrophobic signal sequence at or near its amino terminus and in fact it lacks any sequence of hydrophobic or uncharged amino acids long enough to span a membrane. There is no obvious way to explain how considerable portions of T antigen are translocated across a membrane or where in the cell this process may occur.

To approach this problem, we decided to test whether it would be possible to increase the efficiency with which T antigen could be transported to the cell surface by equipping it with a conventional signal peptide at its amino terminus. We therefore constructed a chimeric gene consisting of the DNA sequences coding for the aminoterminal hydrophobic signal peptide of HA fused in-frame to those coding for SV40 T antigen. The gene was linked to the murine metallothionein promoter and inserted into a plasmid containing a transforming fragment of bovine papilloma virus (BPV) DNA. The resulting vector was used

to establish lines of NIH-3T3 cells that express large quantities (5×10^6 molecules per cell) of the chimeric protein (HA-T antigen). The biochemical properties and intracellular location of HA-T antigen were then compared with those of wild-type T antigen synthesized in an identical vector.

As expected, the great majority of wild-type T antigen is located in the cell nucleus, although a small fraction can also be detected on the cell surface by flow cytofluorometry. In contrast, HA-T antigen is found virtually exclusively in the ER (see Fig. 3) in a form that is neither phosphorylated nor bound to cellular p53. HA-T antigen is cotranslationally translocated across the membrane of the rough ER into the lumen, where the signal peptide is cleaved and a mannose-rich oligosaccharide becomes attached to the polypeptide (T antigen contains one potential N-linked glycosylation site at Asn-154). HA-T antigen does not become terminally glycosylated or acylated, and very little (if any) of it reaches the cell surface. From these results we conclude:

1. The hydrophobic signal peptide of HA acts as a *cis*-dominant marker that overrides other transport signals in SV40 T antigen, e.g., the nuclear transport signals that map in the seven amino acids around Lys-128 (Kalderon et al., *Cell 39:* 499 [1984]). Almost certainly, this dominance stems from the location of the signal peptide at the amino terminus of the nascent protein, where it interacts with the signal-recognition particle and prevents further synthesis of the polypeptide until the ribosome is attached to a docking site on the membrane of the rough ER. When translation resumes, the polypeptide is cotranslationally transported through the membrane in a manner that apparently affords no opportunity for downstream transport signals to function.
2. The translocation of T antigen through the membrane is not sufficient to guarantee that it will move further along the secretory pathway. This conclusion is consistent with observations discussed above on chimeric and mutant forms of HA, many of which show defects in transport to the cell surface. The failure of HA-T antigen to move from the ER can therefore be explained in two ways. First, HA-T antigen is a protein that has been removed from its normal milieu and placed in a foreign environment. There would be no evolutionary reason why the cellular structures that comprise the exocytic pathway should be equipped to deal with HA-T antigen. Second, the presence of a bulky glycan may not be conducive to proper folding of the polypeptide or efficient oligomerization of the protein.
3. Because HA-T antigen fails to move along the secretory pathway and because its biochemical

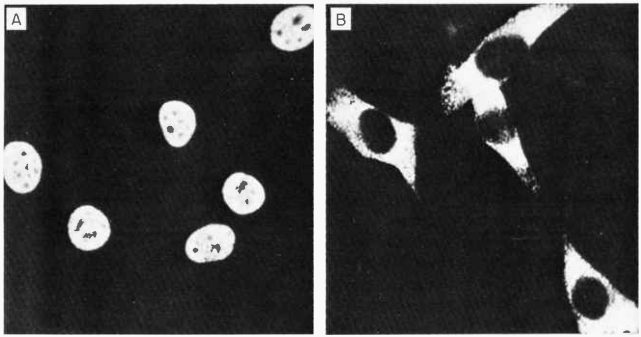

FIGURE 3 Immunofluorescent staining of cells expressing wild-type T antigen or HA-T antigen. NIH-3T3 cells expressing wild-type T antigen or HA-T antigen from BPV vectors were fixed and stained by indirect immunofluorescence. (*A*) Cells expressing wild-type T antigen; (*B*) cells expressing HA-T antigen.

properties are very different from those of wild-type T antigen, it seems unlikely to us that wild-type T antigen reaches the cell surface by traveling along the conventional exocytic route. We are therefore left with a paradox. At some stage in its life, surface T antigen must cross a membrane in order to be presented on the outside of the cell: Yet, it has none of the hallmarks of an integral or transmembrane protein. We therefore postulate (see Fig. 4) that in SV40-transformed cells, all newly synthesized T antigen enters the nucleus, where it becomes associated either directly or via intermediate molecules with the inner wall of the nuclear membrane. At cell division, this membrane is known to break down into small vesicles that become dispersed throughout the cytoplasm. If after cytokinesis, a minority of these vesicles fuses directly with the plasma membrane or with other vesicles that are bound for the surface, their contents could be everted onto the outer face of the plasma membrane. If this hypothesis stands up to experimental test, it will be interesting to determine whether normal cellular proteins travel along this novel pathway from the nucleus to the cell surface.

MUTANTS OF MAMMALIAN CELLS THAT ARE DEFECTIVE IN TRANSPORT OF PROTEINS

A complete understanding of the exocytic pathway will require not only mutational analysis of proteins like HA, but also the identification and analysis of cellular components involved in the establishment and maintenance of the pathway. A good way to identify such components would be to isolate and characterize mutants that specifically affect particular steps in the cell's exocytic machinery. This approach, which has been extraordinarily successful in studies of the secretory pathway of *Saccharomyces cerevisiae* (for review, see Schekman and Novick, in *The Molecular Biology of the Yeast* Saccharomyces cerevisiae, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York [1982]), has not yet been fully exploited in mammalian systems, partly because of formidable technical difficulties and partly because of the lack of a good selective system to isolate mutants of the correct type.

In an effort to solve some of these problems, we have recently isolated and characterized sets of murine and hamster cell lines that constitutively express large quantities of HA on the cell surface. An important feature of these cell lines is that they carry multiple copies of the HA gene (in BPV vectors) in either an integrated state or an episomal state. Because it seems unlikely that all of these copies could be mutated simultaneously, we should be able to avoid the problem of isolating mutants that fail to express HA on their surface merely because of mutations in HA itself.

These cell lines were originally selected for their ability to express large quantities of HA on their surface (approximately 10^7 molecules/cell in the best case). This amount is more than 100 times

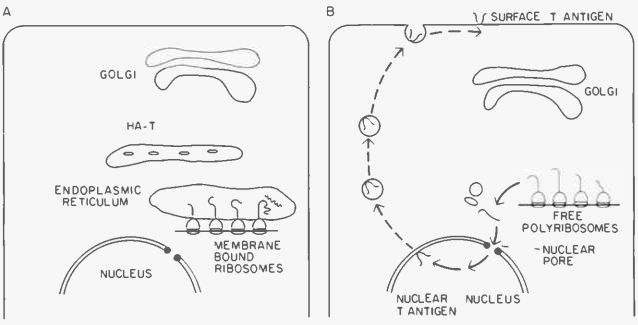

FIGURE 4 A model to illustrate the intracellular location of HA-T antigen (*A*) and a possible scheme for the transport of wild-type T antigen through the nucleus to the cell surface (*B*).

the threshold required for detection of HA by surface immunofluorescence and 10 times more than the amount needed to bind erythrocytes. However, a puzzling characteristic of the original cell lines was the variation in expression of HA from cell to cell, as detected by immunofluorescence. This problem was solved in September, 1984, when we obtained a Coulter Epics C fluorescence-activated cell sorter (Fig. 5). This machine has been invaluable in allowing us to obtain derivatives of the original cell lines that homogeneously express very high levels of HA on their surfaces. Examples of typical data are shown in Figure 6. We are at present analyzing these cell populations to determine why they express HA so efficiently. Among the more obvious possibilities are (1) an increased number of vector molecules per cell, (2) an increased amount of mRNA coding for HA, and (3) an increase in the half-life of HA on the cell surface. Whatever the mechanism, the elevated expression of HA seems to be a stable characteristic of these cell lines: Some of the earlier isolates have now been maintained in continuous culture for several months without a noticeable decrease in HA production and without a return to heterologous expression of HA. Surprisingly, many of these cell lines bind red cells very poorly. Apparently, they secrete a glycocalyx containing a large amount of neuraminic acid that shields HA expressed on the cell surface. After the glycocalyx is removed by mild digestion

FIGURE 5 Eric Hunter, Linda Rodgers, and Janet Hearing working with the Coulter Epics C fluorescence-activated cell sorter.

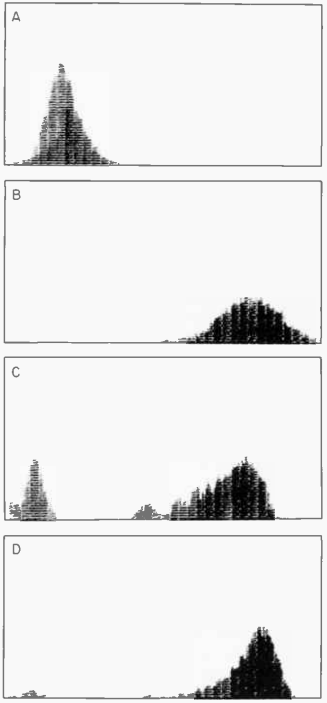

FIGURE 6 Analysis by flow cytometry of cell lines that express large amounts of HA on their surfaces. Each panel shows the number of cells (vertical axis) that fluoresce with a given intensity (horizontal axis). The intensity is displayed in arbitrary units on a logarithmic scale stretching over three log units. (*A*) Autofluorescence of a line of hamster cells expressing HA from a BPV vector. (*B*) Surface fluorescence of the same cells stained with fluorescein-conjugated anti-HA serum. (*C*) Binding of fluorescent erythrocytes to a line of murine cells that show heterogeneous expression of HA on its surface. The peak at the left consists of cells that have not bound any fluorescent red cells; the complex series of peaks at the right represents cells that have bound increasing numbers of fluorescent erythrocytes (1, 2, 3,.. .etc). (*D*) Same cells after six rounds of selection and growth, in which cells that bind erythrocytes efficiently were isolated and grown to mass population. The proportion of cells that fail to bind red cells is greatly reduced; the vast majority of the population bind more than seven red cells.

with trypsin and/or neuraminidase, virtually all of the cells in the population bind erythrocytes very efficiently. In all of the murine and hamster lines, HA is transported to the cell surface more slowly than in simian cells infected with SV40-HA recombinant vectors. There is also an approximately threefold difference in the rate of transport of HA in different cell lines. The reasons for these variations are under investigation.

We are currently using these cell lines to isolate and identify mutant cells that display HA on the cell surface in a temperature-conditional fashion. Cells are mutagenized with ethylmethanesulfonate, and after a period of growth at 32°C, they are shifted to 39°C. Several of the selection procedures that we are using will work only if HA displayed on the surface at the time of the temperature shift is removed. This is done by incubating the cells in the presence of excess anti-HA antibody and allowing the cells to remove the cross-linked antigen during a period of brief incubation at 39°C. Preliminary evidence obtained with one of the HA-producing cell lines indicates that the HA-antibody complexes rapidly become "capped" and then disappear from the cell surface. Cells that are unable to reexpress HA during further incubation at 39°C may then be identified and physically separated from the rest of the population by flow cytofluorometry. These cells are then grown into mass culture, and the process is repeated to enrich for mutants in the secretory pathway.

A second method for the isolation of cellular secretory mutants takes advantage of the hemagglutinating and membrane-fusing activities of HA. In collaboration with J. White and co-workers (Yale University), we have shown that human erythrocytes loaded with small proteins in vitro can be made to fuse with lines of hamster and murine cells expressing HA. The contents of the red cells are then delivered into the cytoplasm. We are using this method to introduce the toxic A chain of the lectin ricin into cells that express HA on their cell surface. Mutant cells that survive this selection should be either resistant to the action of the toxin or defective in the biosynthesis and transport of HA.

Finally, we have devised several schemes to isolate transport mutants of mammalian cells based on the action of aminoglycoside-3′-phosphotransferase, an enzyme that renders eukaryotic cells resistant to the antibiotic G418. Under normal circumstances, this antibiotic kills cells by binding to ribosomes and inhibiting protein synthesis. Aminoglycoside-3′-phosphotransferase is a cytoplasmic enzyme that modifies the antibiotic and appears to block its entry into the cell. We postulate that if the enzyme is directed to a different cellular compartment, entry of the antibiotic will not be blocked and the cell will once again be sensitive to G418. By linking specific dominant signal sequences (e.g., the hydrophobic signal sequences of HA) to the amino terminus of the *neo*^{r} protein, we expect to synthesize a chimera that (like HA-T antigen) crosses the ER. However, cellular mutants that fail to compartmentalize the chimera in this way should be resistant to the antibiotic. We are at present characterizing a series of HA-*neo*^{r} protein fusions to determine their localization and activity within cells. This approach can, in principle, be applied to select mutants in other cellular transport pathways, by coupling to *neo*^{r}, signals that direct proteins to other cellular locations like the nucleus or the mitochondria.

MECHANISM OF HA-MEDIATED CELL FUSION

Influenza viruses, like other enveloped animal viruses, enter and infect cells by a process involving fusion of the viral membrane with a cellular membrane. Following binding to sialic-acid-containing receptors on the cell surface, the virions are taken into the cell by endocytosis; entry into the cytoplasm occurs through the membranes of intracellular vesicles (like endosomes) where the endogenous pH is low. Experimentally, this activity can be manifested as cell-cell fusion when monolayers of cells displaying HA on their plasma membranes are transiently exposed to low pH. Alternatively, as described above, fusion induced by low pH can be assayed by red-cell-mediated delivery of horseradish peroxidase (HRP) into cells expressing surface HA. We have previously shown, using cells expressing HA either transiently from a recombinant SV40-HA viral vector or constitutively from a recombinant BPV-HA 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]; Sambrook et al., *EMBO J. 3:* [1985] in press). This fusion activity requires a

posttranslational proteolytic cleavage of the hemagglutinin 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 hydrophobic amino terminus, which has been implicated in the fusion activity and which is highly conserved in HAs from different virus strains, is generated on the HA_2 subunit. A working hypothesis for the mechanism of HA-mediated fusion is shown in Figure 7. At low pH (<5.2), the protonation of one or more amino acid side chains in the subunit interface causes a partial dissociation of the HA trimer. As a result, the aminoterminal "fusion peptide," which is normally tucked inside the trimer, becomes exposed and is inserted into the target lipid bilayer. Thus, HA becomes an integral component of two membranes, presumably bringing them close enough together to fuse.

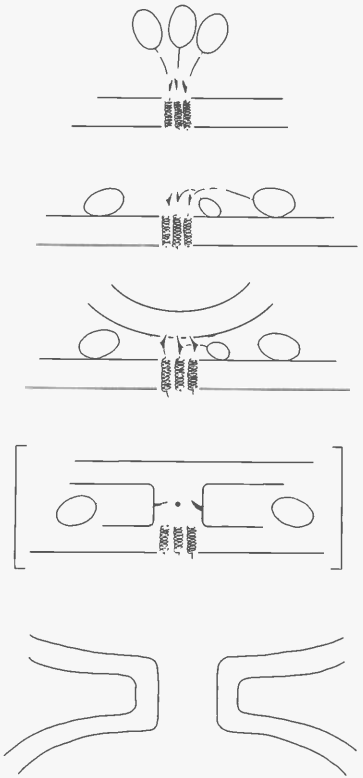

FIGURE 7 A model for the low-pH-induced fusion activity of HA. This model is based largely on the work of A. Helenius (Yale University) and his collaborators.

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. Three mutants have been constructed that contain single base alterations that result in nonconservative amino acid changes in the fusion peptide. These changes interrupt the hydrophobic stretch with charged amino acids or extend the hydrophobic sequence to 18 amino acids. 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, protease sensitivity, and fusion assays (in collaboration with J. White and R. Doms, Yale University) have been used to establish the phenotype of the mutants. The substitution of glutamic acid for the glycine residue at the amino terminus of HA_2 abolishes all fusion activity, although a conformational change in the protein (monitored by protease sensitivity) and interaction of the fusion peptide with liposomes can take place at pH 4.8. Substitution of glutamic acid for the glycine residue at position 4 in HA_2 shifts the threshold pH for cell-cell fusion or erythrocyte delivery from approximately 5.2 (wild type) to approximately 5.6. The efficiency of fusion is significantly less than that observed with the wild-type protein. Finally, extending the hydrophobic stretch by replacement of the glutamic acid with glycine at position 11 yields a mutant protein that induces red-cell-mediated delivery of HRP with the same efficiency and pH profile as the wild-type protein; however, this mutant was unable to induce cell-cell fusion at any pH.

In a separate experiment, we have cloned and sequenced the gene encoding a naturally occurring variant of HA that induces cell-cell fusion at an elevated pH of 5.6. Three amino acid alterations were found when the deduced amino acid

sequence of the variant was compared with that of wild-type HA. Two of the altered amino acids are located on the external surface of HA_1 and are unlikely to be involved in the altered fusion activity. The third change involves the substitution of an uncharged glutamine residue for a glutamic acid residue at position 132 in HA_2. Exchange of restriction fragments between the cloned genes encoding the wild-type and mutant proteins, and expression of the reassorted genes using SV40-HA recombinant vectors, confirmed that this substitution was indeed the cause of fusion at elevated pH. In the wild-type protein, this glutamic acid residue is linked by a salt bridge to an arginine at position 185 in the HA_2 of an adjacent subunit of the trimer. The fact that abolition of this salt bridge allows the conformational change to occur at pH 5.6 suggests that it may play a crucial role in stabilizing the HA trimer.

These results suggest that fusion takes place in at least three stages—a conformational change in HA induced by low pH, which exposes the fusion peptide; binding or insertion of this peptide into the target membrane; and, finally, destabilization and coalescence of the closely apposed lipid bilayers. Partial destabilization resulting in small areas of membrane coalescence may be sufficient for delivery of red cell contents; destabilization and coalescence of more extended lengths of membrane are required for cell-cell fusion and polykaryon formation.

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In Press, Submitted, and In Preparation

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CELL-CYCLE-SPECIFIC EXPRESSION

J.A. Lewis D.A. Matkovich

Genetics of Growth-phase-dependent Thymidine Kinase Gene Expression

J.A. Lewis, D.A. Matkovich

Our laboratory is fundamentally interested in defining the genetic determinants that govern the S-phase specificity of thymidine kinase (*tk*) gene expression in higher eukaryotic cells. To this end, we have (1) cloned the Chinese hamster *tk* gene in a recombinant phage vector, (2) cloned a nearly full-length cDNA to the hamster *tk* mRNA in a λGT10 vector and characterized this clone by dideoxynucleotide sequencing, and (3) sequenced

selected restriction fragments of the hamster *tk*-gene clone. This progression of experiments has led us to a nearly complete resolution of the structure of the Chinese hamster *tk*-gene presented in Figure 1. In brief, the hamster *tk* mRNA sequences are distributed through the 11.2 kb of genomic DNA in at least seven exon segments that range in size from the 32-bp Exon II to the 680-bp Exon VII. These exons are segregated by intervening sequences that range in size from the 5.4-kb intron which segregates Exons IV and V to the 92-bp intervening sequence which segregates Exons I and II. The nucleotide sequences that comprise the exon-intron boundaries show extended homology with the consensus sequences described for splice-donor and splice-acceptor nucleotides.

In an attempt to resolve the structural details at the extreme 5′ end of the hamster *tk* gene and to propose a site for *tk*-gene transcription initiation, we have constructed a *tk* minigene, designated pHaTK·2, that efficiently transforms a variety of Tk⁻ cells to Tk⁺ and have prepared Exo III/S_1 deletion mutants of pHaTK·2 through the promoter region. The endpoints of the deletions are shown in Figure 1.

We find that mutant derivatives of pHaTK·2 that retain at least 121 bp 5′ to the translation initiator AUG transform LTk⁻ cells as efficiently as the pHaTK·2 gene, whereas those that retain only 79 bp transform with a nearly 100-fold reduction in efficiency. It seems clear by this analysis that elements important to *tk*-gene transcription are contained within the 42 bp between −79

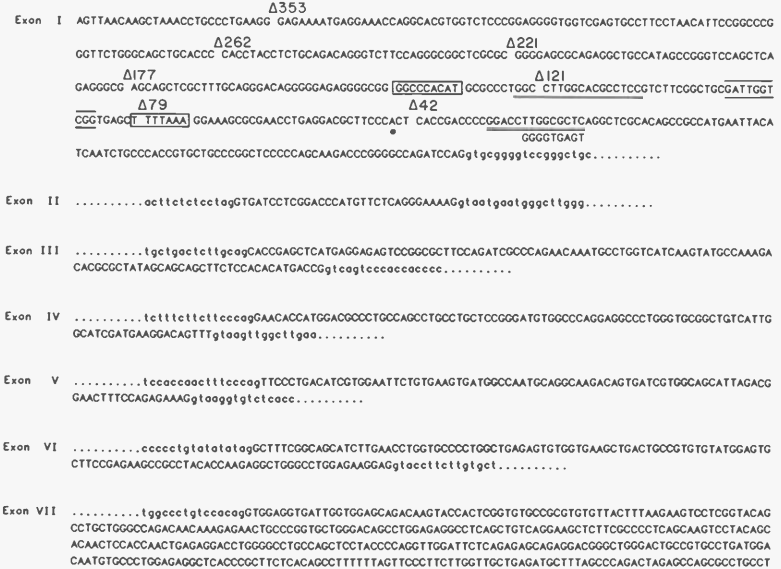

FIGURE 1 The nucleotide sequence of the 380 bp 5′ to the translation initiator AUG of the hamster *tk* gene, the seven *tk*-gene exons, and bordering intron sequences. The endpoints of the Exo III/S_1 5′ deletions described in the text are indicated by a triangle, designated to reflect the number of nucleotides remaining 5′ to the initiator AUG codon.

and −121 and that *tk*-gene transcription initiation must occur within 121 bp 5′ to the translation initiator AUG. Within this region, we find a T/A-rich sequence at −72/−79 that we suspect is a hamster *tk*-gene homolog to the TATA sequence described by Goldberg and Hogness. If we are correct in this assumption, we propose that *tk*-gene transcription is initiated at the A residue at −44, since this residue, by lying in a pyrimidine-rich sequence within 29–33 bp of the TATA sequence, strictly fulfills the consensus criteria for a site of transcription initiation.

In addition to pHaTK·2, we have constructed a variety of other hamster *tk* minigenes shown in Figure 2. These genes were developed to analyze our unexpected finding that minigene pHaTK·1

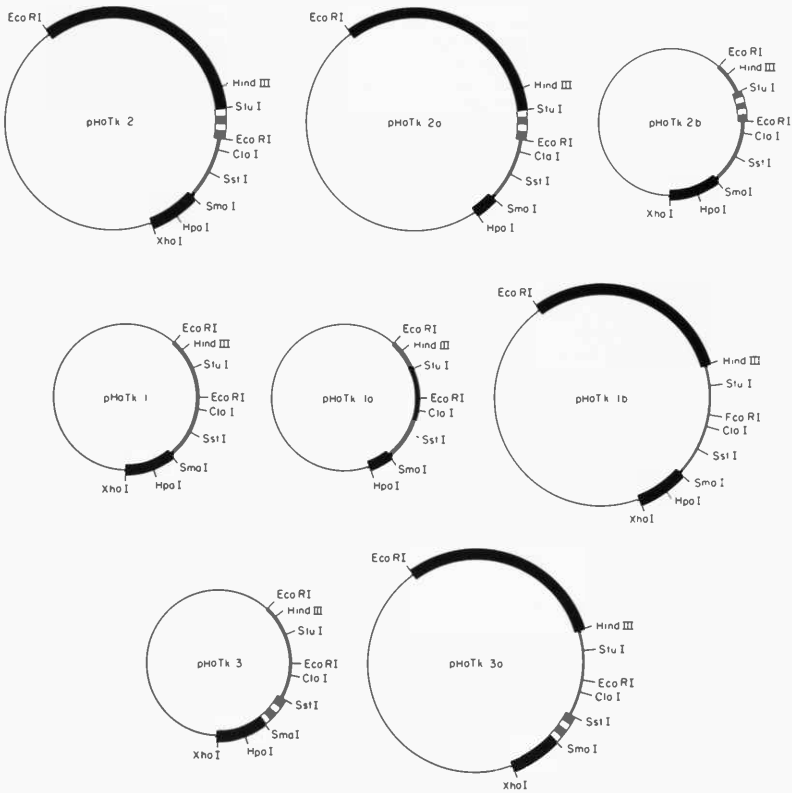

FIGURE 2 Partial restriction maps of various Chinese hamster *tk* minigenes. Thin lines represent sequences derived from the Chinese hamster *tk* cDNA λcTK·90; heavy lines represent sequences derived from the hamster *tk*-gene isolate λHaTK·5. Intervening sequences are indicated as open boxes within the heavy lines.

transforms LTk$^-$ cells 25-fold less efficiently than pHaTK·2. Since pHaTK·1 is composed only of hamster *tk* cDNA sequences fused to the hamster *tk*-gene promoter, it differs significantly in structure from pHaTK·2, which contains all of the sequences of pHaTK·1, introns *e* and *f*, as well as 3.5 kb of genomic DNA derived from the 3′ end of the hamster *tk* gene. To consider the contribution of each of these variables to the transformation efficiency of pHaTK·2, we introduced introns *e* and *f* into pHaTK·1 to create pHaTK·2b and in so doing developed a *tk* minigene that transforms LTk$^-$ cells to Tk$^+$ with a 15-fold greater efficiency than pHaTK·1. At the same time, we introduced introns *a* and *b* of the hamster *tk* gene into pHaTK·1 to create pHaTK·3, which transforms LTk$^-$ cells nearly 8-fold more efficiently than pHaTK·1 and thereby demonstrated the generality of the intervening sequence Tk$^+$ transformation-enhancement phenomenon. Both pHaTK·2b and pHaTK·3 genes, however, were measurably less efficient (2–3-fold) than pHaTK·2, a difference we attributed to the 3.5 kb of genomic DNA contained in pHaTK·2. To test the effects of this DNA segment in other *tk*-gene constructions, we introduced this DNA into pHaTK·1 to create pHaTK·1c and into pHaTK·3 to create pHaTK·3a and found that through these manipulations, we developed derivatives that transformed 3-fold and 1.9-fold more efficiently than the parental *tk* minigenes.

We do not yet understand the mechanistic basis for the intervening sequence enhancement phenomenon that makes the major contribution to pHaTK·2 minigene transformation efficiency. Curiously, this phenomenon has been described previously in transformation studies with dihydrofolate reductase (*dhfr*) minigenes, in which context the introduction of *dhfr* intervening sequences into *dhfr* minigene constructions improved transformation efficiencies as much as 30-fold. We imagine that intervening sequences either promote or facilitate the integration of the transfected DNAs into host genomic DNA, thereby promoting the number of stable Tk$^+$ transformants, or alternatively, intervening sequences in primary gene transcripts may increase the efficiency with which these transcripts mature to cytoplasmic mRNA.

We are just as ignorant about the basis of the relatively minor 3.5-kb enhancement phenomenon. We propose that sequences in this fragment improve the efficiency with which the polyadenylation signals contained in these minigenes are recognized, which leads to an improvement in the efficiency of primary transcript maturation.

Our compelling interest in the construction of *tk* minigenes is of course to exploit them to define the location and function of the genetic determinants governing the growth-phase dependence of *tk*-gene expression. Since the genetic determinants of interest may be contained in 5′-flanking DNA, 3′-flanking DNA, noncoding, coding, or intervening sequences, our experimental plan is to construct a minimal chimeric *tk* gene, containing only Chinese hamster coding and 3′ noncoding sequences, and to analyze the pattern of *tk*-gene expression in Rat 4 Tk$^+$ cells stably transformed with this gene. If necessary, we intend to elaborate this minimal gene systematically, supplying it with structural elements of the hamster *tk* gene, until we develop a *tk* minigene whose expression is growth-phase-dependent.

As a Tk recipient cell for the analysis of *tk*-gene expression from our various *tk*-gene constructions, we have adopted the Rat 4 Tk$^-$ cell line derived here at Cold Spring Harbor Laboratory by W. Topp. This cell line can be readily transformed to Tk$^+$, grows rapidly with a doubling time of 14–16 hours, is strictly growth-arrested at high density, is stable as a confluent monolayer for 96–120 hours without serum refreshment, and can be induced to resume logarithmic cell division if passed through fresh media at lower densities. We have generated Rat 4 Tk$^+$-transformed lines using either the hamster *tk*-gene clone, λHaTK·5, the *tk* gene of herpes simplex virus I, or a hamster/herpes chimeric gene designated pHaΔ6He (Fig. 3). This chimeric gene consists of the herpes *tk*-gene promoter fused to the coding and 3′ noncoding nucleotides of the Chinese hamster *tk* cDNA. As we expected, *tk*-gene expression in Rat 4 lines transformed with the hamster Tk genomic clone λHaTK·5 is growth-phase-dependent by the following criteria. (1) Specific Tk activity in confluent growth-arrested monolayers is only 5–10% the specific Tk activity of logarithmically growing cultures; (2) the specific Tk activity of growth-arrested cultures can be increased five- to tenfold by serum refreshment or by replating at lower density; (3) the specific Tk activity of growth-ar-

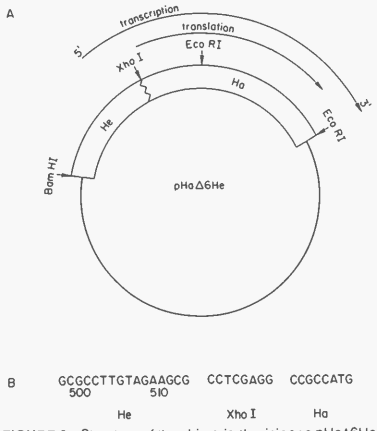

FIGURE 3 Structure of the chimeric *tk* minigene pHaΔ6He. This gene was constructed by combining a 700-bp *Bam*HI-*Xho*I fragment from the HSV *tk*-gene mutant Tk × 81 containing the HSV *tk*-gene promoter and cap site with an Exo III/S_1 deletion mutant of pHaTK·1 that contains the complete coding and 3′ noncoding nucleotides of the hamster *tk* mRNA and six nucleotides 5′ to the translation initiator AUG codon.

rested cultures can be increased five- to tenfold by infection with adenovirus type 5. These findings are characteristic of the pattern of endogenous *tk*-gene expression that has been documented throughout a literature of nearly 20 years. In contrast, the specific Tk activity in Rat 4 cell lines transformed to Tk$^+$ with the *tk* gene of HSV-1 is growth-phase-independent, being largely invariant under the variety of culture conditions described above, results consistent with an extensive literature as well.

We were intrigued to find that *tk*-gene expression in Rat 4 Tk$^+$ lines transformed with the chimeric pHaΔ6He gene is growth-phase-dependent, by all the criteria established for the λHaTk·5-transformed lines above. These findings constitute the first evidence that the determinants of *tk*-gene growth-phase-dependent expression are contained exclusively within sequences of the mature *tk* mRNA. We have yet to establish precisely the location of these determinants and the manner in which they function, although experiments to consider these fundamental issues are presently under way.

In Press, Submitted, and In Preparation

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Lewis, J.A. and D.A. Matkovich. Tk gene expression in Rat 4 cells transformed with TK mini-genes is growth phase dependent and responsive to adenovirus infection. (Submitted.)

HORMONAL AND DEVELOPMENTAL CONTROL OF GENE EXPRESSION

D.T. Kurtz W. Addison, J. MacInnes, D. Danna, J.-Z. Li, E. Nozik, A. Louis

For the past several years, we have been studying the hormonal and developmental control of the expression of a rat gene family that codes for a protein called α_{2u} globulin. This protein was first described as a very abundant protein (1–2% of total protein synthesis) in adult male rats. The synthesis in male rat liver is under complex hormonal control: Androgens, glucocorticoids, growth hormone, and insulin all induce hepatic α_{2u}, whereas estrogens repress the synthesis of this protein. We have previously shown that α_{2u} is encoded by a multigene family (18–25 genes per haploid complement) clustered on chromosome 5.

TISSUE-SPECIFIC EXPRESSION OF THE α_{2u} GENE FAMILY

We have found that α_{2u} is expressed in several other tissues in rats besides the liver. The α_{2u} pro-

teins that are produced in the various tissues are distinguishable by differences in isoelectric points. We have shown that this is not due to posttranslational modification of the same primary translation product by translating mRNA from different α_{2u}-producing tissues, immunoprecipitating the α_{2u} made in vitro, and subjecting it to isoelectric focusing. The same protein isoforms found in vivo are found in α_{2u} produced in the cell-free translational systems. It appears that different α_{2u} genes are being transcribed in different tissues.

Liver α_{2u} represents a family of four to five different proteins (pI 4.8–6.0). The hormonal control of hepatic α_{2u} is well documented by our laboratory and several others. All of the different α_{2u} genes expressed in the liver rise and fall coordinately; i.e., the multihormonal control of rat liver α_{2u} synthesis is apparently not due to different hormones inducing different genes.

Salivary gland α_{2u} consists of four to five protein species with distinctly more acidic pIs than liver α_{2u}. The salivary gland proteins run as a cluster between pH 4.0 and pH 4.8 on isoelectric focusing gels. α_{2u} represents about 0.03% of total protein synthesis in salivary gland, i.e., about 1/20 to 1/30 the level found in liver. The synthesis of salivary gland α_{2u} seems constitutive: Identical levels are found in both males and females, and the levels do not seem to change during several endocrine manipulations in intact animals.

Lachrymal gland α_{2u} comprises a set of proteins that look identical to those of salivary gland α_{2u}. α_{2u} represents about 0.2% of total protein synthesis in lachrymal gland, i.e., about 1/5 the level in liver. Male rats produce about three to five times more lachrymal α_{2u} than do female rats. This difference is apparently due to androgen induction: Female rats given androgens produce lachrymal α_{2u} at the same rate as do males. All isoelectric forms are induced coordinately.

Mammary glands in pregnant female rats at day 18 or 19 of gestation produce α_{2u} at a very low level (about 1/50 the level in male liver). Mammary gland α_{2u} is represented by a very large number of protein isoforms: 15–18 different α_{2u} proteins can be distinguished (pI 4.0–8.0). The hormonal modulation of mammary gland α_{2u} is currently under investigation. The synthesis peaks sharply at day 18 or 19 of gestation and then falls rapidly until, by day 21 and following parturition, no mammary gland α_{2u} is detectable. This timing is consistent with a model in which progesterone is inducing α_{2u} and then a surge in prolactin, which is known to occur about day 17 or 18, represses α_{2u} synthesis.

The preputial gland is the most abundant source of α_{2u} proteins that we have found. It represents about 5–10% of total protein synthesis in this tissue, and the synthesis seems to be constitutive. Preputial gland α_{2u} proteins separate into the same complex protein isoforms that are found in mammary glands. The number of different protein isoforms distinguishable in the preputial gland is close to the number of α_{2u} genes that we have estimated to be in the genome. It thus appeàrs that the preputial gland (and perhaps mammary gland) transcribes nearly the entire complement of α_{2u} genes present in the genome, whereas the liver, salivary gland, and lachrymal gland transcribe much smaller and distinct subsets.

TISSUE-SPECIFIC EXPRESSION OF CLONED α_{2u} GENES

We have cloned 18 different α_{2u} genes from a library of the rat genome. Identifying which gene is being transcribed in which tissue has proved to be difficult: The protein-coding regions of the different α_{2u} genes are for the most part very highly conserved. Thus, one cloned gene will hybridize to mRNA from all α_{2u}-producing tissues; raising the stringency of hybridization has no discernible effect. Surprisingly, the introns are also highly conserved. Intron-specific probes made from different cloned genes cannot be used to distinguish different α_{2u} transcripts in nuclear RNAs from various tissues. The 5′ and 3′ noncoding regions of the different mRNAs are identical in length, as assayed by S1-nuclease and by primer-extension analyses.

We have used two approaches to identify the tissue of expression of specific cloned α_{2u} genes: We have sequenced the extreme 5′ ends of 12 of the 18 cloned genes. In a stretch of approximately 200 nucleotides from the cap site (essentially the entire first exon), no two genes are exactly identical, although the sequences are very similar. We are currently sequencing the corre-

sponding regions of several tissue cDNAs to match up the sequences in this region. Sequence differences in exon III may also prove to be diagnostic.

Another approach is to use the pI differences in the α_{2u} proteins that are made in the various tissues. We have used COS-2 cells as a linked transcription-translation system to produce α_{2u} protein from the individual α_{2u} genes. COS cells constitutively produce SV40 T antigen and will thus effect the replication of any cloned segment of DNA containing the SV40 origin of replication. We have constructed several SV40-α_{2u} fusion genes (driven by the SV40 early promoter) and have produced α_{2u} protein in COS cells. In the course of this research, we made the surprising discovery that adenovirus VA I and VA II RNAs greatly increase the efficiency of translation of transfected genes in COS cells. If the SV40-α_{2u} hybrid construct is cotransfected into COS cells with an adenovirus fragment containing the VA genes, the level of α_{2u} protein produced is increased 15–20-fold, although there is no discernible difference in the extent of replication of, or transcription from, the SV40-α_{2u} hybrid gene. This finding that adenovirus VA RNA can have a nonspecific enhancing effect on translation has now been seen in other systems.

IN VIVO TRANSFECTION OF CLONED α_{2u} GENES

Once we have identified specific genes as being transcribed in different tissues, we will attempt to determine the molecular basis of this tissue-specific expression, i.e., to identify the specific DNA sequences responsible for the tissue specificity. There are many recent examples of "tissue-specific enhancers" that greatly increase transcription in a given tissue but are not part of the promoter region per se.

To study this, we would need analogs of the various tissues in which α_{2u} is produced. This is not a trivial problem, since "liver cells" that have been put into culture very seldom retain their differentiated properties. It was recently shown that calcium-phosphate-precipitated polyoma DNA, when resuspended in a buffer containing collagenase and hyaluronidase, can be injected directly into the liver and spleen of mice. The polyoma DNA is able to replicate and remains apparently extrachromosomal for several days. We have managed to duplicate this finding (in both rats and mice) and, further, we have found that if a cloned segment of DNA containing the polyoma origin is coprecipitated with the entire polyoma genome, the T antigen produced by the polyoma DNA can act in *trans* and effect the replication of the coinjected plasmid.

We have also had success injecting bovine papilloma virus (BPV)-containing plasmids directly into mouse liver. We have used the "69%" *Bam*HI-*Hin*dIII fragment of BPV, which is known to be sufficient for BPV replication in cultured mouse cells. When cloned DNA containing this fragment is injected into mouse liver, extrachromosomal BPV DNA is detectable on Southern blots 2 days after injection. This should be a very direct and simple method by which to study the tissue-specific expression of cloned liver α_{2u} genes. We can further use this to study which DNA sequences are required for the hormonal modulation of α_{2u} transcription in liver. Our studies of the hormonal modulation of α_{2u} synthesis following transfection into tissue-culture cells have been limited by the lack of cell lines that contain various hormone receptors. If α_{2u} genes injected into liver retain their hormonal inducibility, we should be able to use this system to study DNA sequences that are required for the response of liver α_{2u} genes to many different hormones.

CONTROL OF RAT TROPOMYOSIN GENE EXPRESSION

In collaboration with D. Helfman (Cell Biology Section), we are studying the mechanism by which adenovirus infection shuts down the synthesis of tropomyosin I expression, while not affecting the expression of other tropomyosin forms. We have introduced a cloned rat tropomyosin I gene into HeLa cells using DNA-mediated gene transfer, and we are in the process of determining whether this transfected gene is negatively controlled following adenovirus infection. If so, it should be possible to determine which DNA regions of this tropomyosin gene are responsible for its negative regulation.

GENETICS OF CELL PROLIFERATION

M. Wigler C. Birchmeier, D. Birnbaum, D. Broek, S. Cameron, L. Edlund, S. Efrat, O. Fasano, M. Goldfarb, T. Kataoka, S. Powers, P. Sass, E. Taparowsky, T. Toda, G. Waitches, D. Young

Scientists in our section study oncogenes, genes that drastically alter the proliferative capacity of normal cells. In particular, we have concentrated our efforts on the *RAS* oncogenes, which were among the first genes implicated in human cancer. The study of *RAS* has been facilitated by the recent discovery that close homologs exist in the yeast *Saccharomyces cerevisiae,* an organism readily subjected to thorough genetic analysis. Studies of the *RAS* pathway in yeast have led us to examine the cAMP pathway in fine detail. In addition to the above, we have recently isolated and are now studying three new human oncogenes. Much of the work on the yeast *RAS* was done in collaboration with F. Tamanoi (this section), J. Broach (Princeton University), K. Matsumoto (Tottori University, Japan), and I. Uno and T. Ishikawa (University of Tokyo, Japan); some of the work with associated yeast genes was done in collaboration with M. Zoller (Molecular Genetics Section); and the work on other vertebrate oncogenes was performed in collaboration with J. Fogh (Sloan Kettering Institute, Rye, New York).

Yeast *RAS* Genes

D. Broek, S. Cameron, T. Kataoka, S. Powers, T. Toda, M. Wigler

In the yeast *S. cerevisiae*, there are two genes, *RAS1* and *RAS2*, that are closely homologous to the mammalian *RAS* genes (Defeo-Jones et al., *Nature 306:* 707 [1983]; Dhar et al., *Nucleic Acids Res. 12:* 3611 [1984]; Powers et al., *Cell 36:* 607 [1984]). We have cloned both genes (*RAS1* and *RAS2*) from plasmid libraries and have determined the complete nucleotide sequence of their coding regions. They encode proteins that have nearly 90% homology with the first 80 positions of the mammalian *RAS* proteins, and nearly 50% homology with the next 80 amino acids. Yeast *RAS1* and *RAS2* proteins are more homologous to each other, with about 90% homology for the first 180 positions. After this, at nearly the same position that the mammalian *RAS* proteins begin to diverge from each other, the two yeast *RAS* proteins diverge radically. The yeast *RAS* proteins, like the proteins encoded by the mammalian genes, terminate with the sequence CysAAX, where *A* is an aliphatic amino acid. Thus, the yeast *RAS* proteins have the same overall structure and interrelationship as the family of mammalian *RAS* proteins. The domains of divergence may correspond to functional domains of the *RAS* proteins. Monoclonal antibody directed against mammalian *RAS* proteins immunoprecipitates protein in yeast cells containing high copy numbers of the yeast *RAS2* gene.

We and other investigators have previously shown that at least one functional *RAS* gene is essential for the germination of haploid yeast spores (Kataoka et al., *Cell 37:* 437 [1984]; Tatchell et al., *Nature 309:* 523 [1984]). This was demonstrated by constructing doubly heterozygous diploid yeast cells containing wild-type *RAS1* and *RAS2* alleles and *RAS1* and *RAS2* alleles each disrupted by a different auxotrophic marker. These diploid cells were then sporulated, and the resulting tetrads were analyzed. Only spores containing at least one functional *RAS* gene could germinate. We then introduced into these doubly heterozygous diploid cells a *RAS2* gene under the transcriptional control of the galactose inducible *GAL10* promoter (see Kataoka et al., *Cell 40:* 19 [1985]) linked to a third auxotrophic marker. These diploid cells were then sporulated, and tetrads were germinated on either glucose-containing medium (YPD) or galactose-containing medium (YPGal). The results confirm that at least one functional *RAS* gene is required for germination. *ras1*$^-$ *ras2*$^-$ *GAL10-RAS2* spores germinate only in the presence of galactose, the in-

ducer for the *GAL10* promoter. Cells with the genotype *ras1*$^-$ *ras2*$^-$ *GAL10-RAS2* were grown in YPGal and then shifted to YPD. The growth of these cells was then monitored, and cultures were plated on to YPGal agar to measure cell viability. Within several cell generations, cell growth ceased and cells lost viability. These experiments indicated that the *RAS* genes are needed not only for spore germination, but also for the continued growth and viability of yeast cells.

Certain missense mutations drastically alter the biological activity of mammalian *RAS* genes. In particular, the Ha-*ras*^{Val12} gene, which encodes valine instead of glycine at the twelfth codon of the human Ha-*ras* gene, can induce the tumorigenic transformation of NIH-3T3 cells. To test the consequences of a similar mutation of *RAS2* on the properties of yeast cells, we constructed the *RAS2*^{Val19} gene using site-directed mutagenesis (Kataoka et al., *Cell 37:* 437 [1984]). *RAS2*^{Val19} thus encodes valine instead of glycine at position 19, which corresponds to position 12 of the mammalian Ha-*ras* protein. We then examined the effects of introducing this gene into yeast cells. Our first observation was that diploid cells containing *RAS2*^{Val19} could not be induced to sporulate by incubation under conditions of nutritional deprivation (Kataoka et al., *Cell 37:* 437 [1984]). Next, we observed that haploid cells carrying *RAS2*^{Val19} lost viability if starved for nitrogen, sulfur, or phosphorus and failed to arrest in G_1 under those conditions. Wild-type cells normally arrest in G_1 and retain viability for long periods when nutritionally deprived. In addition to these phenotypes, we also noted that cells carrying *RAS2*^{Val19} failed to accumulate carbohydrate stores as cells entered the stationary growth phase (Toda et al., *Cell 40:* 27 [1985]). Thus, in general, *RAS2*^{Val19} cells appeared to show a defective response to nutritional stress.

The cluster of cellular properties just described closely resemble the phenotype of cells carrying the *bcy1* mutation (Matsumoto et al., *Proc. Natl. Acad. Sci. 79:* 2355 [1982], *Cell 32:* 417 [1983], *Exp. Cell Res. 146:* 151 [1983]; Uno et al., *J. Biol. Chem. 258:* 10867 [1983]). The *bcy1* mutation was first isolated by Matsumoto and co-workers as a mutation that suppresses the lethality that otherwise results from the disruption of adenylate cyclase (Matsumoto et al., *Proc. Natl. Acad. Sci. 79:* 2355 [1982]). Cells carrying *bcy1* appear to lack the regulatory subunit of the cAMP-dependent protein kinase and hence have lost the requirement for cAMP (Uno et al., *J. Biol. Chem. 257:* 14110 [1982]). These observations suggest that the *RAS* genes might be participating in the cAMP pathway. In support of this idea, we found that *bcy1* suppressed the lethality that otherwise results from disruption of both *RAS* genes.

The adenylate cyclase activity of the yeast *S. cerevisiae* is stimulated by guanine nucleotides in the presence of magnesium (Casperson et al., *J. Biol. Chem. 258:* 7911 [1983]). In this respect, yeast adenylate cyclase resembles the adenylate cyclase of mammalian cells, which can be stimulated by a guanine-nucleotide-binding complex called G_s (Gilman, *Cell 36:* 577 [1984]). Since the yeast *RAS* proteins also bind guanine nucleotides (Tamanoi et al., *Proc. Natl. Acad. Sci. 81:* 6924 [1984]), we reasoned that they might also modulate adenylate cyclase. This was tested directly by the assay of membranes from wild-type yeast cells and *ras1*$^-$ *ras2*$^-$ cells. Membranes from either contained appreciable adenylate cyclase activity when assayed in the presence of manganese ion, but *ras1*$^-$ *ras2*$^-$ membranes displayed negligible levels of activity when assayed in the presence of magnesium and a nonhydrolyzable guanine nucleotide analog. These results were confirmed in a striking manner by membrane-mixing experiments. We prepared membranes from *RAS1 RAS2* yeast carrying the *cyr1-1* mutation and membranes from *ras1*$^-$ *ras2*$^-$ yeast. The *cyr1-1* mutation disrupts the catalytic subunit of adenylate cyclase. Membranes from these two sources were assayed separately and together after membrane mixing and fusion. The data indicate that membrane mixing and fusion regenerate a guanine-nucleotide-stimulated adenylate cyclase activity (Toda et al., *Cell 40:* 27 [1985]).

The addition of purified yeast *RAS2* protein to membranes from *bcy1 ras1*$^-$ *ras2*$^-$ cells restores adenylate cyclase activity to about 50-fold above background level (Broek et al., *Cell* [1985] in press). Adenylate cyclase activity in the *bcy1 ras1*$^-$ *ras2*$^-$ membranes can also be increased dramatically by addition of yeast *RAS1* protein. Restoration of adenylate cyclase activity by *RAS* proteins is dependent on the presence of guanine

nucleotide, with the nonhydrolyzable GTP analog, GppNp, yielding twice the activity of that observed in the presence of GDP. Incubation of the *RAS2* protein with GTP prior to mixing with the *bcy1 ras1*$^-$ *ras2*$^-$ membranes results in adenylate cyclase activity comparable to that restored with *RAS2* bound to GDP. In contrast, preincubation of *RAS2*^{Val19} with GTP results in activation of adenylate cyclase to levels identical to that induced by *RAS2* proteins bound to GppNp. These results confirm our genetic studies and indicate that there are proteins in membranes that can distinguish between *RAS* proteins complexed with GTP and *RAS* proteins complexed with GDP; furthermore, they provide for the first time an in vitro bioassay for the effector function of *RAS*.

Yeast Genes in the *RAS*/cAMP Pathway

T. Kataoka, S. Powers, P. Sass, T. Toda, M. Wigler

In our effort to understand thoroughly the function of *RAS* in yeast, we have begun cloning genes which encode proteins that operate along the *RAS*/cAMP pathway. To do this, we have used standard genetic strategies.

BCY1 was cloned from a centromere-linked library (constructed by M. Rose, Massachusetts Institute of Technology, and generously provided by him) by transforming *bcy1* cells with the library and selecting cells capable of resistance to heat shock. *bcy1* cells, like *RAS2*^{Val19} cells, are heat-shock-sensitive (55°C for 30 min), presumably because they cannot enter G_0. We obtained one clone that displays all of the genetic features expected of a *BCY1* clone. Gene disruptions of *BCY1* were constructed and used to transform haploid yeast cells by gene replacement. The transformants display the same phenotype of cells containing the spontaneously occurring *bcy1* mutation.

CYR1, the gene encoding adenylate cyclase, was cloned in a similar manner by transforming *cyr1-2* mutants (temperature-sensitive for adenylate cyclase) with centromere-linked libraries and selecting cells that grew at the nonpermissive temperature. The nucleotide sequence of this gene has been determined (>6000 bp), and an amino acid sequence of the product has been deduced. We are not yet certain which methionine represents the start codon. Aminoterminal deletion mutants of the adenylate cyclase gene give rise to proteins that are catalytically active but do not appear to be regulated by *RAS*. High-level expression of such genes suppresses the lethality of the double *ras1*$^-$ *ras2*$^-$ mutation. We conclude from this that, in yeast, all of the lethal effects due to loss of *RAS* function are mediated through adenylate cyclase. We have succeeded in expressing enzymatically active yeast adenylate cyclase in *Escherichia coli*.

CAK1, a cAMP-dependent protein kinase catalytic subunit, was cloned by complementation of *cdc25*, a cell-division control mutant that arrests in G_1 at the nonpermissive temperature. *cdc25* is suppressed by *RAS2*^{Val19} and by high-level expression of adenylate cyclase. We therefore reasoned that *CAK1* would suppress *cdc25*, which it does. The nucleotide sequencing of this gene is nearly complete, and it shows extensive homology with the sequence of the bovine cAMP-dependent protein kinase catalytic subunit. Gene-disruption experiments indicate that cells lacking *CAK1* are viable. We therefore conclude that cells must contain a second cAMP-dependent protein kinase. Indeed, we observe by hybridization analysis another yeast gene weakly homologous to *CAK1*, which we are now cloning. High-copy plasmids of *CAK1* suppress lethality due to loss of endogenous *RAS* function or loss of adenylate cyclase. These results are formal proof that the effects of adenylate cyclase and hence cAMP are mediated through the cAMP-dependent protein kinase system.

CDC25 was cloned together with *CAK1* by transforming a *cdc25* strain with a centromere-linked library. *CDC25* is not one of the known genes in the adenylate cyclase pathway. We are nearly finished with its nucleotide sequence, and this work and the work on *CAK1* are proceeding in collaboration with M. Zoller (Molecular Genetics Section).

In addition to the genes described above, four recessive mutations, supA, B, C, and D, have been isolated that suppress the phenotype of *RAS2*^{Val19}. One gene, *PSI*, has been isolated that, in high copy, suppresses *RAS2*^{Val19}. The relationship of these genes to the classic cAMP pathway has yet to be determined.

Studies of the Vertebrate *RAS* Proteins

C. Birchmeier, D. Broek, O. Fasano, T. Kataoka, M. Wigler

Some tumor cells contain mutant *RAS* genes that are capable of transforming NIH-3T3 cells. Those genes that have been analyzed arise from the wild-type nontransforming *RAS* genes by mutations producing single amino acid substitutions at position 12 or 61 of the encoded protein. We have performed random bisulfite-induced mutagenesis on the cloned wild-type human Ha-*ras* gene to determine whether mutations at other positions can activate the transforming potential of that gene (Fasano et al., *Proc. Natl. Acad. Sci. 81:* 4008 [1984]). Most mutations are not activating, but mutations that specify single amino acid substitutions at position 12, 13, 59, or 63 of the encoded protein do activate the transforming potential of the Ha-*ras* gene. Some, but not all, mutant *RAS* proteins show an altered electrophoretic mobility in SDS-polyacrylamide gels.

Using the approach described in the previous section, we have been able to test if expression of the normal mammalian Ha-*ras* protein is sufficient for viability in yeast cells lacking their own endogenous *RAS* genes. To this end, we constructed a *GAL10*-Ha-*ras* transcription unit that utilized a full-length cDNA clone of the human Ha-*ras* mRNA under the control of the galactose-inducible *GAL10* promoter. This unit, closely linked to a *LEU2* marker, was inserted into diploid yeast cells that were doubly heterozyous for their endogenous *RAS* genes. Cells were induced to sporulate, and tetrads were examined after germination on YPD or YPGal plates. Approximately 40% of spores with the genotype *ras1*$^-$ *ras2*$^-$ *GAL10*-Ha-*ras* were capable of germination when plated on YPGal, from which we conclude that the human Ha-*ras* protein can supply essential *RAS* function to yeast. Direct biochemical experiments yielded the same result. Adenylate cyclase activity in *bcy1 ras1*$^-$ *ras2*$^-$ membranes was dramatically increased by the addition of purified human Ha-*ras* protein. These results indicate that the effector function of yeast and mammalian *RAS* proteins has been conserved in evolution. In particular, these results suggest that *RAS* proteins may be involved in regulating adenylate cyclase in vertebrates.

To test our ideas about *RAS* function derived from studies in yeast, we have developed a frog oocyte microinjection system. We have found that purified mammalian Ha-*ras* proteins (Gross et al., *Mol. Cell. Biol. 5:* 1015 [1985]) can induce immature oocytes to progress from prophase to metaphase. The Ha-*ras*^{Val12} is 200-fold more potent than Ha-*ras*^{Gly12} in inducing oocyte maturation. This effect is blocked by cholera toxin, which increases cAMP production in oocytes. We have observed no significant changes in cAMP concentrations due to injection of Ha-*ras* protein and conclude that the yeast *RAS* system is not entirely functionally analogous to the mammalian system. Nevertheless, it is clear that the oocyte system provides an excellent model system for testing ideas concerning *RAS* protein function.

Isolation and Characterization of Other Oncogenes

C. Birchmeier, D. Birnbaum, S. Efrat, O. Fasano
M. Wigler, D. Young

The transfer of genomic DNA into NIH-3T3 cells has led to the discovery that genes present in some tumor cells are capable of inducing foci of morphologically transformed NIH-3T3 cells. Most of the transforming genes detected in this way have now been identified as members of the *RAS* gene family, either Ha-*ras*, Ki-*ras*, or N-*ras*. The transforming *RAS* genes detected by the NIH-3T3 focus assay have structural gene mutations that account for their transforming activity. Since the great majority of tumor DNAs tested fail to induce transformed foci, we have speculated that the focus assay has a bias for *RAS* genes containing structural mutations. Therefore, we have begun a series of experiments to explore alternative assays for transforming genes present in NIH-3T3 cells after DNA transfer. The system is a modification of the one described by D. Blair and coworkers (National Institutes of Health). Like theirs, our assay also relies on the ability of transformed NIH-3T3 cells to form tumors in nude mice, but it incorporates methods of cotransfection to heighten sensitivity. Using this assay, we have detected three human transforming genes from the DNA of MCF-7, a human breast carci-

noma cell line. One of these is N-*ras*, which is amplified in MCF-7 cells but does not appear to contain structural mutations. We have not yet established whether the other two genes, which we have called *mcf2* and *mcf3*, are associated with any genetic abnormality in MCF-7.

We are still in the process of characterizing *mcf2* and *mcf3*. *mcf3* appears to be the human homolog of the v-*ros* gene and has the structure predicted for a transmembrane receptor. It has been activated by a gene rearrangement resulting in truncation of the region encoding the putative extracellular domain. In addition, we have isolated and are characterizing a gene called *mas1*, which was isolated from a human mastoid epidermal carcinoma.

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BIOCHEMISTRY OF YEAST *RAS* PROTEINS

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Structural and Functional Analyses of Yeast *RAS* Proteins

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A product of the *ras* oncogene continues to be a source of intellectual excitement from several standpoints. First, it is a membrane-bound protein that contains covalently bound fatty acids, which raises the question of how it travels to and interacts with the membrane. Second, it is a GTP-binding protein, as well as a GTPase, and is a member of the GTP-binding proteins that regulate various biochemical reactions within a cell. Finally, a single amino acid alteration is known to convert a normal protein to a form active in transformation. Why such a small change results in dramatic effects on the physiology of cells has been a tantalizing question to us. Our research effort is focused on carrying out physical and chemical studies of the *ras* protein.

In last year's Annual Report, we described expression of the *ras* gene in *Escherichia coli* aimed at preparing a large amount of the pure protein. During the past year, our emphasis has been to analyze products of *ras*-oncogene homologs in the yeast, *Saccharomyces cerevisiae*. The yeast homologs, *RAS1* and *RAS2*, code for proteins that have at their amino termini more than 90% homology with the mammalian *ras* protein. Furthermore, alteration of a 19th amino acid on the yeast *RAS2* protein from glycine to valine, a mutation analogous to the one found with the transforming *ras* protein, results in dramatic

changes in the growth properties of yeast cells. Thus, mechanistic aspects of the action of *ras* proteins could be elucidated using the yeast homologs. Two advances have recently been made. First, we have shown that the yeast *RAS* proteins exhibit GTP binding and GTP hydrolysis activities similar to those seen with the mammalian protein, which establishes similarities in biochemical activities between the yeast and mammalian proteins. Second, in collaboration with D. Broek and M. Wigler (this section), we have developed an adenylate cyclase system from yeast membranes that is dependent on the *RAS* proteins.

OVERPRODUCTION AND PURIFICATION OF YEAST *RAS* PROTEINS

Since the amount of *RAS* proteins produced in wild-type yeast cells is very low, we decided to overproduce these proteins. To accomplish this, the *RAS2* gene was cloned into a YEp51 plasmid that contained a galactose-inducible *GAL10* promoter. The construct directed the synthesis of a protein with an apparent molecular weight of 41,000 that was immunoprecipitable by a monoclonal antibody raised against the mammalian *ras* protein. The 41,000-molecular-weight protein appears to be a primary translation product, since cell-free translation of mRNA extracted from yeast cells expressing the cloned *RAS2* gene resulted in the production of a protein that comigrated with the 41,000-molecular-weight protein on SDS-polyacrylamide gels. Overproducing the *RAS1* protein, on the other hand, required the manipulation of the gene, since no suitable restriction sites were available close to the start of the gene. By using synthetic oligonucleotides, we created a *Sph*I restriction site at the start of the gene. After recloning into M13mp18 to add a *Sal*I site, the gene was cloned into the YEp51 plasmid DNA. The construct directed the synthesis of a protein with an apparent molecular weight of 34,000.

Expression in *E. coli* provides an alternative and powerful means to produce the *RAS* proteins in large amounts. Furthermore, it is relatively easy to purify a protein from *E. coli*. Our first construction utilized a *lac* promoter that is inducible by the addition of isopropyl-β-D-thiogalactoside. The yeast *RAS2* gene was inserted into the *Eco*RI site of pUC8 plasmid, which directed the synthesis of a protein containing 20 extra amino acids added to its amino terminus. A similar construct was made with the *RAS1* gene, which directed the synthesis of a protein containing five extra amino acids added to its amino terminus. We also constructed an expression vector that produced intact *RAS1* or *RAS2* protein in *E. coli*. The expression is under the control of a *recA* promoter that can be induced by the addition of nalidixic acid.

Extracts prepared from cells carrying the above constructs exhibited a high level of GDP-binding activity. This provided an assay to purify the *RAS* proteins. Several column chromatography methods, such as phosphocellulose, DEAE-cellulose, and gel filtration, are being employed for the purification. The fusion *RAS2* protein has been purified to approximately 70% purity as judged from densitometric scanning of a stained gel. Further attempts to purify the proteins using high-performance liquid chromatography (HPLC) and GTP agarose are being made.

MODIFICATION OF *RAS* PROTEINS

The mammalian *ras* proteins contain covalently bound palmitic acid. The fatty acylation most probably takes place on a cysteine residue located close to the carboxy terminus, since mutation of this cysteine destroys the modification. In contrast to the *ras* protein, a product of another oncogene (*src*) contains myristic acid. In this case, the fatty acylation occurs on a glycine residue close to its amino terminus. Whether there exist two distinct types of fatty acylations and whether they are related to the difference in their functions are two questions we would like to address.

As a first step toward investigating these points, we established that fatty acylation also occurs in yeast *RAS* proteins. First, both *RAS1* and *RAS2* proteins could be labeled with [^{3}H]palmitic acid. Furthermore, we were able to recover the radioactivity on the protein as palmitic acid after mild alkali treatment. Currently, we are investigating the site of modification as well as the nature of linkage. We are also making use of secretory mutants of yeast with the hope of gaining insight into the transport of the protein.

It has recently been reported that yeast *RAS1* protein is produced as a 34K protein that is later

modified to a 39K protein (Temeles et al., *Mol. Cell. Biol. 4:* 2298 [1984]). This suggests that another type of modification is taking place. In agreement with this observation, we found a similar 39K protein using our construction. However, we only detected a minor portion of the *RAS1* protein modified, and the majority of the protein remains as a 34K protein even after a long chase. The cause for this discrepancy is currently being investigated.

BIOCHEMICAL ACTIVITIES OF *RAS* PROTEINS

Using the *RAS2* protein purified from *E. coli*, we have carried out a detailed characterization of its enzymatic activities. First, the *RAS2* protein binds GDP and GTP. The binding was dependent on incubation temperature; very little binding was observed at 0°C, whereas maximum binding was observed at 45°C. The pH optimum was 7.6. Optimum binding was observed in the presence of 0.5 mM Mg^{++}, but some binding was also observed in the presence of EDTA. A Scatchard plot of GDP binding was consistent with single-site binding kinetics, which gave a calculated K_d value of 4.7×10^{-8} M. K_d for a nonhydrolyzable GTP analog, Gpp(NH)p, was 8.7×10^{-8} M. Thus, the *RAS2* protein appears to bind GDP and GTP with a similar affinity. This is in contrast to other GTP-binding proteins, such as elongation factor EF-Tu, which binds GDP much more tightly than GTP. Another biochemical activity that the *RAS2* protein exhibits is a GTPase. The hydrolysis was specific to GTP or dGTP and no hydrolysis of ATP, CTP, dATP, dCTP, or dTTP was observed. The products of hydrolysis were GDP and inorganic phosphate. The maximum level of GTPase activity was obtained at 45°C and in the presence of 0.5 mM Mg^{++}. The rate of GTPase activity was calculated to be 60 mmoles/min/mole of protein.

By incubating at low temperature and using Sephadex column chromatography, we were able to isolate a stable complex between the *RAS2* protein and GTP. Subsequent incubation at 37°C resulted in the conversion of the bound GTP to GDP. The GDP was still bound to the *RAS2* protein as revealed by nitrocellulose assay. We further showed that a GDP bound to the *RAS2* protein could be exchanged with a fresh GTP. Thus, the *RAS2* protein is capable of shuttling between two forms: the GTP-bound form and the GDP-bound form. We are currently interested in determining whether there are any differences, such as protein conformation, between the two forms.

The shuttling between the GTP-bound form and the GDP-bound form appears to be an important aspect of *RAS* protein action, since this is precisely the process that is impaired with mutant *RAS* proteins containing an amino acid alteration at either the 19th (Gly→Val) or 66th (Ala→Thr) residues. Expression of these mutant proteins results in a dramatic change in the growth properties of yeast cells, including decreased sporulation efficiency, increased sensitivity to nutrient starvation, and failure to accumulate carbohydrate reserves. The mutant proteins purified after their expression in *E. coli* exhibited GDP-binding activity similar to that seen with the wild-type protein. In addition, GDP bound to the mutant proteins could be exchanged with free GTP with an efficiency comparable to that seen with the wild-type protein. However, a significant difference was observed in their GTPase activities. The activity of the valine mutant was about 15% of that of the wild-type protein, and the threonine mutant displayed further reduced GTPase activity.

EFFECT OF *RAS* PROTEINS ON YEAST ADENYLATE CYCLASE

Genetic and biochemical studies carried out by M. Wigler's group (this section) have indicated that the yeast *RAS* genes are involved in the control of adenylate cyclase. Thus, membranes of yeast cells lacking both *RAS1* and *RAS2* genes fail to show any detectable level of adenylate cyclase (Toda et al., *Cell 40*: 27 [1985]). In collaboration with D. Broek and M. Wigler, we were able to demonstrate that the purified *RAS2* proteins are capable of revitalizing this adenylate-cyclase-defective membrane. *RAS2* protein purified from *E. coli,* as well as the protein purified from yeast, was capable of complementing the adenylate-cyclase-defective membrane. The complementing activity absolutely required the presence of the nonhydrolyzable GTP analog, Gpp(NH)p. Replacing Gpp(NH)p with GDP reduced the stimulation to 50% of that obtained

with Gpp(NH)p. Thus, *RAS2*-GTP is more effective in stimulating the adenylate cyclase than *RAS2*-GDP. Wild-type *RAS2* protein preincubated with GTP stimulated adenylate cyclase only to the level obtained with GDP, presumably because GTP hydrolysis took place during the preincubation. The valine mutant protein preincubated with GTP, on the other hand, gave the same level of stimulation obtained when Gpp(NH)p was used. Thus, the mutant proteins overactivate adenylate cyclase, which might be responsible for causing changes in the physiology of yeast cells. A large amount of purified *RAS* proteins provide an invaluable tool to investigate their function within yeast membranes.

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MOUSE GENETICS

L. Silver V. Bautch, J. Bahrenberg, L. Cisek, H. Fox, C. Jackson, D. Lukralle, P. Mains, S. Panzer, 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 recently, and it is now possible to focus research efforts in attempts to answer specific questions through the combined use of molecular and genetic techniques. Although we have chosen to concentrate our efforts on the *t* complex, we expect many of our results to be indicative of general principles of mammalian genome structure and function.

Integration of Retroviral Sequences into the DNA of Early Mouse Embryos

V. Bautch, L. Silver

One approach to the study of mammalian development is to identify mutations that disrupt the normal program of embryogenesis. Insertional mutations also provide a molecular marker for the recovery of genes disrupted by the mutation. However, in order to be able to clone the preintegration site and analyze the disrupted gene, the integration event must preserve the linear order of cellular sequences in a recognizable way. Retroviral genomes are particularly useful as potential agents of insertional mutation because retroviruses integrate with only a small (4 to 9 bp) duplication of cellular DNA. We are generating insertions in mice using a breeding scheme (N.

Jenkins and N. Copeland, pers. comm.) that allows for the de novo integration of endogenous retroviral genomes into the DNA of early embryos at a relatively high frequency. A subset of retroviral genomes called ecotropic proviruses has been followed because most inbred strains of mice contain a few (0–6) copies that can be easily followed using molecular techniques.

Mice of the RF/J strain contain three ecotropic proviruses. Two of these loci, *Emv-16* and *Emv-17*, are genetically linked and have been implicated in integration events in early embryos. We have crossed male RF/J mice to females of two different strains (CBA/CaJ and SWR/J) that are negative for endogenous ecotropic proviruses. The F_1 progeny were backcrossed to the ecotropic virus-negative strains. The proviral sequences of the N_2 offspring were analyzed by hybridizing genomic DNAs prepared from tail clips to an ecotropic virus-specific probe. N_2 females carrying *Emv-16* and *Emv-17* were then backcrossed to produce N_3 progeny that were analyzed for newly integrated proviruses.

We have scored a total of 29 ecotropic provirus (EP) fragments not found in parental DNAs from 184 mice (15.8%). Analysis of the results led to the following conclusions: (1) New proviral integration events occur when *Emv-16* and *Emv-17* are carried in the maternal genome but not when carried in the paternal genome. (2) Crossing *Emv-16* and *Emv-17* onto the SWR/J strain for three generations results in a higher frequency of integration events (15.8%) than the equivalent crosses onto the CBA/CaJ strain (0%). (3) N_3 integration events appear to be distributed nonrandomly with respect to breeding pairs and litters. (4) The presence or absence of *Emv-16* and *Emv-17* in the embryonic genome does not affect the probability of a de novo integration in that genome. These findings suggest that both genetic and epigenetic events contribute to this phenomenon. The results are consistent with a mechanism of reintegration involving infection of the egg or early embryo by maternal tissues shedding virus, although other possibilities exist.

The EP fragments were visualized at 5–50% the intensity of homozygous bands run on the same gel, with an average at approximately 20%. This heterogeneity shows that there is variation in the stage of embryonic development during which the integration event occurs, with those fragments at 50% intensity of homozygous bands most probably integrating at the one-cell stage. In practical terms, we can predict, assuming that genomic DNA from tails is representative of germ-line DNA, that we will see germ-line transmission of the new loci at these levels. We have been able to pass 12 of the EP fragments through the germ line to date, and preliminary results suggest that in fact germ-line transmission parallels the representation of these fragments in tail DNA. Therefore, we should be able to pass most of the scored EP fragments through the germ line.

We are currently establishing mouse lines containing these new proviral loci and plan to analyze them for insertional mutations. Unfortunately, mutations cannot be easily selected for in mice; however, we can check several parameters. First, visible structural or behavioral mutations cosegregating with new loci can be identified as dominant mutations if found in heterozygotes and as recessive mutations if found in homozygotes. We can also check for recessive lethal mutations by using molecular and genetic techniques to score a homozygous class of offspring. The absence of this class in the appropriate cross will be indicative of a recessive lethal mutation. Finally, we can score any X-chromosome-linked lethal mutations by deviations in the sex ratios of offspring.

A Partial *t* Haplotype Contains Both Copies of a Polymorphic Genomic DNA Sequence

V. Bautch. L. Silver

We have been using short low-copy genomic DNA sequence clones (microclones) to dissect the structure of the partial *t* haplotypes. Partial *t* haplotypes are the result of rare recombination events between complete *t* haplotypes and wild-type chromosome 17. Because *t* chromosomes contain an inversion in the distal region of the complex and a hypothesized inversion in the proximal region, it is proposed that these rare recombination events result in duplication of some

DNA sequences and deletion of other sequences. One of these partial *t* haplotypes, t^{w18}, contains the proximal portion of the *t* complex and carries a recessive lethal mutation that maps in the vicinity of the breakpoint between *t* DNA and wild-type DNA. Recently, one of the microclones, designated 89S, was shown to hybridize to genomic sequences that also map near the breakpoint in this chromosome. This was done using a restriction-fragment-length polymorphism (RFLP) between *t* DNA and DNA from mice of the 129 inbred strain, which carries the t^{w18} haplotype. Genomic DNAs from mice with the t^{w18} chromosome and either the wild-type chromosome 17 (+ 129/t^{w18}) or a complete *t* haplotype (t^{w5}/t^{w18}) were analyzed with a probe made from the 89S microclone. As expected, the (+ 129/t^{w18}) DNA contained fragments specific for both the wild-type chromosome 17 and *t*-haplotype DNA; however, the (t^{w5}/t^{w18}) DNA also contained both sets of fragments. Since (+ 129/ + 129) DNA contains only wild-type fragments hybridizing to the 89S probe, and since DNA of mice with two complete *t* haplotypes (t^{w5}/t^{0}) contains only the *t*-DNA-specific fragments, the wild-type bands in the (t^{w5}/t^{w18}) DNA must have been contributed by the t^{w18} chromosome. Therefore, the recombination event generating this partial *t* haplotype was probably an unequal crossing-over resulting in the presence of both sets of these fragments on the t^{w18} chromosome. Subsequently, several other investigators (H. Fox, this section, and H. Lehrach, European Molecular Biology Laboratory, Heidelberg) have shown that three other independently derived microclones are also duplicated in the t^{w18} chromosome, suggesting that this chromosome contains a substantial amount of duplicated DNA. Current studies are aimed at determining the extent of the duplication. Preliminary results from Lehrach's laboratory indicate that the 89S microclone maps to the TLA region of the *H-2* complex on wild-type chromosome 17. We are currently analyzing the t^{w18} chromosome with *H-2* DNA probes to determine whether any of these sequences are also duplicated. Because the recessive lethality carried by the t^{w18} chromosome is associated with the recombination breakpoint, it is possible that either the duplication or the proposed corresponding deletion of sequences could be responsible for the lethal phenotype.

Characterization of *t* Haplotypes with *t* Complex Clones

H. Fox, L.M. Silver [in collaboration with G.R. Martin, University of California, San Francisco]

Last year, we described the mapping of DNA clones obtained by chromosome microdissection (by H. Lehrach et al., European Molecular Biology Laboratory, Heidelberg) to the *t* complex by identifying restriction fragment length polymorphisms with these clones in the DNA of mice congenic for *t* haplotypes. Specific restriction fragments identified by these clones were found to be *t*-specific, since they were present in all complete *t* haplotypes but replaced by other restriction fragments in all of the wild-type inbred strains tested. However, there were no restriction fragments present in wild-type or *t* that were not found in the other, and the level of polymorphism observed between *t* haplotypes and wild type is roughly comparable to that observed between inbred strains. Thus, the *t* haplotypes appear to have a similar origin and have not diverged greatly from other mice.

These clones have been used to classify partial *t* haplotypes, which are derived from rare recombinant events between complete *t* haplotypes and wild-type chromosomes. Partial *t* haplotypes were found to contain subsets of the *t*-specific restriction fragments, and each could be classified according to the *t*-specific fragments it contains. This is the first molecular evidence that independent partial *t* haplotypes contain different lengths of *t* haplotype DNA and confirms the genetic studies of M. Lyon (Harwell), who has used partial *t* haplotypes to elucidate the genetic basis of the *t* haplotype phenotypes.

In a recent survey of wild mice, J. Klein (Tubingen) has identified *t* haplotypes at a frequency of approximately 25% in mouse populations throughout the world. We have analyzed these new *t* haplotypes with our molecular probes and found that all of the mice resemble the previously characterized *t* haplotypes except one. This *t* haplotype, originally isolated in Haifa, is unique in that it only carries a subset of the *t*-specific markers. Genetic studies indicate that it similarly only expresses a subset of the *t*-specific phenotypes. Several lines of evidence support the the-

ory that this haplotype may be an intermediate in the formation of *t* haplotypes in the wild.

Evidence for a Proximal *t* Haplotype Inversion

N. Sarvetnick, L.M. Silver

Recently, the product of one rare recombination event between a *t* haplotype and wild type (named t^{wLub2}) was characterized and found to express two unusual phenotypes not associated with either of the parental chromosomes from which it was derived. First, the t^{wLub2} chromosome suppresses the dominant, short-tail phenotype expressed by mutations at the Brachyury (*T*) locus so that animals doubly heterozygous for *T* and t^{wLub2} are born with tails of normal length. Second, the t^{wLub2} chromosome expresses a dominant lethal effect (*Tme*) in all embryos that inherit the mutant chromosome from their mother, so that heterozygous females cannot transmit t^{wLub2} to their offspring. Embryos that receive either mutant chromosome from their mother develop in an apparently normal fashion until late in gestation when death inevitably occurs. At the time of death, mutant embryos are edematous and show an increase in weight relative to their normal littermates. In contrast, when embryos receive either mutant chromosome from their father, development proceeds normally to the birth of healthy and fertile individuals.

To gain a better understanding of the genetic basis for the various phenotypes expressed by the t^{wLub2} chromosome, we have used several molecular probes to analyze the structure of this unusual chromosome. Our genetic and biochemical analyses of t^{wLub2}-containing mice have generated evidence that the chromosome contains a deletion. First, t^{wLub2} expresses a new lethality not expressed by its parental *t* haplotype. Second, the chromosome fails to complement the lethality associated with another *t* haplotype, t^{w73}, that maps in the proximal region. Finally, the chromosome fails to express either known form of the TCP-1 protein. Clustered mutations are most simply explained by a deletion.

We have further investigated the structure of the t^{wLub2} chromosome by performing a genetic analysis with a cDNA clone (pMK174) that hybridizes to sequences located in the proximal portion of chromosome 17. The results indicate that the t^{wLub2} chromosome must contain both *t*-specific and wild-type alleles at this locus. These data demonstrate that the t^{wLub2} chromosome carries a duplication of genetic material, with both wild-type and *t*-specific forms of the pMK174 locus.

The accumulated data indicate that a recombination event between a *t* haplotype and a wild-type chromosome caused the duplication of certain genetic loci (*T* and pMK174 sequences), as well as the deletion of other loci (*Tme, Tcp-1*, and the t^{w73} lethal locus) in the generation of the t^{wLub2} chromosome. However, a simple, unequal recombination event between homologous chromosomes would cause either a duplication or a deletion but not both. To explain the generation of t^{wLub2}, we postulate the existence of an inversion over the proximal region of *t* haplotypes, extending from the *T* locus and pMK174 sequences to the loci of *Tcp-1* and *Tme*. A correctly oriented recombination event between these two sets of inverted loci would produce a chromosome with all of the genetic properties of t^{wLub2} (see Fig. 1 below).

Studies performed in a similar manner indicate that the Tt^{Or1} haplotype appears to have a structure reciprocal to that of t^{wLub2}. Our model for the generation of both t^{wLub2} and Tt^{Or1} predicts that the crossover event occurred while the two chromosomes were paired in this type of linear, nonhomologous fashion. The demonstration of nonhomologous chromosomal pairing in *t* heter-

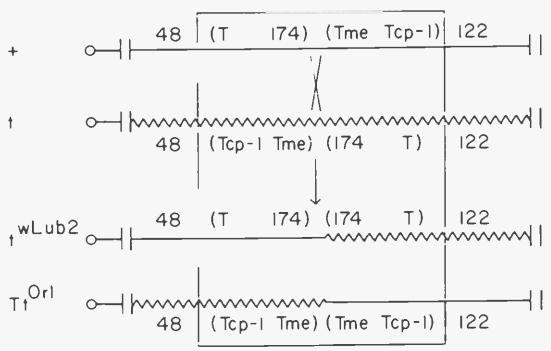

Figure 1 Representation of the proximal region of mouse chromosome 17. The loci inverted in *t* haplotypes relative to wild type are indicated, with the postulated recombination event that generated t^{wLub2} and Tt^{Or1}.

ozygotes makes it possible to explain the mechanism by which rare recombination occurs in this region. The accumulated molecular data suggest that many rare recombination events have occurred within the well-characterized inverted regions, with the production of partial *t* haplotypes that have both duplicated and deleted loci. These events can be explained by the occasional, chance pairing and recombination of nonallelic members of a repetitive DNA family. It is important to emphasize that such events will occur only rarely, and only a fraction will produce viable recombinant products.

Genetic Analysis of a Mouse Dominant Maternal Effect

N. Sarvetnick

The detailed structural analysis of t^{wLub2} and another partial *t* haplotype, Tt^{Or1}, has implied that the two chromosomes represent reciprocal recombination products. Thus, a duplication of a functional *Tme* locus may be present on the Tt^{Or1} chromosome. If the lack of maternal transmission of t^{wLub2} were purely a dosage problem, then the duplication could be utilized to complement the defect. We designed a series of breeding protocols to explore the potential of the Tt^{Or1} chromosome to alter the penetrance of the t^{wLub2} maternal effect.

When Ttf/t^{wLub2} females were mated with wild-type males, 94% of the 188 animals born had the short-tail phenotype indicative of the *T*-carrying chromosome. The remaining 6% of the animals had normal tails and appeared healthy; all that could be analyzed were found to have a recombinant chromosome that carried neither t^{wLub2} nor *T*. Hence, with this mating, there is no evidence for the survival of any t^{wLub2}-carrying individuals to the time of birth.

When Ttf/t^{wLub2} females were mated to $Tt^{Or1}tf/+tf$ males, a third phenotypic class of neonatal mice was observed. Of 195 offspring, 16% had a tail of normal length, but were much larger than their littermates and appeared edematous; all died within 48 hours after birth. This phenotype is reminiscent of that observed in utero for maternally derived t^{wLub2} embryos from other crosses. Another 5% of the offspring had tails of normal length, but were healthy, and when tested, they were found not to carry the t^{wLub2} chromosome. The total fraction of mice born with normal tails from this cross (21%) is too large to be accounted for by crossing-over alone, and the majority of these mice express the characteristic t^{wLub2} phenotype. It appears that the presence of a Tt^{Or1} chromosome in the father allows a percentage of the maternally derived t^{wLub2} individuals to survive to birth.

To determine whether the presence of the Tt^{Or1} chromosome in the mother's genotype has any effect on the survival of t^{wLub2} individuals, Tt^{Or1}/t^{wLub2} females were mated to wild-type males. No animals were born with the t^{wLub2} phenotype. Only 1 (2%) of the 45 offspring born had a normal tail, and when tested, this animal was found to have a recombinant chromosome without the t^{wLub2} haplotype. Hence, the presence of Tt^{Or1} in the primary oocyte is not sufficient to rescue embryos that receive the alternative t^{wLub2} homolog from a doubly heterozygous mother.

There are two possible mechanisms by which the t^{wLub2} maternal effect could be expressed. The first mechanism is dependent on an absolute requirement for a functional *Tme* locus during the haploid phase of oogenesis; this condition is not met when t^{wLub2} is inherited maternally. The second mechanism requires the differential functioning of the maternally and paternally inherited forms of *Tme* at some critical stage of embryogenesis; e.g., if the paternal locus is inactive, embryos having no maternal form will die.

Several facts argue against the first mechanism described above. First, it seems unlikely that a molecule produced (or not produced) during oogenesis could begin to have a dramatic effect on an embryo that is 10,000 times larger at 15–19 days after conception. Second, nuclear transplant results obtained by J. McGrath and D. Solter (Wistar) indicate that a wild-type cytoplasm and membrane are not sufficient to rescue a mutant nucleus. Finally, our results indicate that a specific paternal genome can alter the penetrance of the t^{wLub2} phenotype. This experiment provides support for the model of a differential functioning of the *Tme* locus dependent on the parent from which it is inherited. If the maternal allele is thought of as fully activated and the paternal allele as relatively inactive, then the embryo hav-

ing a maternally derived t^{wLub2} chromosome must survive solely on the paternal allele, resulting in lethality. In our experiment, the double dose of the relatively inactive *Tme* paternal locus partially complements the deficiency and increases the mean life span, so that a large percentage of the t^{wLub2}-containing embryos are born.

Cis-Trans Analysis of *t* Haplotype Lethal Genes

P. Mains

It has long been noted that the viability of mice heterozygous for complementing *t* haplotypes generally ranges from 18% to 85%. A previous study tested whether this viability could be improved by placing two lethal mutations together on one chromosome (in *cis*) rather than on separate chromosomes (in *trans*). It was found that the complementation between two different lethals was increased to 100% if they were on the same chromosome when they are heterozygous with a fully wild-type chromosome. This would indicate that the wild-type alleles work more efficiently when they are on one chromosome (in *cis*) than when they are on separate chromosomes (in *trans*). Other investigators interpreted this *cis-trans* effect as indicating that the two loci were part of a single functional unit, even though they map 10–20-cM apart. They proposed that the necessity for the alleles at widely separated locations to be on the same chromosome in order to function efficiently may be indicative of a genomic rearrangement.

An alternative interpretation of the *cis-trans* test is that *t* haplotypes may carry additional "suboptimal" alleles at loci other than their unconditional lethal mutations. The incomplete complementation of two lethal haplotypes could result from a requirement for a *cis* interaction of wild-type alleles or from an effect of other deleterious genes associated with the *t* haplotypes. To control for this, a *t* chromosome lacking either lethal is required to provide both "wild-type" alleles in a *cis* arrangement. This chromosome would be the reciprocal crossover of the *t* chromosome carrying the two lethals in *cis*. However, because of the limited genetic resolution in the mouse, precise, or even nearly precise, reciprocal crossovers cannot be easily isolated. Any two reciprocal pairs will contain overlapping regions that can complicate the interpretation of the data. We repeated the *cis-trans* test using several independent recombinant chromosomes in different pairwise combinations. The different combinations are likely to share different regions of overlap. We found that some combinations improve the complementation of two *t* lethal alleles, whereas others did not. We believe that the data can be explained simply by the presence of a slightly deleterious gene(s) within the overlap, or they may reflect the effect of genetic background.

The minor differences in the viability of the *cis* and *trans* heterozygotes are unlikely to be caused by a rigorous requirement that they be carried on the same chromosome to function efficiently. Instead, it may be due to additional deleterious genes carried by *t* haplotypes. *t* haplotypes are effectively isolated from the gene pool of mice due to the suppression of recombination with the wild-type chromosome 17. This may allow the *t*-bearing chromosomes to acquire deleterious mutations at loci in addition to their unconditional lethal mutations. In fact, some *t* chromosomes have been found to carry multiple lethal alleles. These other deleterious alleles may be unique to certain *t* haplotypes, or they could be shared by all, and could account for the decrease in the viability of the *trans* heterozygote compared with the *cis*.

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TRANSGENIC MICE

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S. Efrat V. Bautch

Cold Spring Harbor laboratory has established and is expanding a group that is studying various aspects of gene expression and its consequences using the mouse as an experimental organism. Currently, the major approach of this group involves transferring cloned and often-altered genes back into the mouse germ line, so as to examine their effects in that context. This is being accomplished by injecting a solution of DNA into a pronucleus of a one-cell embryo, which is then reimplanted into a foster mouse and allowed to develop. Mice that heritably carry the new genetic information (so-called transgenic mice) arise from about 10% of those born from embryos injected with the DNA. Transgenic mice are being used to examine control of gene expression, the consequences of oncogene expression, and the prospects for using insertional mutagenesis by DNA microinjection as a genetic tool.

Recombinant Insulin SV40 Oncogenes in Transgenic Mice

D. Hanahan, S. Alpert, S. Efrat, M. Lacy

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

Transgenic mice were produced that harbor two different insulin/SV40 T-antigen genes. One (called RIP1-Tag) carries the insulin gene promoter aligned so as to transcribe the T-antigen gene. The second (called RIR-Tag) has the insulin promoter inverted with respect to the SV40-coding information. Both recombinant genes elicit the heritable formation of solid β-cell tumors in transgenic mice that harbor them, suggesting that the tissue specificity element(s) can act bidirectionally. Expression of T antigen is detected only in the insulin-producing β cells. The hybrid genes are expressing in virtually all β cells prior to tumor formation, which occurs in only a few of the several hundred islets of Langerhans that comprise the endocrine pancreas. This indicates that secondary events are necessary to convert a collection of islet cells expressing SV40 T antigen into a solid, well-vascularized tumor. It is possible that a cellular oncogene is involved. The other major cell types normally found in islets—glucagon-producing α cells and somatostatin-producing δ cells—are rare and disordered in these transgenic mice.

In summary, sequences located within 660 bp 5′ to the transcribed portion of the rat insulin gene are sufficient to elicit cell-type-specific expression in transgenic mice, and the synthesis of T antigen produces islet disruption and tumor for-

mation. These transgenic mice should provide further insight into the development of the endocrine pancreas and on the mechanisms of oncogenesis.

Analysis of DNA Microinjection into Mouse Embryos to Create Insertional Mutants

V. Bautch, D. Hanahan

It is known that insertion of DNA into the mouse germ line via microinjection can result in insertional mutations. Microinjected DNA is usually found as 5–50 copies in a head-to-tail tandem array at a single integration site, and the 5′ and 3′ ends of the array are sometimes missing sequences. Limited analyses by several investigators indicate that approximately 10% of transgenic mice carry a recessive-lethal mutation that cosegregates with the inserted DNA. However, in no line has the integrity of the flanking cellular sequences been adequately analyzed. This parameter is important with respect to insertional mutagenesis, because only if integration of DNA preserves the linear order of cellular sequences or gives rise to small deletions or rearrangements (e.g., no larger than cosmid size) will it be possible to clone the preintegration site easily and analyze the disrupted gene(s). Therefore, we plan to investigate both the rate of generating recessive-lethal mutations and the integrity of flanking sequences after integration of microinjected DNA.

We have therefore constructed a plasmid vector that can be used to rescue, by transformation of *Escherichia coli*, the inserted DNA and flanking cellular sequences. Although this vector is derived from pBR322, it has been altered so that deletion of some plasmid sequences from the end of a tandem array by "nibbling" prior to integration should not affect the ability to rescue this plasmid. The vector contains three different drug resistance genes, and each gene contains several restriction enzyme sites unique to the plasmid. In addition, a suppressor tRNA gene has been inserted that can complement bacteriophage λ mutations and provide an additional selection. A polylinker containing unique restriction sites has been inserted between two of the drug resistance markers in order to linearize the plasmid prior to microinjection. It is expected that the DNA will integrate in a head-to-tail tandem repeat so that the linear order after integration will be $-kan^R$-*ori*-sup^F-amp^R-tet^R-. The genomic DNA will be digested with a restriction enzyme whose site is in a drug resistance gene and religated. Any internal copies of the tandem DNA should recircularize to yield plasmids resistant to all three drugs upon transformation; however, each end should contain some cellular DNA and also be uniquely sensitive to the drug whose resistance gene was originally cleaved. For example, digesting with an enzyme that cleaves the tet^R gene should yield a DNA fragment containing a truncated tet^R gene at one end and flanking cellular DNA at the other end. This fragment will ligate to form a plasmid with cellular DNA that is amp^R, kan^R, and tet^S. Moreover, in this scheme, a second rescue using an enzyme that cleaves the kan^R gene should yield a plasmid containing cellular DNA from the opposite end of the tandem array. In the event that plasmid sequences are altered by the integration so as to make plasmid rescue impossible, it should be possible to retrieve the flanking DNA by cloning into phage containing a nonsense mutation and selecting for the suppressor tRNA gene. Finally, if alteration of suppressor tRNA sequences makes phage rescue impossible, the flanking DNA can be retrieved by making a library of genomic DNA from the transgenic mouse and screening with the plasmid as a probe.

We plan to generate and analyze 10–20 lines of transgenic mice containing this vector. The flanking sequences will be cloned and their colinearity with the preintegration site will be assessed. We will also use breeding and molecular analysis to determine the efficiency of generating insertional mutants in mice using this technique.

Insulin/SV40 T-antigen Hybrid Gene Expression in the Developing Mouse Pancreas

S. Alpert, D. Hanahan

Transgenic mice containing an insulin/SV40 T-antigen hybrid gene will be used to investigate the developmental control of insulin. In mammals,

there are two developmental phases of insulin secretion. The first stage is a low level of insulin production detected at about day 14 of gestation in mice. This correlates with the early development of the pancreas. The second phase is a striking increase in insulin synthesis at approximately the 16th day of gestation; β cells are first apparent at this time. During the last 4 days of gestation, insulin synthesis remains high. The molecular mechanism controlling this developmental sequence has yet to be explained.

Our experimental approach is to compare the developmental expression of the endogenous insulin genes to that of the recombinant insulin gene. This hybrid gene contains 660 bp of the 5'-flanking region of the insulin gene joined to the coding region for SV40 large T antigen. Analysis of transgenic mice carrying this gene has shown that the sequences required for tissue-specific regulation are present in the 5'-flanking region of the insulin gene (see above report by D. Hanahan et al.). The mouse pancreata will be excised and examined throughout pre- and postnatal development. Immunohistology to sections of the pancreata will be used to characterize the expression of insulin and other hormone products of the endocrine pancreas. If the sequences that elicit proper developmental expression of the insulin gene are contained in this recombinant gene, we would expect to see T antigen expressed in the same temporal manner as insulin. Various constructions of the hybrid gene containing different pieces of the insulin gene and its flanking regions should allow us to identify the sequences required for the developmental regulation of insulin. The influence of the altered β cells on the development of the islets of Langerhans will be assessed. This will provide more information of the biogenesis of the disordered islets found in the transgenic mice.

Establishment of β Cell Lines from Transgenic Mice

S. Alpert, D. Hanahan

Permanent lines of the β cells of the pancreas, as well as biochemical studies on insulin gene expression, can provide a useful tool for diabetes research. Islet-cell cultures have been maintained for fairly long periods of time in culture (Farkas and Joo, *Diabetes 33:* 1165 [1984]), but established cell lines do not arise from these cultures. Rat insulinomas have been used to create insulin-secreting β cell lines. However, these cell lines do not appear to be glucose-inducible, which is an important attribute of authentic β cells (Gazdar et al., *Proc. Natl. Acad. Sci. 77(6):* 3519 [1980]). The use of a hybrid gene containing a tissue-specific promoter to regulate an oncogene could be a tool for expanding different primary cell types into established cell lines.

As transgenic mice containing the insulin/SV40 T-antigen hybrid gene express T antigen specifically in the β cells of the pancreas, we plan to culture the islets of Langerhans in an attempt to establish authentic glucose-inducible β cell lines. This will involve verification of cultured cell identity by determining (1) that the endogenous insulin gene is glucose-inducible, (2) that the transgene is glucose-inducible, and (3) that glucagon and somatostatin are not secreted by the cells. Further RNA and protein analyses will be performed to quantitate the basal- and glucose-challanged levels of insulin and T antigen.

Bovine Papilloma Virus in Transgenic Mice

S. Alpert, D. Hanahan

Bovine papilloma virus (BPV) is an 8-kb double-stranded DNA virus. In the natural host, BPV induces epithelial or fibroepithelial tumors by abortively infecting the epidermal germinal cells. BPV usually remains a free circular molecule, but it can integrate into the genome in certain cases. Although there is no in vitro system for virus production, C127, an epithelial mouse cell line, can support BPV replication. This replication requires both *cis*- and *trans*-acting factors.

We have injected a BPV plasmid containing a partial tandem repeat of the BPV genome (BPV 1.69; a gift from D. DiMiao, Yale University) into mouse embryos. The injections produced two mice positive for the BPV molecule. Only one of these mice, containing approximately five copies of BPV 1.69 integrated into high-molecular-

weight DNA, survived to adulthood. We have performed analyses on the offspring of this mouse, and have observed no episomal BPV molecules in total DNA preparations, or low-molecular-weight (Hirt) DNA extractions, from 16 mouse tissues. We are now assessing the expression of the BPV genome in this transgenic mouse line, and preliminary results indicate that BPV transcripts are present in the testes of BPV-positive mice. We also plan to try to superinject circular BPV plasmids into this transgenic mouse line to maintain extrachromosomal BPV molecules by complementation with the integrated copies.

MOLECULAR GENETICS

Classic genetics experiments conducted at Cold Spring Harbor Laboratory in the early 1940s on bacteriophage by Delbrück, Luria, and Hershey and on maize by McClintock have paved the way for the field of molecular biology. In recent years, the tools of genetic engineering have allowed the analysis of genes at the nucleotide level. These tools consist of DNA-mediated transformation, gene cloning by complementation, site-directed DNA mutagenesis, transposon tagging, and phenotypic analysis of in-vitro-produced mutations. They have been applied at Cold Spring Harbor Laboratory to dissect the genetic mechanisms, in molecular terms, of various biological systems. We summarize here the results of the past year, involving various eukaryotic organisms (the yeast mating types and cell-cycle genes, maize transposable elements, and somatic-cell genetics of tobacco) and the prokaryotic organisms *Clostridium* sp., *Rhodopseudomonas, E. coli*, and *Azotobactor* sp.

YEAST GENETICS

A. Klar	J. Ivy	S. Kakar
J. Hicks	M. McLeod	M. Kelly
D. Beach	G. Livi	C.-I.P. Lin
	R. Booher	L. Miglio
	J. Gould	C. Stevens

Research by the Delbruck Laboratory Yeast Group during 1984 continued to focus on the mechanism of mating-type switching and its relationship to the cell-division cycle, but changes were developing in the profile of the group and the building. In the Spring, Jeff Strathern and his family left Cold Spring Harbor Laboratory for the Frederick Cancer Research Center in Frederick, Maryland. Jeff was appointed Director of Eukaryotic Genetics and is charged with developing a large research effort on eukaryotic microorganisms. Our group and the Laboratory as a whole will miss Jeff scientifically and socially. He was constantly acting as a catalyst for interdisciplinary discussions and used his broad background in biology to see the relationships among seemingly disparate observations. We wish him well in his challenging new job.

Another development occurred when the plans for the Plant Group to move to nearby Uplands Farm were altered and it was realized that a new addition to Delbruck Laboratory might be required, similar to that which allowed the Yeast Group to have permanent quarters in 1982. Plans have been drawn for a north wing that would provide laboratory space for the Plant Group to double in size while retaining the Uplands Farm land and buildings as a working field station. The next step is to develop funding for this planned expansion in addition to the support for Uplands Farm that was provided by the National Science Foundation last year.

Finally, 1984 was the last full year that the research group studying the the cell-division cycle of fission yeast occupied the Delbruck Laboratory. This group, headed by D. Beach, will move to the newly renovated quarters in the Demerec Laboratory in 1985. The growth of this research program, along with the cell-cycle studies of yeast oncogenes in M. Wigler's laboratory, will keep

Cold Spring Harbor Laboratory at the forefront of efforts to use these simple microorganisms to uncover the reasons behind the unchecked growth of human cancer cells.

SUM1: Regulator of the *HML* and *HMR* Loci of *Saccharomyces cerevisiae*

A. Klar, J. Hicks, S. Kakar, J. Ivy, G. Livi,
C.-I.P. Lin, L. Miglio

In previous years' reports, we have shown that both *MAT***a** and *MAT* code for two transcripts divergently transcribed from within the Y region (Fig. 1), suggesting that their promoters probably lie within Y. The structure of the silent counterparts of *MAT*, *HML*, and *HMR* is, but for a few bases, identical to that of the constitutively expressed *MAT* locus, yet the *HM* loci are unexpressed. The task of keeping the *HM* loci unexpressed is assigned to at least four *trans*-acting *MAR/SIR* loci and *cis*-acting E and I sites (see Fig. 1).

To determine possible interactions between the *MAR/SIR* gene functions and to search for new loci required for regulating the *HM* loci, we sought extragenic suppressors of the *mar1-1* mutation. The following selection scheme was used: The *HML***a** *MAT*α *HMR***a** *mar1-1* strain exhibits a sterile phenotype because of the simultaneous expression of **a** and α information. A mutant that exhibits the α phenotype, and therefore has repressed the *HM* loci, was isolated. The mutation is unlinked to *MAT*, to the *HM* loci, or to the known *MAR/SIR* genes. We designate the new locus as defined by the mutation as *SUM1* (*SU*ppressor of *M*ar). The mutation *sum1-1* is allele nonspecific because it also suppresses a *mar1* deletion mutation. Even more interesting, the *mar2* (*sir3*) and *sir4* mutations are also suppressed. To account for these data, we suggest that the *MAR/SIR* functions negatively regulate

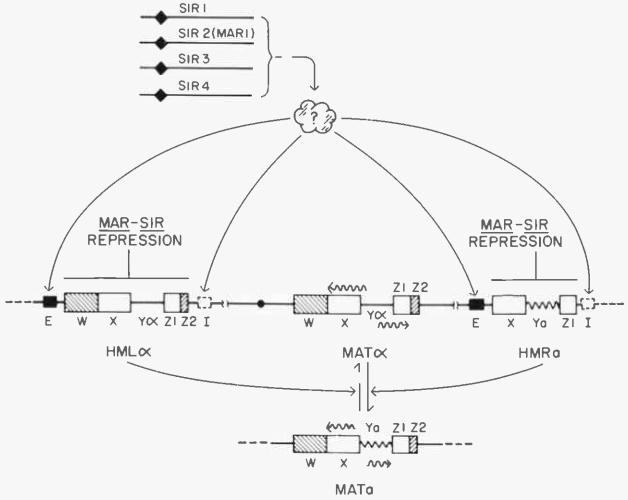

FIGURE 1 Structure of chromosome III showing the mating-type cassettes. The *HML* and the *HMR* copies of the mating-type genes are normally kept silent by the *MAR/SIR* genes and the *cis*-acting E and I sites. Interconversion of *MAT***a** and *MAT*α involves a unidirectional transposition-substitution event from *HML* or *HMR* to *MAT*.

the *SUM1* gene function, which in turn is required to "turn on" the *HM* loci by a positive control. Similarly, we have been successful in isolating suppressors of the *mar2* (*sir3*) mutation. Future genetic and molecular studies will allow us to understand more fully the regulation of the *HM* loci.

Analysis of *MAR/SIR* Gene Clones

J. Ivy, G. Livi, C. Stephens, M. Kelly, J. Hicks, A. Klar

As described in previous Annual Reports, we have isolated and identified the *MAR/SIR* genes of *S. cerevisiae* from within a cloned genomic library of DNA fragments by their ability to complement *MAR/SIR* mutations in vivo. *MAR/SIR* mutant strains, which are normally nonmaters due to expression of *HML* and *HMR*, become mating proficient when they contain an extrachromosomal, independently replicating plasmid bearing a wild-type *MAR/SIR* gene. Using the four *MAR/SIR* gene clones, we have examined the possibility of a cascade of regulation among them. RNA blot analysis, however, has ruled out a cascade of transcriptional regulation.

One observation that multiple copies of *SIR3* will suppress *SIR4* mutations has suggested the possibility that Sir4 combines with Sir3 to alter it sterically and to enhance its activity. Overproduction of Sir3 in the high-copy-number *SIR3* transformants might obviate the need for the steric enhancement of Sir3 activity. In support of the idea that Sir4 combines with other *SIR* gene products in a dimer or multimer is the observation that a cloned, truncated *SIR4* gene has an Antimar activity; transformation of wild-type *SIR* strains with Antimar results in expression of the silent cassettes.

We have constructed null mutations in the four *MAR/SIR* genes by altering and transplacing such disruptions into the genome. Null mutations in all four genes are viable, and *mar1(sir2)*, *mar2(sir3)*, and *sir4* null mutations have a nonmating phenotype. Cells with a null mutation in *sir1*, however, still retain partial regulation of the silent cassettes. We conjecture that a regulatory complex still forms in the absence of Sir1 but that it is not stable.

MAR1(SIR2) and *MAR2(SIR3)* have been mapped by standard genetic analysis, taking advantage of the fact that crosses can be made with *mar*-mutant spores if the spores came from a heterozygous *MAR/mar* diploid. The cloned genes have provided us with an alternate means of mapping these loci. By "tagging" the *SIR1* and *SIR4* loci by chromosomal integration of plasmids and mapping the plasmids' selectable marker, we have made unnecessary the mapping of mutations that normally render the strain a nonmater. *SIR1* has been assigned to the tip of the right arm of chromosome XI and *SIR4* has been assigned to the middle of the right arm of chromosome IV; *MAR1* is on the left arm of chromosome IV and *MAR2* is located distal to *URA4* on chromosome XII.

To understand more fully the mechanisms involved in regulating silent mating-type gene expression, we have begun experiments to identify and purify each of the *MAR/SIR* gene products. DNA fragments containing functional sequences of *SIR1, SIR2*, and *SIR3* have been subcloned into the open-reading-frame vectors constructed by M. Berman and colleagues (Weinstock et al., *Proc. Natl. Acad. Sci. 80:* 4432 [1983]), which allow the expression (in *Escherichia coli*) of open-reading-frame DNA as a trihybrid protein containing sequences from the *E. coli* proteins *ompF* and β-galactosidase. Antisera raised against purified fusion proteins will be used to purify the yeast *MAR/SIR* gene products and to study *MAR/SIR* protein synthesis, localization, and DNA-binding properties.

In our screen of the gene bank for sequences able to suppress *MAR/SIR* mutations in vivo, we found one other cloned gene that could do so. Disruption in this locus is lethal to the cell, indicating that it encodes an essential function. It suppresses mutation in all four *MAR/SIR* loci, but it does not correspond to any of them. Genetic evidence indicates that the suppression of *MAR/SIR* mutations is not caused by restoration of *MAR/SIR* regulation. Rather, it appears that regulation of mating functions by *MAT* is disrupted. Since all transformants have displayed a weak α mating type, this sequence formally behaves as an antagonist of *MAT***a**1 function; hence, our working designation for the locus is antagonist of **a**1 (ANA). We are presently trying to distinguish between the possibilities that either

a truncated gene product or the cloned sequences themselves disrupt regulation of mating functions.

To determine if the E and I sites are able to regulate expression of genes other than the mating-type genes, we replaced the mating-type a information normally found at *HMR* with the *HIS3* gene. We found that in one orientation, E and I could repress the expression of the *HIS3* gene. These results indicate that promoters other than the mating-type promoters can come under the control of E and I.

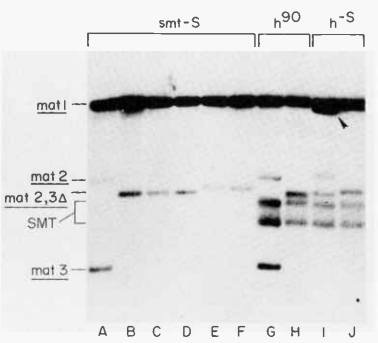

FIGURE 2 Southern blot analysis demonstrating deletions of *MAT2* and *MAT3* loci. *smt-s* is a *cis*-acting mutation that reduces the level of the double-stranded break at *MAT*. h^{90} is a wild-type strain and is shown to contain a double-stranded break at *MAT1* in about 20% of its DNA (marked by *SMT*, lane *G*). h^{-S} is a strain that contains a fusion between *MAT2* and *MAT3*. DNA displayed in *B–F*, *H*, and *J* show deletions of *MAT2* and *MAT3*.

Behavior of the Double-stranded DNA Breaks at *MAT* in *Schizosaccharomyces pombe*

A. Klar, D. Beach, L. Miglio

During the past year, we reported three classes of mutants in fission yeast that are defective in mating-type switching: (1) those that lacked the ability to make double-stranded breaks at *MAT*, (2) those that had the break but apparently did not switch, and (3) those that were defective in resolution. The second class is quite paradoxical—either they switch only homologously or this yeast can heal the broken ends without obligately switching. To distinguish between these possibilities, we deleted both *MAT2* and *MAT3* in Swi$^-$ (*swi*tching-deficient mutants) and Swi$^+$ strains.

The deletions were made by first cloning a bit of sequence proximal to *MAT2* and a small sequence distal to *MAT3*. Between these sequences, a selectable marker (the *Saccharomyces cerevisiae LEU2* gene, which complements the *leu1* defect of *Schizosaccharomyces pombe*) was inserted. The in-vitro-made construct was then cut out of the vector and transformed into appropriate strains by selecting for the Leu$^+$ phenotype. As shown in Figure 2, *MAT2* and *MAT3* were deleted, and a new fusion band of the construct was found. A quite interesting result is that Swi$^+$ strains with the deletion maintain a stable mating type, still generate the wild-type level of DNA cut at *MAT*, and do not exhibit lethality. The presence of the *swi* mutation in these strains has no additional phenotype. Thus, this yeast can easily heal the broken ends without switching. We therefore propose that mutations in the second class (which comprise three complementation groups) are defective in using the double-strand broken ends for switching.

In both *S. cerevisiae* and *S. pombe*, we have shown that double-stranded breaks are highly recombinogenic during mitotic growth (see previous years' reports). Most of the recombination studies in fungi, however, are conducted in meiosis. The question arises as to whether the double-stranded DNA breaks are also recombinogenic in meiosis. To address this question, we used the *MAT2*, *MAT3* deletion strains of *S. pombe*. In these strains, the double-stranded breaks at *MAT* cannot be healed by switching because of the lack of the donor loci. We tested these breaks to determine whether they can be used for recombination in meiosis. Tetrad dissection of appropriate hybrids of these deletion strains has shown that *MAT* gives gene conversion of the 3:1 and 1:3 types in about 20% of the asci, whereas standard wild-type strains hardly generate such events. If we reduce the amount of the in-vivo-cut DNA, either by using the appropriate *swi* mutations or by using the *cis*-acting speckled mutation, the gene conversion fre-

quency is correspondingly reduced. Thus, the double-stranded breaks can also promote efficient gene conversion in meiosis. Similar observations have been made recently in our collaborative work with A. Kolodkin and F. Stahl (University of Oregon) in the *S. cerevisiae* system.

Control of the Mitotic Cell Cycle and Meiosis in Fission Yeast

D. Beach, M. McLeod, R. Booher, J. Gould

This year's work concentrated on two key genes of fission yeast, *cdc2* and *ran1*. *cdc2*$^+$ is required for progression through both the G_1 and G_2 phases of the mitotic cell cycle, and *ran1*$^+$ inhibits the transition from mitotic cell division to meiosis. In the absence of *ran1*$^-$, a haploid cell undergoes premeiotic S phase, partial meiosis, and sporulation, despite the lack of all the normal meiotic requirements (e.g., diploidy, heterozygosity at the mating-type locus, and nutritional starvation).

The *cdc2* gene, which is homologous to the *cdc28* gene of *S. cerevisiae*, is predicted to encode a protein kinase on the basis of homology with known mammalian kinases. An intron-less form of the *cdc2* gene has been constructed in an *E. coli* expression vector, and a new kinase activity is currently being sought in bacterial extracts.

In vitro mutagenesis has been used to create nonphosphorylatable residues at two potential sites of phosphorylation in the *cdc2* protein. One site (Tyr-166→Phe; identified by homology with the major site of phosphorylation in *src* and *fps*) was mutated with no effect. Mutation of the

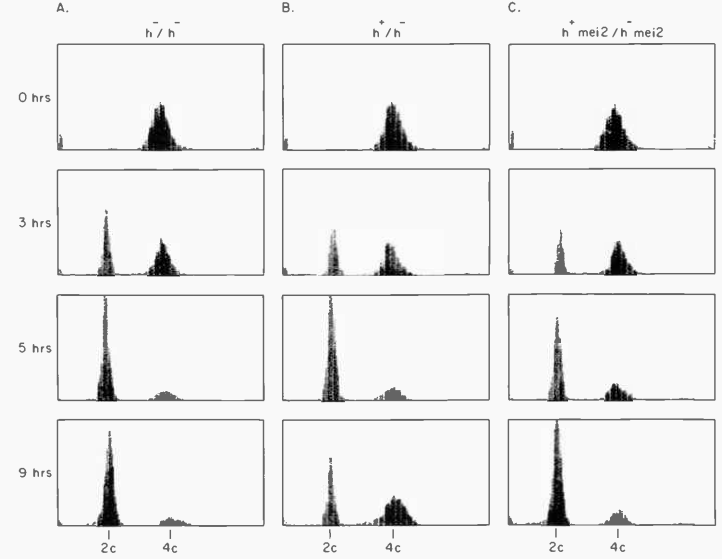

FIGURE 3 Analysis of propidium-iodide-stained cells on the EPICS cell sorter. Three strains, h⁻/h⁻, h⁺/h⁻, and h⁺ *mei2*/h⁻ *mei2*, were starved of a nitrogen source and analyzed at intervals. In h⁻/h⁻, cells accumulate in G_1, and in h⁺/h⁻, cells accumulate in G_1 but then undergo premeiotic DNA synthesis. In the *mei2* mutant (C), premeiotic DNA synthesis does not occur.

FIGURE 4 Sequence homologies between fission yeast *ran1*, *cdc2*, and bovine cAMP-dependent protein kinase. The region of homology contains the known site of ATP binding in the mammalian kinase.

neighboring site (Thr-167→Val; identified by homology with the major site of phosphorylation in bovine cAMP-dependent protein kinase) gave a phenotype of cell-cycle arrest. It is unknown at present whether Thr-167 is indeed a site of phosphorylation in *cdc2*, but the inability of cells carrying a mutation at this site to undergo DNA synthesis raises the interesting possibility that phosphorylation of *cdc2* may be required for cell-cycle progression.

The EPICS cell sorter (James Laboratory) has been used to characterize the transition of cells from the mitotic cell cycle to meiosis (see Fig. 3). It has been confirmed that initiation of meiosis is signaled by a round of DNA replication (premeiotic S phase) that does not take place in nonsporulating cells. We have found that the premeiotic S phase and meiosis I, induced in a temperature-sensitive *ran1*$^-$ strain, do not require the activity of the *cdc2*$^+$ gene. This feature distinguishes the premeiotic S phase and meiosis I from the mitotic S phase and mitotic cell division, both of which require *cdc2*$^+$ function. The *ran1*$^+$ gene has been shown to produce a 4.3-kb RNA transcript. The gene has been partially sequenced, and a small region of homology with the *cdc2* gene has been observed (Fig. 4). This region is particularly significant as it represents the site of ATP binding in a family of protein kinases. The homology suggests that *ran1*$^+$ probably encodes a protein with ATPase activity.

A mass of genetic evidence suggests that meiosis is initiated by turning off the *ran1*$^+$ product. We have shown that this inhibition does not occur at the transcriptional level. Future work will be directed at identifying the *ran1*$^+$ protein product and attempting to understand its biochemical activity.

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PLANT GENETICS

S.L. Dellaporta A.C. Hiatt J. McIndoo
J.B. Hicks P.S. Chomet J. Wood
R.L. Malmberg

Maize controlling elements have been the subject of genetic studies for more than 40 years. However, work on the molecular biology of maize controlling elements is relatively recent. In a few short years, several controlling elements, including the first controlling element system described, *Activator-Dissociation* (*Ac/Ds*), by McClintock (*Cold Spring Harbor Symp. Quant. Biol. 16:* 13[1951]), have been isolated and characterized at the nucleotide level. Several studies have shown that autonomous controlling elements, such as *Ac*, are transposable elements that share homology with dispersed middle repetitive DNA sequences. Autonomous elements such as *Ac* can transactivate nonautonomous elements such as *Ds* that are genetically inactive in the absence of the autonomous *Ac* element. There appear to be two classes of *Ds* elements: *Ds* elements that are structural derivatives of *Ac* (most appear to be deletion derivatives) and a family of *Ds* elements that shares little homology with *Ac* DNA, except for the 11-bp inverted terminal repeats characteristic of all *Ac/Ds* elements. This type of *Ds* element has been named *Ds1*, since it represents the first *Ds* element cloned (Peacock et al., *Cold Spring Harbor Symp. Quant. Biol. 49:* 347[1984]). Interestingly, the *Ds1* element hybridizes to approximately 40 conserved DNA elements in the maize genome.

Ds1 Element at the *waxy* Locus

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

We have isolated another *Ds1* element which was found as an insertion in the *waxy* mutation, *wx-m1*, first isolated by McClintock (*Carnegie Inst. Wash. Year Book 47:* 155[1948]). This insertion is approximately 400 bp, contains the expected 11-bp inverted terminal repeats of *Ac/Ds* elements, and is flanked by 8-bp direct repeats. The direct repeats represent a target-site duplication caused during integration of *Ds*.

In the presence of an active *Ac* element, the *wx-m1* allele is somatically and germinally unstable. This germinal instability reflects the *Ac*-mediated excision of *Ds1* from the *waxy* locus. We have cloned and sequenced several germinal *Wx* mutations from plants containing an *Ac* element and the *wx-m1* allele. These mutations were selected for partial, rather than full, restoration of the *Wx* phenotype. Sequence analysis showed that these excision events are not precise, leaving the 8-bp direct repeats in modified form (Fig. 1). In each case, however, the modifications represent the addition of nucleotides in multiples of three. We predict that the insertion lies within exon sequences and that the partial restoration of the *Wx*

Wx TARGET SITE		GCATCACC	
wx-m1	GCATCACC	tagggatgaaa...*Ds*...tttcatcccta	GCATCACC
wx-m1(GM1)	GCATCACG		*GCCATCACC*
wx-m1(GM2)	GCATCACC		*ATCACC*

FIGURE 1 Sequence analysis of the *wx-m1* mutation and germinal mutations to partial *Wx* activity. Partial sequence is shown for the insertion termini of the *Ds* element found in the *wx-m1* mutation. The 11-bp inverted terminal repeats (lowercase) are flanked by 8-bp direct repeats (uppercase). *Ac*-mediated germinal mutations of the *wx-m1* allele can be recovered that show partial *Wx* activity. Sequence analysis of the excision sites of two partial *Wx* alleles (GM1 and GM2) are shown. Both mutations have lost *Ds* sequences but leave behind the 8-bp direct repeats in modified form. The modifications result in the addition of nucleotides in multiples of three. This may affect *Wx* gene action by introducing additional amino acids into the primary sequence of the *Wx* protein

phenotype is due to the addition of amino acid residues to the *Wx* protein. We are presently testing this hypothesis by examining a number of *wx-m1* germinal mutations and by cDNA analysis of the region of the *waxy* gene near the *Ds1* insertion.

Gene Cloning by Transposon Tagging

S.L. Dellaporta [in collaboration with S. Wessler, University of Georgia, and I. Greenblatt, University of Connecticut, Storrs]

The autonomous controlling element *Ac* contains internal DNA sequences that are present in as few as 8–10 copies per maize genome, even if the plant does not contain an *Ac* element. The active *Ac* element can be distinguished from these related, but inactive, sequences by structural features such as the presence of unique restriction fragments and the state of DNA methylation (see below). The presence of relatively few sequences related to internal *Ac* DNA and the ability to distinguish the active form of *Ac* from the inactive cryptic *Ac*-like sequences are criteria that can be used to clone genes containing *Ac* insertions.

We have used *Ac* tagging to clone the *R* locus of maize. This locus is one of several epistatic genes involved in the anthocyanin biosynthetic pathway. Our interest in *R* stems from previous genetic analysis of this locus which suggests that *R* conditions tissue-specific expression of antho-

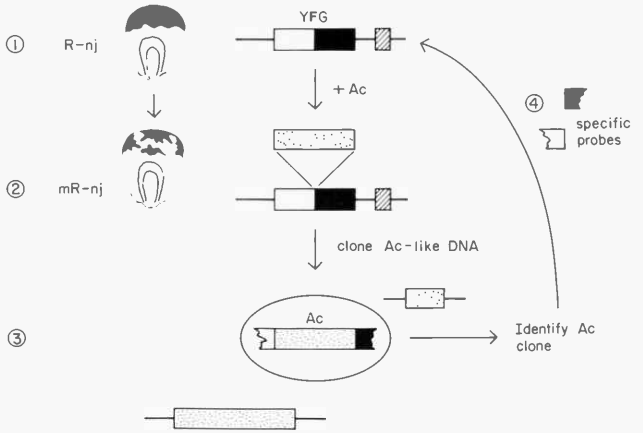


FIGURE 2 Gene cloning strategy using Ac tagging. A functional allele of the *R* locus is coupled with an active *Ac* element by using maize stocks containing reciprocal translocations between a donor allele (*P*-vv locus located on chromosome 1) and the recipient locus (*R*-nj locus located on chromosome 10). Transposition of *Ac* from donor to recipient site results in an unstable *R*-nj phenotype that shows the genetic characteristics of *Ac*-induced mutations. (*1*) The mutation shows a characteristic dosage effect; with increasing copies of *Ac*-induced mutation, the apparent frequency and developmental timing of somatic and germinal mutation is delayed; (*2*) the *Ac*-induced mutation is capable of mediating *Ds* transposition in *trans*. Once an *Ac*-induced *R* mutation is genetically identified, recombinant clones are isolated from genomic libraries with low-copy internal *Ac* DNA probes. The recombinant clone containing the *R* allele is identified by screening recombinants for the canonical *Ac* sequences by restriction site analysis. DNA flanking the *Ac* element is used as an *R*-specific probe to isolate a functional *R* allele.

cyanin in both seed and plant organs. The locus can be fractionated by meiotic recombination into distinct seed and plant components, suggesting that these regions may represent tandem displacements of homologous sequences with divergent function. Hence, the availability of a molecular probe to *R* DNA may prove to be a valuable tool to examine the tissue-specific components and regulation of gene expression in developing plant tissues.

Because the product(s) of the *R* locus is unknown and may be expressed in trace amounts in plant tissues, conventional cloning by immunological cDNA screening seemed inappropriate. Therefore, we used the strategy outlined in Figure 2 to clone the *R* locus. This strategy requires an insertion of the *Ac* element at the *R* locus. This event was dictated by (1) the transposition patterns of an *Ac* element, (2) the availability of the target site to *Ac* insertion, and (3) the ability to identify an *Ac*-induced mutation at the *R* locus. Previous studies demonstrated that the majority of *Ac* transpositions from the *P* locus on chromosome 1 are intrachromosomal events that exhibit a preference for short-range distal events. I. Greenblatt (unpubl.) obtained an *Ac* insertion at *R* by using strains carrying a reciprocal translocation involving breakpoints proximal to the long arm of chromosome 10 (containing the *R* locus) and distal to the *P* locus on chromosome 1 (containing an *Ac*-induced unstable *P* mutation). Several putative transpositions of *Ac* from the *P* locus to the *R* locus were obtained. An autonomous unstable allele of *R* was genetically characterized as *Ac*-induced by the ability of the element located at *R* to transactivate the standard *Ds* element located on chromosome 9. This mutation also exhibited a dosage pattern characteristic of *Ac*-induced mutations.

Several recombinant clones were isolated from genomic libraries, constructed with DNA from plants homozygous for the *Ac*-induced mutable *R* mutation, and screened with a low-copy internal *Ac* probe. One recombinant contained a canonical *Ac* element as judged from restriction enzyme analysis. DNA flanking the *Ac* element was determined to be *R*-specific on the basis of a structural comparison of various transposon-induced *R* mutations and their respective revertants. We are now in the process of determining the structure and are cloning the tissue-specific components of the standard *R* locus.

Active and Inactive *Ac* Elements

P.S. Chomet, S.L. Dellaporta

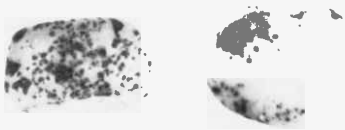
Changes in phase refers to the ability of an *Ac* element to cycle from an active state to an inactive state. In the active state, the *Ac* element is capable of transposition and transactivation of *Ds* elements (Fig. 3). This ability is thought to be due to the production of an unidentified transposase referred to as the *mutator* component of *Ac*. *Ac* can cycle from an active phase to an inactive phase where no *mutator* function is genetically detectable. We examined an *Ac* element at the *waxy* locus, isolated by McClintock (*Carnegie Inst. Wash. Year Book 63:* 592[1964]), in both the active and inactive phases. The active and inactive *Ac* elements were identical in both size and position at the *waxy* locus. However, the *Ac* in the inactive phase has undergone DNA modification. This modification was detected with restriction enzymes sensitive to 5-methylcytosine. The modified *Ac* DNA was found in much reduced amounts during the active phase at *waxy*. Examination of a number of active *Ac* elements at other loci shows that extensive modification was specific for the inactive phase of *Ac*.

Tz Elements of Maize

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

Last year, we described the molecular characterization of several unstable mutations at the *shrunken* locus in maize. These mutations were selected in maize lines descendant from plants systemically infected with barley stripe mosaic virus (BSMV). BSMV infection in maize was associated with high rates of genetic instability, including spontaneous mutations. Three *shrunken* mutations were caused by insertion elements that are structurally unrelated. The insertion sites and elements are shown in Figure 4.

Genomic clones of the mutant *sh-5586* allele



A B C

FIGURE 3 Changes in phase of *Ac* activity at the *waxy* locus. An *Ac* element insertion at the waxy locus [(*Ac*)*wx*-m7] has been identified by McClintock (*Carnegie Inst. Wash. Year Book 62*: 486[1964]) and shown to undergo changes in phase of activity. The cycling activity of *Ac* can be seen in kernels *A–C*. Each kernel has an endosperm genotype: *a1-m3/a1-m3/a1 wx/wx/* (*Ac*)*wx*-m7. In kernel *A*, the *Ac* at *waxy* remained active throughout endosperm development. The activity of *Ac* can be monitored by the instability of *Ds* at the *a1-m3* mutation. In the presence of an active *Ac* element, the *Ds* at *a1-m3* is somatically unstable, giving the kernel a variegated colored phenotype. When the *Ac* cycles from active to inactive, the *Ds* at *a1-m3* is stable, inhibiting *A1* gene action. This can be seen as a colorless sector in kernel *B*. Kernel *C* represents an allele of (*Ac*)*wx-m7* that is both somatically and germinally inactive. This kernel shows no *Ac* activity and is fully colorless. Molecular analyses (see text) of *Ac* at *wx-m7* indicate that DNA modification of *Ac* DNA occurs in the inactive phase. This modification is detectable with restriction enzymes that are sensitive to 5-methylcytosine.

containing the *Tz86* element were characterized by restriction mapping, genomic blot analysis, and M13 dideoxy-sequencing. We have shown that *Tz86* is a 3.6-kb element flanked by 10-bp direct repeats. This 10-bp sequence was found once in the progenitor *Sh* allele, indicating that *Tz86* causes a 10-bp duplication of target *Sh* DNA upon insertion. Unlike other maize transposable elements, *Tz86* does not contain terminal inverted or direct repeats. When subclones of *Tz86* were hybridized to genomic blots of maize DNA, we found that *Tz86* DNA represents dispersed middle repetitive DNA. Internal subclones of *Tz86* hybridize to relatively fewer copies, perhaps 10 fragments or less, depending on the strain examined. This situation is similar to that in other maize controlling elements and suggests that viral infection may destabilize these components in the maize genome and contribute to high rates of genetic instability. Moreover, *Tz86* continues to

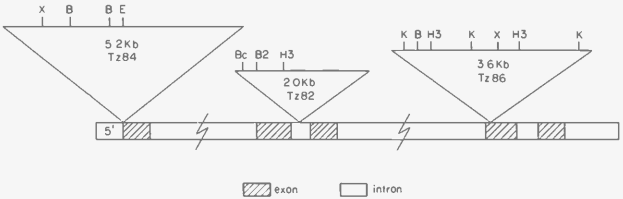


FIGURE 4 Relative position of the *Tz* insertion element at the *shrunken-1* locus. (The intron/exon borders of the *Sh* gene have been previously determined by W. Werr et al., pers. comm.) We position several insertion elements at *Sh* by restriction mapping and dideoxy-sequence analysis of mutant *sh* alleles (*sh-5582, sh-5584, sh-5586*) recovered from virus-infected progeny (see text). The size of *Tz84* is a minimum value and has not been precisely determined. On the basis of restriction mapping and hybridization analysis, we find no significant homology among these elements. Yet each element hybridizes to multiple genomic sequences of maize DNA.

show germinal instability in advanced progeny. This instability often restores the Sh phenotype (plump kernels) and is usually accompanied by excision of *Tz86* from the *shrunken* locus. Sequence analysis of one such event showed a restoration of the DNA sequence of the progenitor *Sh* DNA, except for a single-base substitution in the 10-bp target sequence.

Cell and Developmental Genetics of Tobacco

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

For the past few years, we have been studying the polyamine synthesis pathway in tobacco. Our approach was to focus on genetics: the isolation of mutants that are in some manner altered in the pathway. As documented in the 1982 and 1983 Annual Reports, we have developed successful mutagenesis and selection protocols. These make use of some powerful features of the tobacco somatic-cell system and some useful features of the polyamine pathway. Tobacco is relatively easy to manipulate in somatic-cell culture and can be subsequently regenerated into a whole plant for developmental and genetic analyses. The polyamine pathway has been intensively studied biochemically in mammalian systems, and there exist specific inhibitors for four of the six enzymes in the pathway. Figure 5 shows a simplified diagram of the pathway and the inhibitors. Our research during the past year has been concerned with the molecular and biochemical characterization of the mutants we have isolated previously and with the developmental genetics of the regenerated mutant plants.

DEVELOPMENTAL REGULATION OF ORNITHINE AND ARGININE DECARBOXYLASES

One interesting feature of the pathway is that tobacco synthesizes putrescine in two ways: via ornithine decarboxylase (OrnDC) or arginine decarboxylase (ArgDC). We have analyzed the distribution of these two enzymes in different tissues of the whole plant and in cell cultures. The results, shown in Figure 6, reveal that OrnDC is preferentially found in flowers and in cell cul-

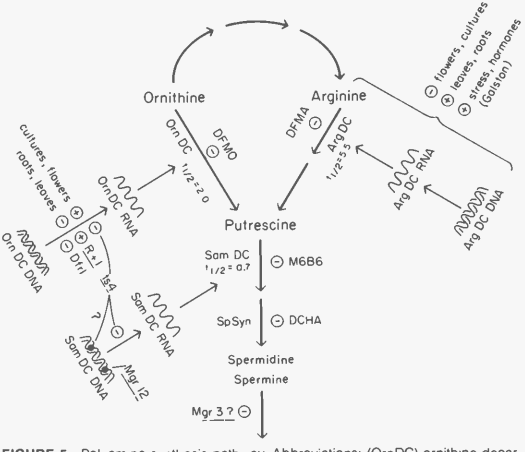


FIGURE 5 Polyamine synthesis pathway. Abbreviations: (OrnDC) ornithine decarboxylase; (ArgDC) arginine decarboxylase; (SamDC) *S*-adenosylmethionine decarboxylase; (DFMO) difluoromethylornithine; (DFMA) difluoromethylarginine; (MGBG) methylglyoxal-*bis*(guanylhydrazone).

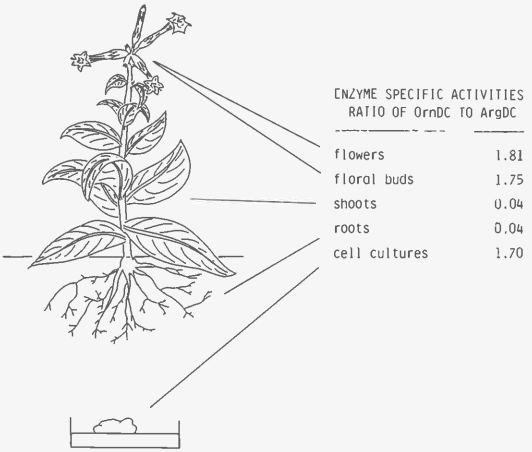


FIGURE 6 Developmental regulation of OrnDC and ArgDC in tobacco.

tures but not in leaves, stems, or roots. ArgDC is found in all tissues. Two independently isolated mutants (*ts4* and *Dfr1*) each have near-zero levels of OrnDC in cell cultures. These two mutants regenerate into very similar plants (dwarf, small leaves), and they have not flowered under normal growth conditions. This suggests that OrnDC is not only a flower-specific enzyme but may actually be required for flowering. We also examined the effects of the inhibitors of these enzymes on cell cultures. DFMO inhibits the growth of cultures after a delay of several generations, but DFMA does not. Thus, even though both enzymes are found in culture, OrnDC is required, but ArgDC is normally not.

We have used the known tissue distribution of OrnDC (and the existence of two mutants with low levels of OrnDC) as an aid to molecular cloning of the gene encoding the enzyme. L. McConlogue and P. Coffino (University of California, San Francisco) have previously obtained cDNA clones for the mouse OrnDC. We have used their clone as a probe against RNA isolated from different tissues of the plant and against RNA from wild-type and mutant cell cultures. When washed at moderate stringency, the mouse OrnDC hybridized in a pattern identical to that predicted by the distribution of the enzyme activities. This suggested that developmental regulation was occurring at a pretranslational level and that we could use the mouse OrnDC cDNA as an interkingdom probe to isolate the tobacco gene. With this strategy, we have found two tobacco genomic clones that hybridize to the mouse probe and to tobacco RNA in the pattern predicted by measurements of enzyme activity. We are currently in the process of sequencing the tobacco genomic clones for OrnDC.

EFFECTS OF MGBG ON ENZYME TURNOVER

We had previously found that wild-type cells grown on sublethal levels of MGBG overproduced a polypeptide of about 35,000 daltons. To examine this increase in abundance, we carried out a series of protein-labeling experiments, with and without MGBG. These experiments demonstrated that the increase in abundance of the 35-kD polypeptide was due not to new synthesis but to an accumulation of the previously made polypeptide. Apparently, the MGBG was stabilizing the polypeptide. This mode of regulation sug-

gested that the polypeptide was in fact *S*-adenosylmethionine decarboxylase (SamDC), since a similar phenomenon has been noted in mammalian cells. To prove this with tobacco, we partially purified SamDC via standard biochemical techniques to an approximate 4000-fold increase in specific activity. Although this was not absolutely pure, the predominant band on an SDS gel was 35 kD, making it likely that the originally observed band was SamDC.

As a further confirmation of the effect of MGBG on SamDC turnover, we measured enzyme specific activities for OrnDC, SamDC, and ArgDC, following the addition of cycloheximide to the tobacco cultures. This allowed us to estimate values for the half lives of the enzymes after shutting down protein synthesis in normal media: OrnDC, 2 hours; ArgDC, 5.5 hours; SamDC (normal media), 0.7 hours; and SamDC (plus MGBG), 9 hours. When the cells were grown in the presence of MGBG, the half-life of SamDC increased from 0.7 hours to 9 hours. Thus, all of our evidence is consistent with a model of SamDC being a 35-kD polypeptide that is stabilized by the presence of its inhibitor MGBG.

WHOLE-PLANT PHENOTYPES OF POLYAMINE SYNTHESIS MUTANTS

Our current mutant collection includes a temperature-sensitive cell line (*ts4*), its revertant (*Rt1*), a DFMO-resistant line (*Dfr1*), 32 MGBG-resistant lines (*Mgr1* through *Mgr32*), and some DCHA-resistant lines (*Dcr*). In cell culture, we are attempting to characterize polyamine synthesis in these lines systematically to determine the nature of the lesions in each case. When these mutants were regenerated into whole plants, they provided an astonishing array of phenotypes, particularly including developmental switches in the flower. A summary of the observed phenotypes is given in Table 1.

The whole-plant phenotypes naturally fall into four groups. Class I plants do not regenerate well but maintain a coral-like morphology on regeneration medium. Class II includes the two lines deficient in OrnDC; these are dwarf plants and do not flower. Class III plants are also dwarf, but they do flower with a phenotype we call puzzle box. These flowers have sepals, petals, and stamen that appear normal; however, the carpel is a

TABLE 1 Whole-plant Phenotypes of Polyamine Synthesis Variants

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

RO indicates plants regenerated from culture, and F1 indicates progeny from a cross of an RO plant to wild type

hollow cylinder that does not appear to have fused properly during development. Inside the hollow carpel is a second, and sometimes a third, row of stamen. Apparently, these mutants cannot finish carpel development correctly and thus repeat the previous stage of stamen.

Class IV phenotypes represent a range of developmental switches, including stamenoid ovules, stigmoid anthers, petaloid anthers, extrapetals, petaloid sepals, and simple sterilities. There is overlap within this group; e.g., one plant may have petaloid anthers, another may have stigmoid anthers, and yet another may have both phenotypes, as well as others. Thus, the class IV phenotypes mutually overlap in a way that suggests they are related. The sterilities generated have hampered sexual crossing and conventional genetic analysis. However, in two cases, *Mgr3* and *Mgr12*, we have generated a small F_1 population from crosses to wild type. In these cases, the polyamine phenotype and the abnormal flower cosegregate as nuclear dominant mutations.

The possible role of polyamines in altering the expression of floral meristems is indicated by the fact that all of the polyamine mutants that do flower have some sort of abnormal flower. Various artifacts are possible: double mutants, cell-culture-induced variation, and nongenetic regeneration abnormalities. Thus, the most critical experiments to be performed in the future will involve further meiotic genetic analysis of the mutants to test the nature of the suggested linkage between polyamine synthesis and floral development.

FLORAL MONOCLONAL ANTIBODIES

Our mutant collection should allow us to dissect floral development genetically, regardless of the physiological connection to the polyamine synthesis pathway. As an aid to analysis of the abnormal structures seen in the mutant flowers, we are making monoclonal antibodies against floral antigens, in collaboration with S. Hockfield (Neurobiology Section). The strategy is the familiar one of immunizing mice with floral extract, and then screening the resulting hybridomas against extracts from individual parts of the flower. In our initial experiment, we were able to find an antibody that recognizes sepal and petal extracts, but not other tissues; we also found two antibodies that reacted with only carpel extracts. We are currently using these antibodies to optimize histoimmunological techniques with sections of tobacco flowers.

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SITE-SPECIFIC MUTAGENESIS

M. Zoller K. Johnson
R. Kostriken

DNA/Protein Interactions: Recognition and Cleavage of *MAT*a DNA by HO Endonuclease

M. Zoller, K. Johnson, R. Kostriken

Our research is aimed at the problem of how a protein recognizes a specific sequence of DNA. Much has been learned from the study of prokaryotic proteins such as the λ repressor and *Eco*RI endonuclease. The interaction between a eukaryotic protein and a specific sequence of DNA has yet to be defined to the same extent. Toward this goal, we are investigating the manner in which the HO endonuclease from the yeast *Saccharomyces cerevisiae* binds to and cleaves *MAT* DNA. HO

endonuclease is one of a number of elements required for mating-type switching in *S. cerevisiae*. A model has been proposed in which the formation of a specific double-strand break in the chromosome at *MAT* is an initiating event in the process. The formation of this break is thought to be catalyzed by HO endonuclease. R. Kostriken and F. Heffron (Research Institute of Scripps Clinic) demonstrated that HO endonuclease cleaved *MAT* DNA in vitro. Through biochemical as well as genetic experiments, the specific site of the HO cut has been localized.

The biochemical events involved in HO-dependent DNA transposition are not fully understood. The problem is being approached from two directions. (1) What are the determinants within the HO cut site that are important for recognition and cleavage? (2) What are the specific amino acid residues within HO endonuclease that are responsible for recognition and cleavage?

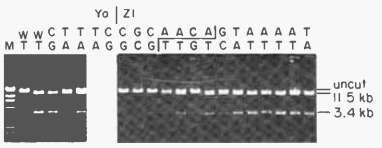

FIGURE 1 Agarose gel analysis of *MAT* substrates cleaved in vitro with HO endonuclease expressed in *E. coli*. (*1*) DNA cleaved with *Hin*dIII for markers; (*2,3*) *Bam*HI-cleaved wild-type *MAT***a** DNA without and with HO endonuclease, respectively; (*4–22*) mutant *MAT***a** substrates cleaved with HO endonuclease. The corresponding wild-type *MAT***a** sequence is shown above each lane.

MUTATIONS IN *MATa* THAT AFFECT HO CLEAVAGE IN VITRO

Since genetic approaches yielded only two point mutations within the HO cut site that affected switching and cutting, we used oligonucleotide-directed mutagenesis to saturate this region with single base changes. The ability of each mutant to serve as a substrate for HO endonuclease was assessed in vitro using a yeast extract from an HO strain and an *Escherichia coli* extract from a strain engineered to express the *HO*-gene product (see below). Qualitatively, both the yeast- and the *E. coli*-produced enzymes cleaved the mutants in a similar manner. Figure 1 shows the analysis of all 17 mutant substrates after incubation with HO endonuclease produced in *E. coli*. The mutant substrates can be grouped into three categories on the basis of the rate with which each is cleaved compared with wild type. Four mutations completed inhibited cleavage, nine mutations resulted in a reduced rate of cleavage, and the remaining four mutations had no effect on the rate of cleavage as compared with wild type. These data reveal that the core of the recognition sequence is CGCNNC, since alteration of any one of the four bases completely inhibited cleavage. Furthermore, the sequence TTTCCGCAACAGT represents the region in close contact with the enzyme, since alteration of noncore positions in this region affected the rate of cleavage. It is significant to note that the extent of the contact region spans the so-called Y/Z boundary into the allele-specific Ya region. Does the enzyme recognize *MAT*α in the same manner? A parallel set of mutations have not been made in *MAT*α to answer this question. However, inspection of the allele-specific DNA sequences of *MAT***a** and *MAT*α in the region of the Y/Z boundary revealed the same sequence (CTT) at positions -5, -4, and -3 (with respect to the Y/Z boundary). As shown in Figure 1 only the mutations at -4 and -3 affected cleavage.

Future studies will assess the effect of each point mutation on the frequency of mating-type switching in vivo. Currently, we are integrating each of the mutant *MAT***a** substrates into the chromosome, thereby replacing the wild-type *MAT***a** sequences. We predict that the frequency of switching will correlate with the efficiency of cleavage in vitro.

CHARACTERIZATION OF THE HO ENDONUCLEASE

The concentration of HO endonuclease in yeast is too low to serve as a source for purified protein. Therefore, we have engineered the *HO* gene to be expressed in *E. coli*. The gene was placed under the isopropyl-β-D-thiogalactoside (IPTG)-inducible *lac* operator/*trp* promoter of the vector pKK233-2 (courtesy of J. Brosius, Columbia University). This construction yielded approximately 50–100 times higher expression per gram

of wet cells compared with yeast. Expression from this vector was 5 times better than that of an earlier plasmid utilizing the λP_r promoter. Currently, we are purifying HO endonuclease. Future experiments will involve (1) determination of native stoichiometry and molecular size and shape, (2) alkylation protection studies using the wild-type and mutant recognition sequences, (3) quantitative binding studies with mutant substrates, (4) investigation of domains by limited proteolysis, and (5) active-site affinity labeling using synthetic recognition sequences.

Oligonucleotide-directed Mutagenesis

M. Zoller

In vitro mutagenesis of cloned DNA has become a standard tool in the functional analysis of nucleic acids and proteins. A number of approaches exist that can be broadly grouped into random and site-directed methods. The repertoire of procedures to mutagenize a fragment of DNA randomly continues to grow each year. These random techniques are used to identify the location and boundaries of a particular function. Once an important region has been identified, site-directed mutagenesis can be employed to determine the role of specific nucleotides (or amino acids). The construction of cloned DNA bearing a specific alteration is best accomplished by either gene synthesis or oligonucleotide-directed mutagenesis.

The availability of reliable procedures for manual oligonucleotide synthesis, along with an increasing access to DNA synthesizers, has made gene synthesis a reasonable method to produce specific mutations. The gene is constructed using segments of oligonucleotides that are ligated together, and thus an entire gene can be synthesized that contains a desired change. In addition, a simple construct can be produced that allows for the replacement of segments by a number of mutated "oligonucleotide cartridges" positioned between restriction endonuclease sites.

Oligonucleotide-directed mutagenesis is similar in principle to mutagenesis by gene synthesis in that an oligonucleotide that bears the desired mutant sequence is inserted into a cloned gene. However, oligonucleotide-directed mutagenesis differs from gene synthesis in the manner in which the cloning is accomplished. In this case, an oligonucleotide consisting of the mutant sequence is hybridized to its complementary sequence in a clone of wild-type DNA, thereby forming a mutant–wild-type heteroduplex. The oligonucleotide serves as a primer for in vitro enzymatic DNA synthesis of regions that are to remain genotypically wild type. A double-stranded heteroduplex is formed, which is subsequently segregated in vivo into separate mutant and wild-type clones. The two can be distinguished by a number of screening procedures.

The basic strategy of oligonucleotide-directed mutagenesis was developed using the single-stranded phage ϕX174. The technique has been successfully applied to genes cloned into either plasmid or phage vectors. Previously, I developed an efficient procedure for oligonucleotide-directed mutagenesis using M13-derived vectors. The major features of this method were the use of single-stranded clone DNA as template, the purification of in-vitro-synthesized covalently closed, circular DNA by alkaline sucrose gradient centrifugation, and the use of the mutagenic oligonucleotide to screen for mutants by hybridization.

I have developed a variation of the original method that is simpler, faster, and of equal efficiency (Zoller and Smith, *DNA 3:* 479 [1984]). It is based on the use of two primers, one of which is the mutagenic oligonucleotide and the other is a standard sequencing primer (see Fig. 2). Both primers are simultaneously annealed to single-stranded template DNA, extended by DNA polymerase I (large fragment), and ligated together to form a mutant/wild-type gapped heteroduplex. *E. coli* is transformed directly with this DNA. Mutants are identified by plaque-lift hybridization using the mutagenic oligonucleotide as a probe. The same procedure can be used without modification to create site-specific insertions as well as deletions. This method obviates the need to isolate covalently closed, circular DNA to obtain mutants in high yield and retains the convenience of the M13 system for template isolation, sequencing, and screening.

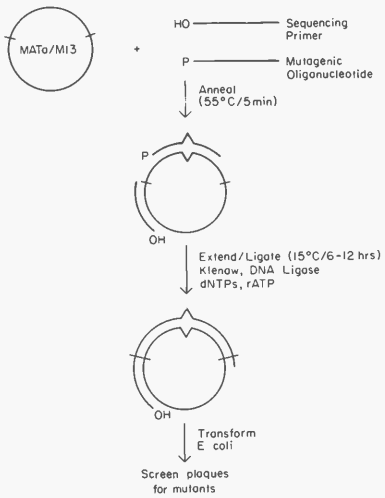

FIGURE 2 Scheme for oligonucleotide-directed mutagenesis using two primers and a single-stranded template.

DNA Synthesis Facility

M. Zoller

Synthetic oligonucleotides have expanded the ability of the molecular biologist to produce cloned DNA with a precisely defined sequence. Applications include the construction of point mutations in a gene, custom-designed linkers, and the precise fusion of promoter and coding sequences. In addition, oligonucleotides have been to used to detect a specific gene within a library. Although procedures exist for the manual synthesis of oligonucleotides, the operations have been automated, thereby freeing the scientist for more important problems. In December of 1983, I began operating an Applied Biosystems DNA Synthesizer, which initially served my laboratory primarily. However, during 1984, about 300 oligonucleotides were synthesized for more than 30 individual scientists at the Laboratory. Oligonucleotides were produced at about one-tenth the cost charged by commercial sources and were obtained much faster, usually within 1 week. Initially, the machine synthesized only a single oligomer at a time; however, the capability was expanded so that three separate oligonucleotides could be synthesized at once. This has decreased the waiting time for an individual oligonucleotide and has made usage of solvents more efficient.

The chemistry of the Applied Biosystems DNA Synthesizer is the solid-phase phosphite triester method based on procedures developed by M. Caruthers (University of Colorado). Currently, the scale is 1 μmole of starting material and synthesis proceeds at 20 minutes per cycle; oligomers as long as 100 bases have been successfully produced on this machine. In the future, a 0.1-μmole scale synthesis will be offered in conjunction with a 6-minute cycle, which will result in faster syntheses and a 50% reduction in the cost per oligonucleotide. The small-scale synthesis will be especially useful for producing short oligonucleotides for sequencing projects. We have found that the efficiency of synthesis is high enough that the crude (nonpurified) sample can be used as a primer for M13 dideoxy sequencing experiments. In addition to the small-scale synthesis, a 10-μmole scale synthesis is available for producing oligonucleotides to be used for structural experiments on nucleic acids.

PUBLICATIONS

Zoller, M.J. and M. Smith. 1984. Oligonucleotide-directed mutagenesis: A simple method using two oligonucleotide primers and a single-stranded template. *DNA* **3:** 479–488.

In Press, Submitted, and In Preparation

Zoller, M.J., K.E. Johnson, M. Smith, and R.G. Kostriken. Point mutations in the yeast *MAT***a** gene that affect cleavage by HO endonuclease. (Submitted).

PROKARYOTIC GENETICS

F. Daldal	G. Freyer	W. Eisner
S. Hinton	J. Applebaum	T. Fischer
P.A. Scolnik	E.J. Bylina	S. Ismail
C. Slaughter	S. Cheng	S. Johann
D.C. Youvan	E. Davidson	J. Schumann

Molecular Genetics of Cytochrome c_2 of *Rhodopseudomonas capsulata*

F. Daldal, J. Applebaum, S. Cheng

Cytochrome c_2 is an electron carrier common to the photosynthetic and respiratory machinery of *Rhodopseudomonas capsulata*. It is structurally homologous and functionally analogous to the cytochrome *c* of mitochrondria and to the plastocyanin of chloroplasts. During photosynthesis, c_2 is the primary electron donor to the reaction center, and during respiration, it is an important component of the *c*-type cytochrome-containing respiratory chain. This implies that c_2, an evolutionarily well-conserved heme-containing protein, must be evolved in such a way that, when required, it can interact with the components of the reaction center, of the ubiquinol:cytochrome c_2 oxidoreductase (bc_1 complex), and of the cytochrome oxidase. Even though cytochrome c_2 of photosynthetic bacteria has been well studied biochemically, biophysically, and immunologically, genetic data are still scarce. Until recently, no mutant deficient solely in cytochrome c_2 was available, and schemes devised to select c_2^- mutants often yielded pleiotropic mutants devoid of all known *c*-type cytochromes. Therefore, the in vivo role of c_2 on photosynthesis has remained untested.

To isolate a c_2^- structural mutant, we have taken a "reverse genetic" approach. First, the c_2 structural gene of *R. capsulata* was cloned by using synthetic mixed oligonucleotide probes and then its nucleotide sequence was determined. The deduced amino acid sequence was found to be in perfect agreement with the known amino acid sequence obtained from the purified c_2 protein. Moreover, the nucleotide sequence of the amino-terminal part of the c_2 gene indicated the presence of a 21-amino-acid "signal sequence." Therefore, in agreement with its known periplasmic location, the c_2 protein is synthesized in the cytoplasm as a precursor and is then processed during its secretion to the periplasm.

Using the cloned gene, we constructed in vitro c_2 genes containing insertion and insertion-deletion mutations, and we used these to replace the chromosomal wild-type allele by genetic crosses mediated by the "gene-transfer agents" (defective phage-like particles specific to *R. capsulata*). This led us to the isolation of the first known c_2 structural mutants of a photosynthetic bacterium. In these mutants, the absence of the c_2 gene was confirmed by Southern analysis using either the cloned c_2 gene or the inserted kanamycin marker as probes. Furthermore, the absence of the c_2 gene product was proved by immunoprecipitation using polyclonal antibodies raised against the purified c_2 protein, by *c*-type cytochrome staining of SDS-polyacrylamide gels of total cell extracts, and by reduced oxidized spectrum analysis.

c_2^- mutants are stable and can grow well by photosynthesis and by cytochrome-*c*-dependent respiration. Considering the fact that during photosynthesis, cytochrome c_2 is the primary electron donor to the reaction center, these mutants show that in the absence of c_2, *R. capsulata* may have alternative ways unknown until now to reduce the oxidized photosynthetic reaction center. To understand this interesting observation, several hypotheses, such as the presence of other cytochromes functionally analogous to c_2 or the nonessentiality of a mobile carrier in photosynthesis, are currently under investigation.

Clostridium pasteurianum Galactokinase

F. Daldal, J. Applebaum

Until now, no gene from a bacterium like *Clostridium pasteurianum,* which has a very high A + T

content (70%), has been isolated. To study the structure of a clostridial gene, to assess the possibility of its expression in other hosts, and eventually to use it as a genetic tool, we have cloned the *C. pasteurianum* galactokinase by complementation of an *Escherichia coli* mutant deficient in galactokinase. Restriction enzyme analysis, subcloning, and Tn*5* mutagenesis indicated that the gene was located on a 1.8-kb *Cla*I-*Sau*3A fragment that encoded a polypeptide with an apparent molecular weight of approximately 40,000. Although they have similar subunit molecular weights, Southern hybridization analysis indicated no strong homology between the *C. pasteurianum* and the *E. coli* galactokinases. In *E. coli*, the Gal$^+$ phenotype provided by this clostridial galactokinase was found to be unstable, and we have shown that spontaneous Gal$^-$ derivatives were originated by insertion sequences (IS*1* and IS*5*) inactivating the clostridial galactokinase. Even though this clone was able to complement a *galK* mutant of *E. coli*, it expressed the clostridial galactokinase at a low level in this host. Using as a probe a DNA fragment internal to the clostridial galactokinase, we isolated by hybridization another clone carrying (presumably) the wild-type gene. This latter clone was not able to grow on galactose-containing media due to the overproduction of the clostridial galactokinase and the concomitant inhibition of the *E. coli* host by "galactosemia."

Comparison of these two plasmids producing low and high amounts of galactokinase revealed that the low-level producer carries a deletion of approximately 300 bp located in the 5′-proximal part of the gene. Operon fusions constructed between this region where the deletion is localized and a promoterless *E. coli* galactokinase gene indicated that these clostridial DNA sequences have promoter activity in *E. coli* and that the overexpression of the clostridial galactokinase is at least partly transcriptional in this latter clone.

The nucleotide sequences of the 5′-proximal part of the galactokinase gene from several clones producing high, low, or undetectable amounts of galactokinase were determined. Their comparison revealed that the clone producing high amounts of galactokinase has clostridial nucleotide sequences very homologous to the *E. coli* consensus promoter sequences. The role of these sequences in *Clostridium* is not yet known, but fusion studies demonstrated that they mediate the expression of the clostridial galactokinase in *E. coli*. Therefore, considering that the transcription of a gene is an important barrier in heterologous expression systems, the A + T richness of the clostridial genome appears to be a lucky situation when the chosen host is *E. coli*. This work shows that the complementation of *E. coli* mutants by the clostridial genes is feasible and is a good way to isolate clostridial genes whenever appropriate mutants are available. It remains to be seen whether the *C. pasteurianum* galactokinase gene will turn out to be a useful genetic marker for the study, by gene fusions, of the structure and the regulation of other clostridial genes in *Clostridium* using the recently developed transformation technique.

Finally, the cloning and characterization of the *C. pasteurianum* galactokinase indicate that in this organism, galactose is used through the "Le-Loir pathway" via galactose-1-phosphate.

Characterization of MT113, a Ps$^-$ Mutant of *R. capsulata* That Lacks a Functional bc_1 Complex and c-type Cytochromes

E. Davidson, F. Daldal

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

Electron paramagnetic resonance (EPR) spectroscopy indicated that membranes derived from MT113 contained the Rieske Fe-S center at a much lower amount (< 10%) than the wild-type membranes. This lack of the Rieske Fe-S center can-

not be attributed to a general defect in the synthesis of Fe-S clusters, since the same mutant contains an almost normal amount of total Fe-S clusters. Similarly, on the basis of EPR spectroscopy, an antimycin-sensitive signal characteristic of the anionic semiquinone $Q_c^{\cdot -}$ was not detectable in MT113 membranes. Absorption spectroscopy showed that, even though an almost normal amount of *b*-type cytochromes was present in this mutant, the antimycin-induced red shift of the cytochrome b_{560} of the bc_1 complex was absent.

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

Cartridge Mutagenesis and Deletion of the Photosynthetic Reaction-center and Light-harvesting Genes from *R. capsulata*

D.C. Youvan, S. Ismail

Our purpose in constructing photosynthetic apparatus deletion strains is to provide backgrounds for the assay of in-vitro-mutagenized reaction-center and light-harvesting genes carried by plasmid vectors (Fig. 1). The nucleotide sequence and deduced polypeptide sequences for all of the reaction-center and light-harvesting structural polypeptides have been determined (Youvan et al., *Cell 37:* 949 [1984]; Youvan and Ismail, *Proc. Natl. Acad. Sci. 82:* 58 [1985]). On the basis of primary sequence data, models have already been proposed for reaction-center quinone binding and light-harvesting bacteriochlorophyll binding. The three-dimensional structure of the

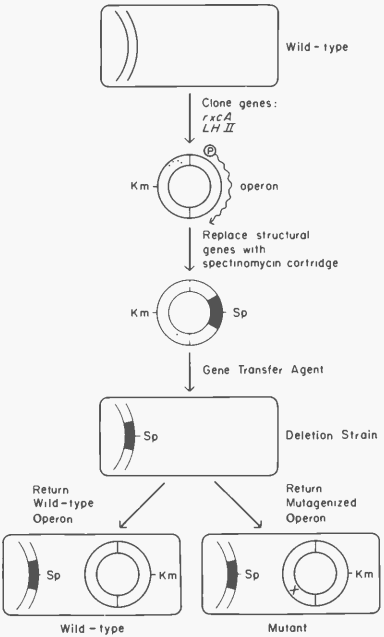

FIGURE 1 Construction and complementation of photosynthetic deletion strains. Deletion backgrounds constructed by cartridge mutagenesis may be complemented by the wild-type reaction-center or light-harvesting operons in *trans*. Alternatively, in-vitro-mutagenized plasmids may be returned to the deletion strain in order to assay the affect of engineered point mutations.

reaction center is being solved by X-ray crystallography. We anticipate that it will be essential to test reaction-center and light-harvesting structure-function models by in vitro mutagenesis of the structural genes. Progress in this rapidly advancing multidisciplinary field has been reviewed recently (Youvan and Marrs, *Cell 39:* 1 [1984]; Youvan and Marrs, in *Molecular Biology of the Photosyntheic Apparatus*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York [1985] in press).

Deletion strains were constructed using the cartridge mutagenesis technique of P. Scolnik (this section). This procedure employs the gene-transfer agent (GTA) that is specific to *Rhodopseudomonas capsulata*. The GTA is similar to a generalized transducing phage, randomly packaging 4.6-kb DNA segments from the chromosome. Plasmids carrying *R. capsulata* genes are mutagenized in vitro by the insertion of small (2–4 kb) restriction fragments specifying resistance to kanamycin or spectinomycin (from Tn*5* or Tn*7*, respectively). These plasmids are then transferred via conjugation from *Escherichia coli* to a GTA overproducer strain. GTA from the overproducer is crossed with a suitable recipient, and transductants are selected for the appropriate antibiotic marker. Antibiotic-resistant transductants have incorporated the mutagenized copy of the gene into their chromosome by homologous recombination, and the corresponding wild-type alleles are lost. Cartridge mutagenesis need not be limited to simple insertional interruption of genes: Deletions may be engineered through ligation of the cartridge in place of a target restriction fragment.

Strains have been constructed wherein all or part of the reaction-center (RC), light-harvesting I (LHI), and light-harvesting II (LHII) structural genes have been deleted. In one series of strains, the 2778-bp *Apa*I fragment bearing more than 90% of the *rxcA* operon (promoter and structural genes for LHI β, LHI α, RC L, and RC M) has been deleted from the chromosome. When the *rxcA* operon is deleted from the wild type, resultant strains possess only LHII and are photosynthetically defective. The *rxcA* deletion in a LHII$^-$ background results in a strain lacking all light-harvesting antennae and reaction-center subunits. As expected, this strain has no near-infrared absorption characteristic of light-harvesting or reaction-center bacteriochlorophyll. The *rxcA* deletion may be complemented by a pBR322 derivative (pU2I) carrying the entire *rxcA* operon. In a second series of deletion strains, the 2500-bp *Bst*EII-*Stu*I fragment, including the β and α structural genes for LHII, has been deleted from the chromosome. In the wild-type background, the functional reaction center and LHI are expressed. LHII may be restored by returning the plasmid pU2 that carries the LHII operon.

Characterization of the Photosynthetic Reaction Center

D.C. Youvan

Photosynthetic reaction centers from the purple nonsulfur bacterium *Rhodopseudomonas capsulata* have been purified to near homogeneity in amounts (100 mg) suitable for biophysical analysis. Photosynthetic membranes (chromatophores) can be prepared by differential centrifugation using a cartridge mutant (U34) lacking the LHII antenna. Otherwise, reaction centers and LHI are wild type. Membrane proteins are solubilized in the anionic detergent lauryl-dimethylamine-*N*-oxide (LDAO), and reaction-center proteins may be purified by affinity chromatography on horse heart cytochrome c_2 coupled to Sepharose 4B. This procedure was originally developed by Brudvig et al. (*Proc. Natl. Acad. Sci. 80:* 683 [1983]) for reaction centers from *Rhodopseudomonas sphaeroides*.

Reaction centers purified by affinity chromatography are very pure and photochemically active. No contaminating light-harvesting polypeptides or bacteriochlorophyll is observed by SDS-PAGE or absorption spectroscopy. The purified reaction centers are capable of the initial photosynthesis reaction involving charge separation: The 870-nm absorption band assigned to the special pair is photobleached by actinic light.

Reaction centers have been crystallized from two species, *Rhodopseudomonas viridis* and *R. sphaeroides*. The detailed biophysics of the photoelectron cycle is best characterized in the latter species. This is somewhat of an unfortunate situation since genetics is best developed in *R.*

capsulata. The amino acid sequence of the photosynthetic reaction-center polypeptides is completed for only *R. capsulata*. We believe that genetics is an essential feature for future structure-function problems; hence, X-ray crystallography should be repeated using *R. capsulata* reaction centers. The ability to prepare large quantities of purified *R. capsulata* reaction centers should help to remedy the problem.

In Vitro Mutagenesis of the Photosynthetic Reaction-center and Light-harvesting I Genes

E.J. Bylina, D.C. Youvan

We are using in vitro mutagenesis to address the fundamental question: How are proteins involved in the primary light reactions of photosynthesis? In the light reactions, membrane proteins and cofactors (chlorophyll, quinone, and carotenoids) interact in a highly efficient way to harvest light energy and to transduce this energy into a biochemically useful reductant. Presumably, the exact placement of the cofactors by the membrane proteins facilitates the unprecedented photochemical efficiency of the photosynthetic apparatus (quantum yield approaching unity). Some of the amino acid residues within the membrane proteins may play more than a structural role and participate directly in the photochemical reactions, facilitating proton and electron conduction.

We have recently developed in vitro mutagenesis procedures and bacterial strains to assay the phenotype of plasmid-borne *rxcA* operons. This 3000-bp operon carries the structural genes encoding four membrane polypeptides: the photochemical core of the reaction center (L and M subunits) and LHI (α and β subunits). The cloned *rxcA* operon may be returned to *rxcA* deletion strains in order to assay the phenotype of the plasmid genes in *trans*.

The plasmid bearing the *rxcA* operon (pU21) has been mutagenized in vitro by oligonucleotide-mediated site-directed mutagenesis. Plasmid pU21 is nicked with *Eco*RI in the presence of ethidium bromide, and exonuclease III is then used to convert the nicked DNA into gapped DNA. The mutagenic oligonucleotide is annealed to this gapped region, and the gap is then repaired with Klenow and ligase. The mutagenized plasmids are transformed, and the transformants are screened by colony hybridization using the mutagenic oligonucleotide as the probe. Direct mutagenesis of the plasmid avoids shuttling, since pU21 may be conjugated from an *E. coli* donor to the deletion background with the help of a mobilizing plasmid. Hence, the phenotype of a light-harvesting or reaction-center mutation may be rapidly assayed. Additionally, revertants may be selected and the sequence of pU21 redetermined.

Experiments have been initiated to test the light-harvesting bacteriochlorophyll-binding model of Theiler and Zuber (*Hoppe-Seyler's Z. Physiol. Chem. 365:* 721 [1984]). The proposed binding site Ala-*X*-*X*-*X*-His has been targeted by in vitro mutagenesis of pU21, assayed in the deletion background. A histidine-to-aspartic acid change in the LHI α-subunit results in the loss of this antenna. This is consistent with the model which proposed that the α and β polypeptides interact through a chlorophyll dimer, each bound to a histidine lone pair on both polypeptides. The loss of LHI also causes the pleiotropic derangement of the reaction center and a photosynthesis-defective (PS^-) phenotype. PS^+ revertants have been subsequently isolated by direct selection. We plan to sequence the site of reversion in order to determine whether histidine is the only functional residue for chlorophyll binding.

Future in vitro mutagenesis targets include the reaction-center putative quinone-binding site Pro-Phe-His-Met-Leu, which is conserved with higher plants. Mutations in this sequence may affect herbicide resistance, since these chemicals are competitive inhibitors of quinone binding. Another possible target of particular interest to spectroscopists is a possible carotenoid binding site in LHII: Tyr-Arg. The arginine residue is believed to be the point charge responsible for the carotenoid electrochromic band shift that is sensitive to membrane potential. As crystallographic data become available, mutagenesis should play an increasingly important role in testing light-harvesting and reaction-center structure-function models.

Cartridge Mutagenesis in *R. capsulata*

P.A. Scolnik

Modern genetic approaches require the capability of selectively inactivating genes by insertions or deletions. I have developed a system for introducing gene cartridges into the chromosome of *Rhodopseudomonas capsulata* by homologous recombination (Scolnik and Haselkorn, *Nature 307*: 289 [1984]). This method takes advantage of a defective transducing phage (gene-transfer agent, GTA) produced only by this bacterium. GTA transduces linear double-stranded segments of DNA of 4.5 kbp, which by homologous recombination are integrated into the chromosome of the recipient cell. I determined that DNA from recombinant plasmids, introduced into the cell by conjugation, are also packaged into GTA heads. Consequently, the following method (Fig. 2) was developed: (1) A gene cartridge is introduced in vitro into a cloned sequence by standard recombinant DNA techniques, generating either an insertion or a deletion. (2) The resulting plasmid is transformed into *Escherichia coli* and then introduced into an *R. capsulata* GTA overproducer by conjugation. (3) GTA is then prepared and used to treat wild-type recipient cells, selecting for the antibiotic resistance of the gene cartridge. (4) Since the incoming molecule is not a replicon, antibiotic resistance cells can only be the product of homologous recombination with the host chromosome.

This method has two main advantages over transposon mutagenesis. First, the insertions or deletions can be engineered in vitro using restriction sites. Second, since this method does not involve the introduction of plasmids into the cell, the mutants obtained can be used for complementation studies with recombinant plasmids. I have successfully used kanamycin resistance from Tn*5* and spectinomycin resistance from Tn*7* as gene cartridges.

Maximum efficiency (10^{-3} with respect to the efficiency of transduction of a point mutation) is obtained when both ends of the molecule contain *R. capsulata* rather than vector DNA, probably reflecting the fact that the free ends are recombinogenic. However, insertions have been obtained with as little as 250 bp of homologous DNA flanking the gene cartridge, indicating that either internal recombination occurs or the linear molecule is attacked by exonucleases, generating free ends.

Cryptic Genes and Gene Rearrangements in Response of a Bacterial Genome to Stress: Precise Excision of a Gene Cartridge

P.A. Scolnik, J. Schumann

Rhodopseudomonas capsulata can fix atmospheric nitrogen through the enzyme nitrogenase. We have cloned the structural genes (*nif* HDK) and have determined that they are organized in a transcriptional unit (Scolnik and Haselkorn, *Nature*

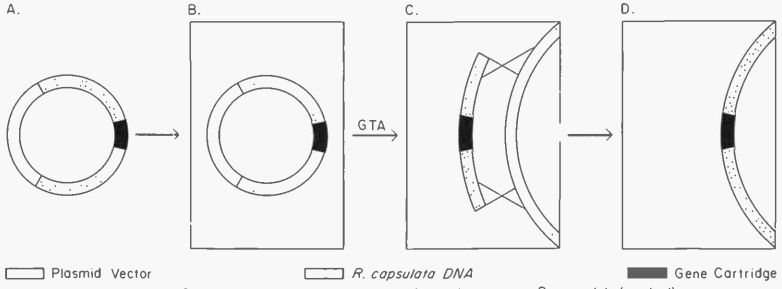

FIGURE 2 Strategy for site-directed gene cartridge mutagenesis in *R. capsulata* (see text)

307: 289 [1984]; *J. Bacteriol. 156:* 251 [1983]), entirely contained within a 12-kbp *Hin*dIII fragment. Insertions and deletions within this fragment (produced by the cartridge mutagenesis method using kanamycin as the selection marker) give rise to a Nif$^-$ phenotype and introduce into the region an additional *Hin*dIII site that is present in the cartridge.

These mutants are stable in their phenotype when grown in medium supplemented with fixed nitrogen. However, Nif$^+$ pseudorevertants can be isolated by challenging these cells to grow in ammonia-free medium. A time-course experiment starting with an insertional mutation grown in ammonia-free medium showed that the first class of pseudorevertants appears within the first few hours of selection. These cells are resistant to kanamycin, and the gene cartridge remains in the original place of insertion, as shown by Southern hybridization; however, a low (10% of wild-type levels) nitrogenase activity can be detected. Kanamycin-sensitive cells, with full nitrogenase activity, are observed after at least 2 days of growth. Southern hybridization shows that the gene cartridge has been deleted from the genome, and two new *Hin*dIII bands (12 and 16 kbp) are observed. The 16-kbp fragment is a transient form, which resolves in the 12-kbp band upon further incubation. Cells containing this 12-kbp band are indistinct from wild types.

We interpret these results as follows: When the *nif* genes are inactivated by cartridge insertion, *R. capsulata* will respond to the need for nitrogen by bringing a cryptic *nif* gene(s) into operation, and this event is responsible for the low level of nitrogenase activity initially observed. That this response does not involve some type of activation of the inactivated *nif* genes is evident because it will occur whenever these genes are deleted. Subsequently, the gene cartridge is excised from the genome, probably by translocation of a 4-kbp element into the site of kanamycin insertion. This event generates a 16-kbp band, which in turn resolves (probably by excision of the 4-kbp element) into a 12-kbp *Hin*dIII band, and the full nitrogenase activity phenotype is restored. The activation of the cryptic gene provides the necessary energy for the process of gene rearrangement to take place.

Since nitrogenase activity is fully restored, the excision of this cartridge must be precise or near precise. The fact that this process occurs indicates that gene cartridges as well as transposons can be excised from the genome, and therefore transposon activity is not required. We are currently characterizing the excision process, and we have sequenced the *nifH* and *nifD* genes in order to be able to determine the nature of the rearrangement observed.

The nature of the cryptic gene remains unclear. Southern hybrydization of *nif* structural genes to total genomic DNA shows several (at least eight) *Hin*dIII bands that contain homology with all three *nifH*, *nifD*, and *nifK* genes. We have cloned one of them and have determined that, although it contains 65–80% homology with *nifH* at the nucleotide level, it does not have the capability of coding for a nitrogenase gene. There are four copies of this element in the genome, and cosmid cloning shows that they are dispersed in the chromosome. Insertions in this region have no phenotype (P. Schumann and P. Scolnik, in prep.). It is possible that this element constitutes a *nif* homologous insertion sequence, a possibility currently being explored.

Cloning Prokaryotic Genes by Complementation with a Cosmid Library

P.A. Scolnik

A situation often exists in which no physical probe to clone a certain gene is available, but a mutant with the desired phenotype has been obtained. A gene complementing such a mutation can be easily isolated from a genomic library, provided a method exists to introduce this library massively into the mutant cells. In most gram-negative bacteria, transformation, at least at high efficiency, is not available. However, wide-host-range plasmids (developed by D. Helinski's group, University of California, San Diego) can be mobilized by conjugation from *E. coli*. I have constructed a genomic library from *R. capsulata* in a cosmid derivative of these vectors and designed a protocol that allows cloning of the mutants by complementation. In conjunction with cartridge mutagenesis, this method can be used to clone and to identify *R. capsulata* genes unequivocally. More than ten different sequences, affecting both

photosynthesis and nitrogen fixation, have been cloned using this approach.

A Study of Bacterioferritin from *Azotobacter vinelandii*

S. Hinton, C. Slaughter, T. Fischer

Bacterioferritin is a *b*-type cytochrome, but it also closely resembles mammalian ferritin because of its quaternary structure (24 identical subunits) and contains an average of 1600 Fe atoms in an oxide-hydroxide-phosphate core. Bacterioferritin differs from mammalian ferritin in that it contains heme and has the ability to take up hundreds of electrons at a low redox potential, which suggests that it may function as an electron storage protein for use in respiration, biosynthesis, and nitrogen fixation. It has been proposed that bacterioferritin may also be an iron storage protein for *Azotobacter vinelandii*.

We are initiating structure-function studies of bacterioferritin to understand how it resembles and differs from ferritin, as well as biochemical studies to establish its physiological role. Bacterioferritin was purified and crystallized. The aminoterminal amino acid sequence was determined, and antibodies were raised against the purified protein. Oligonucleotides were synthesized based on the aminoterminal amino acid sequence of the protein, and antibodies recognizing bacterioferritin are being used to screen various genomic libraries of *A. vinelandii*. The complete primary structure of bacterioferritin will be determined by amino acid sequencing as well as by gene sequencing. Future experiments will include site-directed mutagenesis for structure-function studies to determine the effect mutations have on nitrogen fixation, respiration, and iron metabolism.

Molybdenum Metabolism in *Clostridium pasteurianum*

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

There are two distinct cofactors for various redox enzymes. The molybdenum-iron protein of nitrogenase contains a unique iron-molybdenum cofactor, FeMo-co, whereas all other known molybdoenzymes contain a common molybdenum cofactor, Mo-co, a molybdenum-pterin species. We have proposed a hypothesis that during the biogenesis of Mo cofactors, precursors are protein-bound. We have identified four molybdoproteins in crude extracts of *Clostridium pasteurianum* that have characteristics which suggest that they may play a role in Mo metabolism.

The major Mo-binding protein (Mop) in *C. pasteurianum*, suspected to be involved in Mo metabolism, was purified and biochemically characterized, and the *mop* gene was cloned and sequenced. Our studies have shown that the isolated Mo protein contains Mo as well as a pterin-like fluorescent chromophore. Spectroscopic analysis indicates that the Mo environment and the pterin-derivative associated with Mop are similiar to the components of the Mo-co isolated from clostridial formate dehydrogenase. The Mo-co dissociated from formate dehydrogenase was used to reconstitute the activity of Mo-co-deficient nitrate reductase (biological assay for catalytically active Mo-co). The molybdopterin species dissociated from Mop was not active in the Mo-co reconstitution assay. The results suggest that the catalytically inactive molybdopterin species associated with Mop might be a precursor to Mo-co.

We identified an expression clone in *Escherichia coli* cross-reacting with Mop antibodies by immunoscreening genomic libraries of *C. pasteurianum*. Western blot analysis showed that the clone produced a protein recognized by the antibody with the same effective electrophoretic mobility as purified Mop. DNA sequence analysis of the expression clone revealed an open reading frame coding for 67 amino acids (m.w. ~8000). The aminoterminal amino acid sequence of the protein showed perfect alignment with the first 12 codons in the open reading frame. Amino acid sequence analysis also showed that there appear to be two amino acid residues at six different positions. The DNA sequence predicts only one of the amino acid residues identified at each of these positions. This ambiguity suggests that there are two or more related proteins in the purified protein preparation. Southern blot analysis of *C. pasteurianum* chromosomal DNA showed that the original (Mop) clone hybridized to two DNA

fragments. The results indicate that the *mop* gene has been duplicated and the two variant Mo proteins copurify. We have cloned and are presently sequencing what appears to be the second copy of the *mop* gene.

Amino acid sequence analysis of Mop maintained under anaerobic conditions showed that at positions where the DNA sequence predicted a glutamic acid residue, an amino acid phenylthiohydantoin (PTH) derivative was identified that did not correspond to any known standard. The modified glutamic acid appears to be oxygen-sensitive because glutamic acid PTH was identified in the positions predicted by the gene sequence when the protein was not handled under anaerobic conditions. It is interesting to note that Mo binding to Mop was also oxygen-sensitive. Currently, we are determining the structure of the glutamic acid derivative by fast-atom bombardment mass spectroscopy and nuclear magnetic resonance analysis.

Biogenesis of the Molybdenum Cofactor in *Escherichia coli*

S. Hinton, S. Johann

The molybdenum cofactor, Mo-co, common to a variety of molybdoenzymes, contains an as yet undefined pterin derivative associated with a molybdenum (Mo) atom. The products of at least five genes (*chl A*, *B*, *D*, *E*, and *G*) in *Escherichia coli* are needed for Mo-co biogenesis. It has been proposed that the gene products of *chlA* and *chlE* are involved early in Mo-co synthesis, possibly modifying the pterin. The *chlD*, *chlG*, and *chlB* gene products are thought to be involved in stabilizing the pterin-derivative in the absence of Mo, inserting Mo into the cofactor, and inserting the Mo-co into the apoenzyme, respectively. Our goal is to understand the biology and bioinorganic chemistry in the transformation of molybdate, $MoO_4^{=}$, into Mo-co. To achieve this end, we are studying the role the *chl* gene products have in Mo metabolism by identifying the gene products, determining the DNA sequence of the genes, and studying the effect each *chl* mutant has on Mo accumulation in *E. coli*.

Genomic libraries of *E. coli* DNA were constructed in a pUC expression vector, several clones that complemented the *chl* (Mu insertion) mutants were identified, and complementation was shown to be dependent on the inserts of *E. coli* DNA. Southern hybridization analysis showed that the clones contained fragments of *E. coli* DNA that have sequence homology with chromosomal DNA flanking the Mu insertion in their respective *chl* mutant. Maxicell experiments showed that the plasmids which conferred complementation encoded proteins with apparent molecular weights of 24,000 and 10,000 with the *chlB* clone, 26,000 with the *chlD* clone, 44,000 and 23,000 with the *chlE* clone, and 25,000 and 6,500 with the *chlG* clone. Restriction analysis, subcloning, and site-specific mutagenesis have been performed to localize the *chl* genes further for future maxicell and DNA-sequencing experiments. DNA-sequencing analyses of the *chlD* and *chlG* genes are in progress.

Radioisotope ^{99}Mo-labeling experiments in conjunction with native (anaerobic) multiphasic gel electrophoresis are being used to identify the molybdoproteins in crude extracts of *E. coli*. We are interested in determining whether any of the *chl* gene products are molybdoproteins possibly binding a precursor of Mo-co.

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CELL BIOLOGY

The focus of the Cell Biology Group has slowly evolved from solely the analysis of cell structure to the analysis of cell growth control. During the past year, virtually all of our work involved some aspect of normal- or transformed-cell growth-control pathways. The biochemical and cell biological roles of the *ras* proteins have been studied in great detail. Analysis of the effects of adenovirus E1A proteins and genes and those of the human oncogene, c-*myc*, in living cells has just begun. Analyses of these proteins and their effects on cells are also being studied by computerized two-dimensional gels. The role and properties of protein kinase C (and of the inositol phospholipids) in cellular functions are also areas of active research by the Group. Investigations into the molecular components of the heat shock or stress response of cells, which characteristically leads to a temporary shutoff of growth, are continuing at a fast pace. In addition, cloning, genetic organizational studies, and protein biochemical analysis of the actin-binding proteins, the tropomyosins, are being pursued with vigor. The expression of the tropomyosins is rapidly and dramatically changed during the transformation process. Progress is being made, but the complexity of the molecular events that must be involved in mammalian cell-growth control is so great that the field will continue at a high pitch here and elsewhere for some time.

GENES FOR MAJOR STRUCTURAL PROTEINS

D.M. Helfman Y. Yamawaki-Kataoka
L. Finn

Isolation and Characterization of cDNA Clones Encoding Nonmuscle Tropomyosin

Y. Yamawaki-Kataoka, L. Finn, D.M. Helfman

Rat embryonic fibroblasts contain 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 ($M_r = 32{,}000$). The structure and functional significance of the different forms of tropomyosin in nonmuscle cells remain to be determined. In addition, recent studies have indicated significant alterations in the pattern of tropomyosins expressed in cells transformed by various DNA and RNA tumor viruses. These alterations in tropomyosin expression may in part be responsible for the reduction of microfilament bundles (stress fibers) and alterations of cell shape characteristic of transformed cells. The mechanism(s) responsible for the altered patterns of tropomyosin synthesis in transformed cells is unknown. With these questions in mind, we have been studying tropomyosins in nonmuscle cells (rat embryonic fibroblasts) and the genes that encode these proteins. To isolate cDNA clones encoding nonmuscle tropomyosins, we constructed a rat embryonic fibroblast cDNA expression library using the plasmid expression vectors pUC8 and pUC9. Using both an immunological screening procedure and ^{32}P-labeled cDNA probes, we have identified and isolated cDNA clones encoding three of the five tropomyosins found in rat embryonic fibroblasts. By hybrid-selection translation and analysis of the translation products by one- and two-dimensional gel electrophoresis, we have identified clones that contain sequences complementary to TM-1, TM-2, and TM-

4. Northern blot analysis indicated transcripts of 1.1, 1.8, and 2.2 kb for TM-1, TM-2, and TM-4, respectively. We have identified overlapping clones for the entire coding region of rat TM-1 and have determined the complete amino acid sequence of the protein deduced from nucleic acid sequence analysis (see below). In addition, we have sequenced cDNA clones encoding a portion of TM-2 and TM-4. Comparison of the nucleic acid sequences of clones for TM-1, TM-2, and TM-4 indicates differences in their coding regions, although their predicted amino acid sequences show more than 85% homology. Southern blot analysis of rat DNA showed several unique fragments hybridizing to each probe made from cDNA clones encoding TM-1, TM-2, or TM-4. These results indicate that these proteins are encoded by separate but related genes and that tropomyosin species present in rat embryonic fibroblasts are encoded by at least three separate genes. We are currently analyzing genomic clones that encode nonmuscle tropomyosins (see below).

Rat Embryonic Fibroblast Tropomyosin 1: cDNA and Complete Primary Amino Acid Sequence

Y. Yamawaki-Kataoka, D.M. Helfman

A first step in understanding the molecular basis for the differences in the various forms of tropomyosin in rat embryonic fibroblasts will be a determination of their primary structure. Using overlapping cDNA clones for TM-1, we have determined the sequence of the entire coding region of 852 bp, 61 bp of 5′-untranslated sequences, and 73 bp of the 3′-untranslated region (Yamawaki-Kataoka and Helfman, *J. Biol. Chem.* [1985] submitted). Since the 3′-untranslated sequence contains a poly(A) track and the polyadenylation signal AATAAA is found 14 bp from the poly(A) track, we conclude that our clones contain the complete 3′-untranslated sequence. Thus, TM-1 has a relatively short (73 bp) 3′-untranslated region. Comparison of the deduced amino acid sequence of the rat TM-1 with those of tropomyosins isolated from chicken smooth muscle, chicken and rabbit skeletal muscle, and equine platelet indicates extensive homology among the various tropomyosin isoforms. TM-1, like smooth- and skeletal-muscle tropomyosins, was found to contain 284 amino acids. On the other hand, platelet tropomyosin has been reported to contain only 247 amino acids. The tropomyosin most homologous to rat TM-1 is chicken smooth-muscle α-tropomyosin (95% homology); in contrast, rat TM-1 shows only 77% and 78% homology with skeletal-muscle and platelet tropomyosins, respectively. Interestingly, TM-1, smooth-muscle, and platelet tropomyosins were found to be highly homologous at their carboxyterminal domains, compared with skeletal-muscle tropomyosins. Whether all nonmuscle and smooth-muscle tropomyosins share this feature remains to be determined. In this respect, we have also determined the sequence of the carboxyterminal end of rat embryonic fibroblast TM-4. This sequence is also highly homologous to TM-1, smooth-muscle, and platelet tropomyosins. These differences in sequence correlate well with work from other laboratories showing that skeletal-muscle troponin T binds strongly to the carboxyterminal end of skeletal-muscle tropomyosin. In contrast, troponin T interacts poorly with smooth-muscle and nonmuscle tropomyosins. Work is currently under way to isolate and characterize cDNA clones encoding the other nonmuscle tropomyosins.

A Single Rat Tropomyosin Gene Specifies Two Different Tissue-specific mRNAs

D.M. Helfman, L. Finn

To isolate the gene encoding rat embryonic fibroblast tropomyosin 1 (TM-1), we screened a rat genomic library for sequences complementary to our cloned tropomyosin cDNAs. Analysis of genomic clones indicates there are two separate loci in the rat genome that contain sequences complementary to TM-1. One appears to contain a functional gene and the other represents a processed pseudogene. The functional gene is approximately 10 kb long. Northern blot analysis

using ^{32}P-labeled cDNA probes encoding TM-1 revealed that rat embryonic fibroblasts and smooth muscle (stomach or uterus) contain a single transcript of 1.1 kb. In contrast, skeletal muscle contains a single transcript of 1.3 kb. S1 protection experiments indicate that the skeletal-muscle transcript differs from both the fibroblast and smooth-muscle messages only at the 3′ end (corresponding to a region from approximately amino acid 190 to 284). This is supported by sequence comparisons demonstrating that skeletal-muscle β-tropomyosin is virtually identical with rat TM-1 from amino acid 1 to 190, but differs substantially at the carboxyterminal end. We have now identified two distinct exons at the 3′ end of the gene; one is specific for the 1.1-kb transcript in rat embryonic fibroblasts and smooth muscle and the other is specific for the 1.3-kb transcript in skeletal muscle. In addition, there are at least two exons common to the 1.1-kb and 1.3-kb transcripts. Thus, a single gene encodes two different transcripts, whose expression is regulated by a tissue-specific mechanism. We are continuing to characterize the genomic clones fully and plan to study *cis*- and *trans*-acting elements involved in the regulation of this gene.

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BIOLOGY OF THE CYTOSKELETON

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Physical and Chemical Properties of Normal- and Transformed-cell Tropomyosin

F. Matsumura, S. Yamashiro-Matsumura

We have previously shown that multiple forms of tropomyosin are differentially expressed upon many types of oncogenic transformation (Matsumura et al., *J. Biol. Chem. 258*: 13954 [1983]; Lin et al., *Cancer Cells 1:* 57 [1984]). Generally, the levels of tropomyosin with higher apparent molecular weights (prominent in normal cells) are decreased in transformed cells, whereas the levels of tropomyosin with lower apparent molecular weights (minor tropomyosins in normal cells) become predominant. Of the five forms of rat cultured-cell tropomyosin (TM-1–TM-5) (Matsumura et al., *J. Biol. Chem. 258*: 6636 [1983]), TM-1 (M_r = 40,000) and TM-2 (M_r = 36,000) are high-molecular-weight tropomyosins (prominent in normal cells) and TM-4 (M_r = 32,400) and TM-5 (M_r = 32,000) are low-molecular-weight tropomyosins (prominent in transformed cells). To elucidate how the changes in tropomyosin expression affect the altered organization of microfilaments observed in transformed cells, we have examined the physical and chemical properties of these normal- and transformed-cell tropomyosins. We have also tested whether the changes in tropomyosin pattern in microfilaments alter the interactions between actin and other actin-bind-

ing proteins, which may cause the alteration in the assembly state of microfilaments in transformed cells.

Five forms of rat cultured-cell tropomyosins are found to share many physical and chemical properties, including (1) similar isoelectric focusing points (pI ~4.5), (2) heat stability, (3) similar amino acid compositions, (4) homodimer formation, (5) rod-like molecular shapes, and (6) cross-reactivities to polyclonal antibodies to muscle or nonmuscle tropomyosin. However, we have found several important differences in the properties of high-molecular-weight tropomyosins (TM-1, TM-2, and TM-3) and low-molecular-weight tropomyosins (TM-4 and TM-5).

First, these tropomyosin variants show different extents of polymerization. Table 1 shows the Stokes' radii and sedimentation coefficients of the five forms of tropomyosin measured by gel filtration and by sucrose density gradient centrifugation, respectively. Low-molecular-weight tropomyosins (TM-4 and TM-5) show a lesser extent of salt-dependent polymerization than high-molecular-weight tropomyosins (TM-1, TM-2, and TM-3). The Stokes' radius of high-molecular-weight tropomyosins increased from 7.5 nm to 12.3 nm when the NaCl concentration was lowered from 0.6 M to 0.1 M, whereas that of low-molecular-weight tropomyosins changed from 6.2 nm to 8.2 nm. The sedimentation coefficients also showed the difference in salt dependency between high- and low-molecular-weight tropomyosins. With a decrease of NaCl concentrations, the sedimentation coefficients of high-molecular-weight tropomyosins changed from 3.4 to 3.7, whereas those of low-molecular-weight tropomyosins remained constant ($S_{20,w}$ = 3.4). These results were also confirmed by electron microscopy using a rotary-shadowing technique. Although rod-like molecules (~40 nm long; characteristic of tropomyosin) were observed in specimens from both kinds of tropomyosins, linear polymers as long as 500 nm were frequently seen only in specimens from high-molecular-weight tropomyosins.

Second, we have found that high-molecular-weight tropomyosins show a higher affinity to actin than low-molecular-weight tropomyosins (Fig. 1). The lower actin-affinity and the lower polymerizability of the low-molecular-weight tropomyosins (prominent in transformed cells) suggest that these tropomyosins do not stabilize the structure of microfilaments in transformed cells as high-molecular-weight tropomyosins do in normal cells. Such changes in stability may result in the disorganization of actin cables observed in transformed cells.

We next examined if different forms of tropomyosin affect the activity of other actin-binding proteins. For this purpose, we first chose a new actin-binding 55-kD protein purified from HeLa cells because this protein makes actin filaments aggregate into bundles (Yamashiro-Matsumura and Matsumura, *J. Biol. Chem.* [1985] in press) and may function as a structural component of the actin bundles present in stress fibers

TABLE 1 Physical Properties of Tropomyosin Variants

	TM-1, TM-2, TM-3		TM-4, TM-5	
	0.1 M NaCl	0.5 M NaCl	0.1 M NaCl	0.5 M NaCl
Stokes' radius (nm)	12.3	7.5	8.2	6.2
Sedimentation coefficient	3.7	3.4	3.4	3.4
M, on SDS gels	40K-35K		32K	

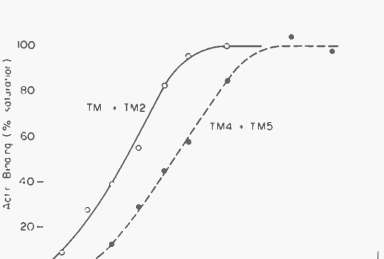

FIGURE 1 Actin binding of tropomyosin variants. Actin (1 mg/ml) was incubated with different isoforms of tropomyosin at the indicated concentrations and ultracentrifuged at 140,000*g* for 20 min. Both supernatants and pellets were analyzed by SDS-PAGE. The amounts of tropomyosin bound to actin were quantitated by densitometry and plotted as a percentage of full binding. The actin affinity of high-molecular-weight tropomyosins (TM-1 and TM-2) is higher than that of low-molecular-weight tropomyosins (TM-4 and TM-5).

or philopodia. The bundling activity of the 55-kD protein was measured by low-speed centrifugation (Fig. 2, lane 1), where bundles of actin filaments are pelleted (lane 1b) while free actin remains in the supernatant (Fig. 2, lane 1a). Actin was first complexed with either high- or low-molecular-weight tropomyosins and then the 55-kD protein was added. When the 55-kD protein was mixed with the actin/TM-4/TM-5 complex, it chased out TM-4 and TM-5 from actin and made bundles of actin filaments free of tropomyosin (Fig. 2, lane 2). In contrast, when the 55-kD protein was mixed with the actin/TM-1/TM-2/TM-3 complex, it made bundles of actin filaments containing these tropomyosins (Fig. 2, lane 3). We have further examined the effect of skeletal-muscle tropomyosin on bundling activity. Interestingly, skeletal-muscle tropomyosin inhibited the bundling activity of the 55-kD protein. As lane 4 shows, most actin remains in the supernatant (lane 4a).

The modification of the interaction between actin and the 55-kD protein by the multiple forms of tropomyosin may be involved in the regulation of microfilament assembly. We are now studying whether multiple forms of tropomyosin change the interaction of actin with other types of actin-binding protein (actin-filament-severing protein such as gelsolin).

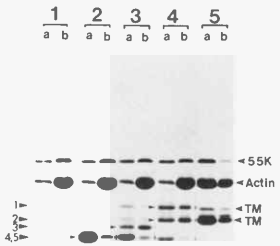

FIGURE 2 The interaction between the 55-kD protein and different forms of tropomyosin. Actin (1 mg/ml) was first complexed with different forms of tropomyosin (0.5 mg/ml) and then the 55-kD protein (0.2 mg/ml) was added. The activity of actin bundling was examined by low-speed centrifugation (12,000*g* for 15 min). Both pellets (lane *a*) and supernatants (lane *b*) were analyzed on SDS-polyacrylamide gels. (*1*) Control without tropomyosin (actin bundles were precipitated with the 55-kD protein [lane *b*]); (*2*) actin plus TM-4 and TM-5; (*3*) actin plus TM-3, TM-4, and TM-5; (*4*) actin plus TM-1 and TM-2, (*5*) actin plus rabbit skeletal-muscle tropomyosin. Note that TM-4 and TM-5 were chased out by the 55-kD protein, whereas TM-1 TM-2, and TM-3 bind to actin bundles made with the 55-kD protein. Skeletal-muscle tropomyosin, in contrast, inhibits the bundling activity of 55-kD protein

Intracellular Localization of a New Actin-bundling 55-kD Protein in Cultured Cells

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Last year, we reported the purification and characterization of a new actin-bundling 55-kD protein from HeLa cells (Yamashiro-Matsumura and Matsumura, *J. Biol. Chem.* [1985] in press). To determine the intracellular localization of this protein in cultured cells, we have made both polyclonal and monoclonal antibodies to the 55-kD protein. Indirect immunofluorescence using both polyclonal and monoclonal antibodies shows the predominant localization of this protein in philopodia and retraction fibers, as well as in stress fibers (Fig. 3B).

The 55-kD protein is very similar to fimbrin in that both proteins are actin-bundling proteins and show similar localization in cultured cells. Although biochemical characterization suggests that they are different proteins, we have tested the 55-kD protein by immunoblot analysis to determine if it is immunologically related to fimbrin. Anti-55-kD protein antibody was reactive with the 55-kD protein but not with fimbrin. Likewise, anti-fimbrin antibody reacted with fimbrin but not with the 55-kD protein. Furthermore, indirect immunofluorescence of chemically transformed human fibroblasts double-stained with polyclonal anti-fimbrin and with monoclonal anti-55-kD protein showed similar but different localization. Although both antibodies stained microspikes, the anti-55-kD protein antibody stained the stress fibers, but the anti-fimbrin staining was very weak. These results indicate that the 55-kD protein and fimbrin are not related to each other

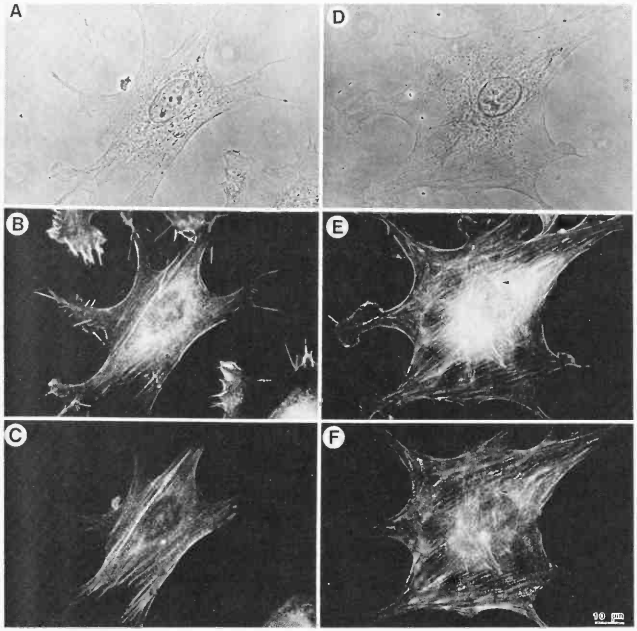

FIGURE 3 Phase-contrast (*A,D*) and immunofluorescent (*B,C,E,F*) micrographs of CCL-gerbil fibroma cells double-stained with mouse monoclonal antibody to the 55-kD protein (*B,E*) and with rabbit polyclonal antibody to tropomyosin (*C,F*). Arrowheads in *E* and *F* show the center of polygonal networks, where the 55-kD protein is present but tropomyosin is absent.

and that both proteins are structural components of microspikes in cultured cells.

As described above, the interaction of 55-kD protein with actin is modified by tropomyosin. The localization of the 55-kD protein was therefore compared with that of tropomyosin by double-labeled immunofluorescence, using mouse monoclonal antibody against the 55-kD protein and rabbit polyclonal antibody against tropomyosin. The staining patterns of these two antibodies are different, although both antibodies stained the same stress fibers (Fig. 3). The staining patterns of stress fibers are different in the fine structure. While tropomyosin antibody gives clear periodic staining of stress fibers, such periodic staining is not as clear with the anti-55-kD protein antibody. Furthermore, as Figure 3, E and F, shows, the center (arrow) of polygonal networks is stained with the anti-55-kD protein antibody but not with the anti-tropomyosin antibody.

It is tempting to speculate that the 55-kD protein, together with the differential expression of tropomyosin isoforms, regulates the microfilament assembly in normal and transformed cells. Immunofluorescence shows that tropomyosin is localized in stress fibers but is absent in the movable portions of cells, such as ruffling membranes and microspikes. This observation, coupled with the ability of this protein to stabilize

actin filaments, suggests that tropomyosin may regulate the organization of microfilaments into stress fibers. In normal cells with well-spread morphology, where high-molecular-weight tropomyosins are predominant, the 55-kD protein probably makes bundles of actin filaments containing high-molecular-weight tropomyosins. These tropomyosin-containing bundles may be stable enough to form stress fibers. In contrast, in transformed cells where high-molecular-weight tropomyosins are substituted by low-molecular-weight tropomyosins, the 55-kD protein chases out the tropomyosin from actin and makes actin bundles without tropomyosin. Such tropomyosin-free bundles may not be stable enough to form stress fibers but may be used for the formation of microspikes that are prominent in many transformed cells. We are currently examining this hypothesis through the combined use of biochemical, cell biological, and immunological methods.

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BIOCHEMISTRY OF TRANSFORMING PROTEINS

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Intrinsic GTPase Activity of the *ras* Proteins: A Biochemical Analogy to the "G" Protein Family

T. Kamata, G. Binns, J.R. Feramisco [in collaboration with R.W. Sweet, S. Yokoyama, M. Rosenberg, and M. Gross, Smith, Kline and French Laboratories, Pennsylvania]

Ha-*ras* is a member of a multigene family in man that encodes highly related proteins of 189 amino acids (p21). In vitro, *ras* proteins bind GTP and p21 mutants with threonine at position 59 and autophosphorylate at that residue. Mutation (at amino acids 12 or 61) and elevated expression of *ras* genes result in cell transformation in culture and are also observed in many types of human tumors. Normal and mutant transforming *ras* proteins show no differences in localization, lipidation, or GTP binding. However, mutations at position 12 in recombinant (Thr-59) p21 molecules were observed to affect autophosphorylation. We have expressed the full-length normal and T24 transforming (Gly→Val at position 12) Ha-*ras* proteins in *Escherichia coli* and have pu-

rified them to homogeneity; these proteins bound GTP with approximately molar stoichiometry and with an affinity comparable to that of partially purified mammalian proteins. Microinjection of the T24 protein into quiescent rodent fibroblasts resulted in a rapid alteration in cell morphology, stimulation of DNA synthesis, and cell division; in contrast, little response was observed with the normal protein. We now report that the normal *ras* protein has an intrinsic GTPase activity, yielding GDP and P_i, whereas the T24 transforming protein is reduced tenfold in this activity (Fig. 1). We suggest that this deficiency in GTPase is the probable cause for the transforming phenotype of the T24 protein.

The tenfold reduction in GTPase activity of the T24 protein represents the first major biochemical differentiation between the products of a normal and a transforming mutant *ras* gene. It is tempting to speculate on the consequence of this altered activity by drawing a functional analogy to transducin and the G stimulatory protein—both of these proteins are activators of enzymes affecting cyclic nucleotide pools. This activation requires GTP binding (or exchange of GTP for bound GDP) and appears to terminate on hydrolysis to GDP. If the *ras* proteins have an analogous regulatory function in whichever proliferative control pathway they participate, then the deficiency in GTPase activity of the T24 mutant could result in prolonged stimulation of an otherwise regulatable activity. As shown for the G stimulatory protein, perhaps modulation of *ras* activity itself occurs through interactions with other proteins, and these interactions may be sensitive to whether GTP and GDP (or neither) occupies the nucleotide binding site.

Molecular Characterization of Ha-*ras* Proteins: Proteolytic Substructure

D. Bar-Sagi, J.R. Feramisco

Oncogenic *ras*-encoded proteins differ from their normal homologs by an amino acid substitution for Gly-12, Ala-59, or Gln-61. To provide insight into the structural requirements involved in the oncogenic activation of *ras* proteins, we have initiated studies concerning the characterization of their structural and functional domains. From this analysis, we hope to build a framework to study the function of *ras* proteins.

We have purified to homogeneity the protooncogenic and oncogenic (T24) forms of Ha-*ras* proteins produced in *Escherichia coli*. Both proteins were subjected to digestion by trypsin under mild conditions. Figure 2A displays the products of limited tryptic digestion of the proteins at different times during proteolysis. The results indicate a progressive disappearance of the intact Ha-*ras* proteins (24 kD) and the appearance of 23.5-kD and 21-kD polypeptides. The 23.5-kD polypeptide is likely to be an intermediate in the hydrolysis of Ha-*ras* protein as its appearance precedes that of the 21-kD polypeptide. It then declines in amount, and by 2 hours it has vir-

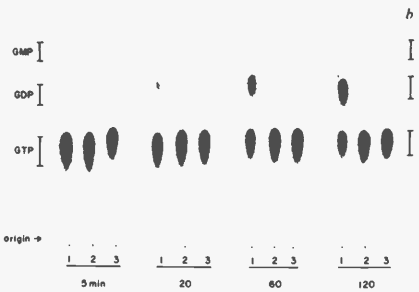

FIGURE 1 GTP hydrolysis catalyzed by the *ras* proteins. PEI-cellulose thin-layer analyses of incubations of [α-^{32}P]GTP with the normal *ras* protein (lane *1*), with the activated oncogene *ras* protein (lane *2*), or with BSA (lane *3*) are shown. Notice the low GTPase activity of the oncogene protein.

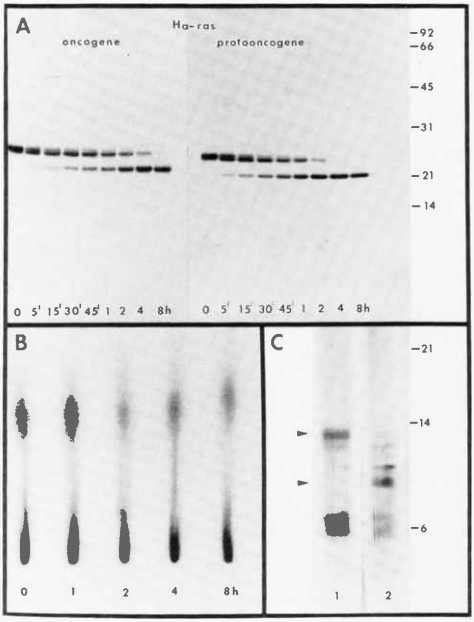

FIGURE 2 (*A*) Time course of proteolysis of Ha-*ras* protein by trypsin. Purified Ha-*ras* proteins (normal and oncogenic) were digested with trypsin at a ratio of enzyme to substrate of 1:100. The digestion was terminated at the indicated intervals by boiling the samples in SDS-solubilizing buffer. The samples were electrophoresed on a 17% SDS gel and polypeptides were visualized by Coomassie blue stain. (*B*) Effect of tryptic digestion of normal Ha-*ras* protein on the GTPase activity. The conditions for proteolysis were the same as those described in *A*. At the times indicated, the proteolysis was terminated by the addition of trypsin inhibitor. Aliquots from each sample were incubated with [α-^{32}P]GTP for 1 hr and analyzed for GTPase activity by thin-layer chromatography. (*C*) V8 protease cleavage of the intact Ha-*ras* protein (*1*) and the trypsin-resistant fragment (*2*). Samples were digested with 5 μg of V8 protease for 90 min. The cleavage products were resolved on 17% SDS gel, and polypeptides were visualized by silver stain.

tually disappeared from the digestion mixture. The 21-kD polypeptide accumulates during the digestion, accounting for approximately 90% of the stained material on the gel, and is resistant to further cleavage even at higher concentrations of the enzyme. Recently, we and others have observed that normal *ras* protein exhibits GTPase activity that is significantly reduced by the oncogenic substitution Val-12. As shown in Figure 2B, the 21-kD trypsin-resistant fragment of the normal *ras* protein retains GTPase activity. To determine which portion of the Ha-*ras* protein is represented in the 21-kD trypsin-resistant fragment, we analyzed the protein by hydrolysis with *Staphylococcus aureus* V8 protease. Cleavage of the Ha-*ras* protein with V8 protease generates a major 13-kD peptide that is derived from the carboxyterminal half of the molecule. Figure 2C shows that the major V8 peptide of the trypsin-resistant fragment is smaller than the V8 peptide of the intact protein by approximately 3 kD. It therefore appears that the 21-kD proteolytic resistant fragment is derived from the Ha-*ras* protein by the loss of the 3-kD fragment from the carboxy terminus of the molecule.

In summary, we have demonstrated that the carboxyterminal 3 kD of Ha-*ras* protein is not essential for the catalytic activity of the protein. Our results indicate that the catalytic domain of the protein has a conformation that confers protection against limited conditions of hydrolysis. In view of the fact that the Ha-*ras* protein is associated with the plasma membrane, it is intriguing to speculate that sequences located within 3 kD of the carboxyl terminus have a structural role in membrane anchorage of the protein, whereas

the GTPase activity is carried by a portion of the amino terminus.

Microinjection of the *ras* Oncogene Protein into Quiescent Cells Results in Rapid Proliferation

J.R. Feramisco, N. Kronenberg, C. Hallaran, G. Binns, T. Kamata [in collaboration with M. Gross, M. Rosenberg, and R.W. Sweet, Smith, Kline and French Laboratories, Pennsylvania]

Although the function of the *ras* proteins is not yet known, several observations concerning their possible role in cell proliferation have been described. Epidermal growth factor or insulin stimulates the GTP-dependent phosphorylation of the murine v-*ras* protein and the guanine-nucleotide-binding activity of the *ras* proteins in *ras*-transformed cells or membranes derived thereof. A second observation is that (with only limited information available) there is some structural homology between the *ras* proteins and the G protein, which is known to regulate the hormone-sensitive adenylate cyclase activity. These observations suggest that the *ras* proteins may be involved in growth-factor receptor activity, be it at the level of the receptor(s) itself or in the signal transduction that may follow receptor activation with a mechanism that may employ a G-like activity, and/or may be directly regulating the adenylate cyclase system as deduced by M. Wigler and his colleagues (Molecular Genetics of Eukaryotic Cells Section).

To study the biochemical and biological activities of the human protooncogenic and oncogenic forms of the Ha-*ras* proteins, we have begun to examine the effects of the purified Ha-*ras* proteins in living, normal cells by needle microinjection. Here, we show that the oncogenic form of the human Ha-*ras* protein (T24) produced in and purified from *Escherichia coli* causes rapid morphological changes and induces cell division when injected into quiescent, contact-inhibited cells (see Fig. 3). In certain established cell lines, the protein completely obviates the need for growth factors in the proliferation process. In contrast, microinjection of the wild-type protooncogenic form of Ha-*ras* exhibits little, if any, effect on these cells.

Cooperation of the *ras* Oncogene Protein with the Adenovirus E1A Oncogene Protein following Microinjection into Quiescent Nonestablished Cells

J.R. Feramisco, D. Bar-Sagi, W.J. Welch, T. Kamata [in collaboration with N.F. Sullivan, R.W. Sweet, T.L. Chao, B.Q. Ferguson, M.S. Gross, and M. Rosenberg, Smith, Kline and French Laboratories, Pennsylvania]

We have shown that early-passage nonestablished rat embryo fibroblasts respond to microinjection of *Escherichia coli*-made *ras* oncogene protein by synthesizing DNA but not undergoing division. This effect is specific to *ras* oncogene protein since no response is observed upon injection of comparable levels of the *ras* protooncogene protein. In contrast, microinjection of *ras* oncogene protein into late-passage REF cells caused both DNA synthesis and cell division. Early-passage REF cells could be stimulated to divide in response to oncogenic *ras* by either preincubation in conditioned medium from late-passage cells or coinjection experiments using the adenovirus E1A 13S mRNA gene product in combination with *ras* oncogene protein. The localization of the two proteins in single cells after microinjection is shown in Figure 4.

Of the two known growth-factor pathways, i.e., competence as induced by platelet-derived growth factor (PDGF) and progression as induced by epidermal growth factor (EGF), insulin, and somatomadin C, only the progression pathway can be blocked by cycloheximide. Since cycloheximide blocks DNA synthesis in response to microinjected oncogenic *ras*, we believe that the *ras* protein must be involved in the progression pathway of cell growth. In addition, *ras* must act before the proposed restriction point between G_1 and S, since after this point DNA synthesis cannot be blocked by cycloheximide. Furthermore, since oncogenic *ras* stimulates DNA synthesis with only trace amounts of serum, it can apparently bypass the need for exogenous competence and progression factors. Previous studies have indicated that EGF and insulin treatment of cell membranes leads to effects on the guanine-nucleotide-binding activity of the *ras* protein, observations that tend to support some role for *ras* in these growth-factor pathways.

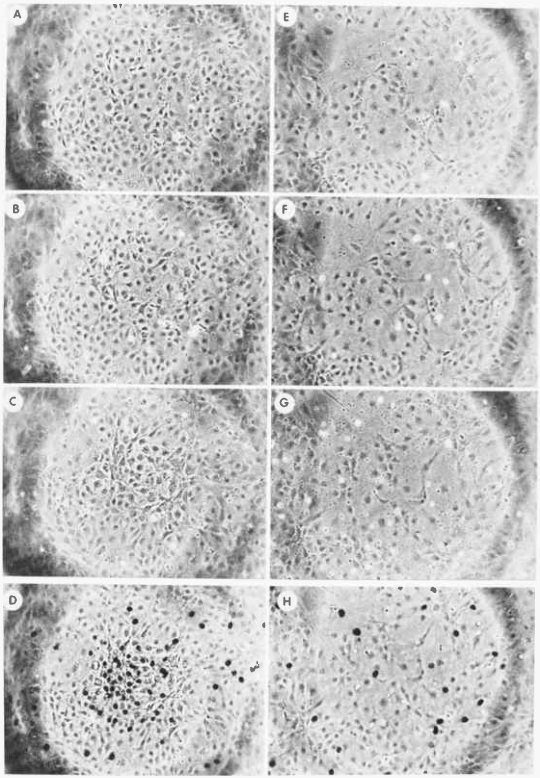

FIGURE 3 Stimulation of DNA synthesis and cell proliferation by microinjected *ras* oncogene protein. Confluent NIH-3T3 cells were injected with the activated *ras* oncogene protein (*A–D*) or with the normal *ras* protein (*E–H*) and incubated with [^{3}H]thymidine. The fields were photographed at 0, 8, and 24 hr postinjection (*A,B,C* or *E, F,G*, respectively). At 24 hr the cells were fixed, processed for emulsion autoradiography, and rephotographed (*D* or *H*, respectively). Notice the proliferation induced by the oncogene form of the *ras* protein.

Most strikingly, our microinjection studies have shown that late-passage REF cells are more responsive to *ras* oncogene protein than their early-passage counterparts. This may be due to the de novo secretion of a growth factor(s) induced by continued passage. We have not yet classified this molecule(s), but it might be similar to β-TGF, since microinjection of *ras* oncogene protein into early-passage cells grown in serum-free media supplemented with EGF and β-TGF results in cell proliferation. These results suggest that upon removal from native tissue culture in vitro, the cells

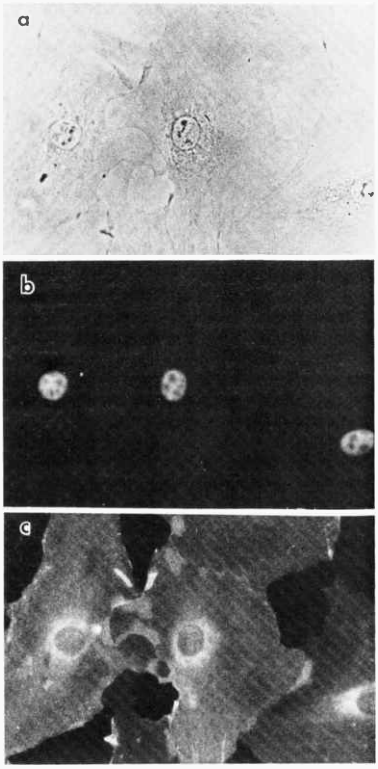

FIGURE 4 Localization of the adenovirus E1A protein and the *ras* oncogene protein after comicroinjection into living cells. Purified adenovirus E1A protein and the *ras* oncogene protein (both purified from *E. coli* after expression) were comicroinjected into primary rat embryo fibroblasts. Two hours later, the cells were fixed and analyzed by double-label immunofluorescence to detect the E1A protein (*b*) or the *ras* protein (*c*).

immediately begin to change with respect to their growth-responsive properties and continue to change as passage number increases. Whether this reflects a decrease in the cellular division potential remains to be determined. Our results suggest a possible explanation for previous results which indicated that rat embryo fibroblasts can be transformed at low frequency by overexpression of the *ras* oncogene alone. Perhaps complete transformation of REF cells in tissue culture (even at early passage) may result from cooperation between oncogenic *ras* and the induced growth-factor response described here.

Inactivation of the *ras* Oncogene Protein in Living Transformed Cells via Antibody Microinjection

J.R. Feramisco [in collaboration with R. Clark, G. Wong, N. Arnheim, R. Milley, and F. McCormick, Cetus Corporation, California]

To obtain a biochemical probe capable of distinguishing the normal and oncogenic forms of the *ras* proteins, antibodies were raised (by the Cetus group) against *ras* (p21)-related peptides that differed at position 12. The antibody designated anti-p21-ser was affinity-purified from the serum of rabbits immunized with the peptide Lys^{6}-Leu-Val-Val-Gly-Ala-Ser^{12}-Gly-Val-Gly-Lys^{16}-Cys-Ser (serine at position 12) covalently coupled to carrier protein. The production and characterization of this antibody will be described in detail by R. Clark et al. (in prep.). Anti-p21-ser was found to bind the v-Ki-*ras* protein (serine at position 12) but not the v-Ha-*ras* protein (arginine at position 12) or the *ras* protein containing glycine (normal) at position 12. In addition, biochemical studies showed that this antibody blocked both the GTP-dependent autophosphorylation and GTP-binding activities of the v-Ki-*ras* protein, suggesting that the antibody and GTP bind to the same region of the protein.

With this high degree of specificity displayed by the affinity-purified antibody preparation toward the oncogenic v-Ki-*ras* protein, as well as the ability of the antibody to inhibit the GTP-binding activity of the v-Ki-*ras* protein, it seemed possible that introduction of this antibody preparation into living, transformed cells would block the biochemical action of the *ras* oncogene protein in vivo, without affecting the function of the protooncogene product. Using microneedle injection procedures, we injected approximately 5×10^5 molecules of purified IgG (either anti-p21-ser or nonimmune) into the cytoplasm of NRK

cells transformed by the v-Ki-*ras* oncogene (called Ki-NRK cells). This represents a five- to tenfold excess of antibody over the estimated amount of the *ras* protein in these cells. Within 15 hours, and persisting for 48 hours after injection of the anti-p21-ser antibodies, the Ki-NRK cells showed a flattened, normal morphology, and after 48 hours, the cells eventually returned to their rounded morphology. In contrast, cells on the same dish injected with control IgG showed no obvious change in morphology. (Control IgGs included goat anti-mouse IgG and rabbit nonimmune IgG.) Inspection of the photographs taken from these types of experiments indicated that in addition to the reversion of the transformed phenotype, the flattened cells appeared to grow at a slower rate than cells injected with control IgG or in surrounding uninjected cells. Considering the data from the genetic analysis of the yeast *ras* genes which indicated that complete elimination of *ras* gene expression leads to nonviable cells (see M. Wigler et al., Molecular Genetics of Eukaryotic Cells Section), we might predict that antibodies that block the function of both the normal and oncogenic forms of the *ras* proteins would kill or prevent growth of microinjected, transformed mammalian cells. However, the cells injected with the anti-p21-ser preparation are capable of growth, a result that is in keeping with the biochemical experiments which showed that this antibody only binds to the oncogenic form of the *ras* protein with serine at position 12.

By utilizing immunofluorescence microscopy, we determined exactly which cells were inoculated with the two antibody preparations. An exact correlation between the cells inoculated with the anti-p21-ser preparation and the morphologically normal, flattened cells was seen (Fig. 5). Again, cells inoculated with the control IgG retained their rounded, transformed appearance.

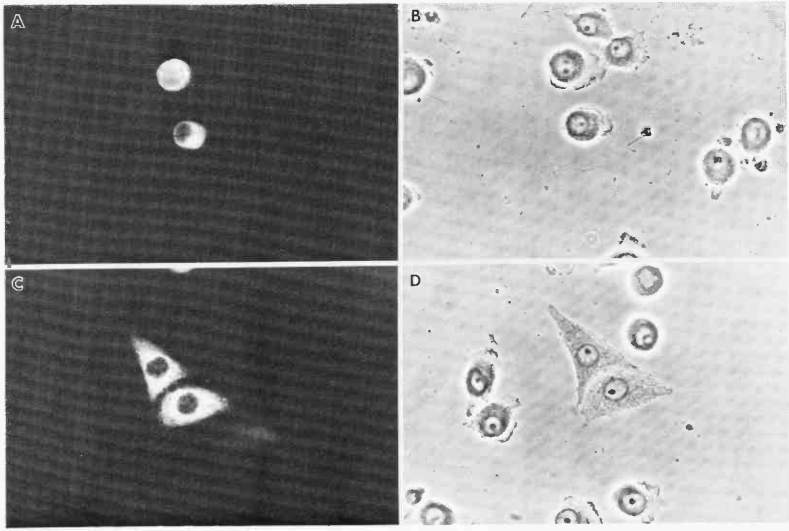

FIGURE 5 Injection of antibodies specific for the *ras* oncogene protein causes reversion of the transformed phenotype of *ras*-transformed cells. Ki-*ras*-transformed NRK cells were injected either with nonimmune IgG (*A,B*) or with IgG specific for the Ki-*ras* oncogene protein (*C,D*). Twenty-four hours later, the cells were fixed and analyzed by immunofluorescence to detect the injected IgGs. Notice the flat, normal appearance of the cells injected with the anti-*ras* IgG.

Protein Kinase C

K. Matlack, T. Kamata, M. Wooten, D. Bar-Sagi,
D.M. Helfman, S. Smith, J.R. Feramisco

As our interest in the molecular mechanisms of growth control widens, we have undertaken studies to examine the role of phospholipids and protein kinase C in the cell. A wide variety of experiments have suggested regulatory roles for protein kinase C, phospholipids, and their breakdown products in growth-factor action and thus have captured our attention. Here and in the following report, we describe the purification, characterization, and cloning of protein kinase C.

One of the major problems one faces in working with protein kinase C is a lack of purified, stable enzyme. To overcome this problem, we designed a protocol applicable to many tissues and sources that enabled us to generate relatively large quantities of purified protein kinase C. The principal feature of the purification procedure is an affinity column utilizing protamine attached to a solid support. Protamine is the one known substrate that is utilized by protein kinase C in the absence of Ca^{++} or phospholipids. Thus, protein kinase C binds tightly to the affinity support in the absence of any added activators of the enzyme. It is eluted from the column when MgATP is added, which most likely reflects a lower affinity of the enzyme to protamine after phosphorylation (i.e., product release). By combining this step with several steps taken from the original, lengthy procedure of Y. Nishizuka (Kobe University, Okazaki, Japan), we can easily purify relatively large quantities (~500 μg) in less than 1 week (Fig. 6).

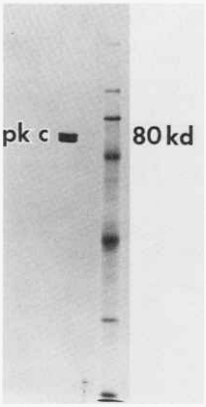

FIGURE 6 Purified protein kinase C. Approximately 5 μg of rat brain protein kinase C was applied to an SDS-polyacrylamide gel; the resultant gel was stained with Coomassie blue.

Isolation of cDNA Clones Encoding Protein Kinase C

D.M. Helfman, T. Kamata, M. Wooten, S. Smith, M. Zoller, C. Slaughter, T. Fischer, J.R. Feramisco [in collaboration with J.F. Kuo, P. Girard, and I.C. Gonsalus, Department of Pharmacology, Emory University School of Medicine]

To isolate cDNA clones encoding protein kinase C, we constructed a cDNA expression library of approximately 80,000 members from adult rat brain using the plasmid expression vectors pUC8 and pUC9. Using antisera for brain protein kinase C, we have identified and isolated six bacterial colonies producing protein products antigenically related to protein kinase C. The plasmids were isolated, and the cDNA inserts ranged in size from 0.6 kb to 2.7 kb. The fusion proteins produced in bacteria have a molecular weight ranging from 17,000 to 80,000. The size of the cDNA inserts correlated with the size of the fusion proteins. Three independent colonies were found to produce a fusion protein with a molecular weight of about 80,000, approximately the size of authentic protein kinase C. Comparison by peptide mapping of the 80,000-molecular-weight bacterial fusion proteins with authentic protein kinase C isolated from rat brain indicated that the two proteins were indeed related. In addition, the cDNA clones encoding the large fusion protein were found to cross-hybridize to a synthetic oligonucleotide probe prepared from direct amino acid sequence analysis of purified brain protein kinase C. We are continuing to characterize these putative cDNA clones encoding protein kinase C.

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MAMMALIAN STRESS RESPONSE

W.J. Welch G. Binns N. Kronenberg
J.R. Feramisco C. Hallaran J.P. Suhan

Cell Biology of the Mammalian Stress Response

W.J. Welch, J.R. Feramisco, G. Binns,
N. Kronenberg, C. Hallaran

Mammalian cells, when confronted with adverse environmental conditions, undergo what is referred to as "stress response." This universal response is characterized by the rapid, increased transcription and translation of a small number of genes, resulting in the increased synthesis and accumulation of a group of proteins, the stress proteins. Although the function of any one of the individual stress proteins is unknown, collectively, these proteins appear to serve some protective capacity both during and after recovery of a particular environmental insult. We have continued to study and define many of the basic changes occurring in the stressed cell. In addition, using both biochemical and immunological approaches, we have paid considerable attention to the structure, properties, and intracellular location of the individual stress proteins with the eventual aim to determine the function these proteins serve in the cell.

Morphological Studies

W.J. Welch, J.P. Suhan

To get a better feeling of the many events occurring in the stressed cell, we have been examining basic morphological changes that accompany induction of the stress response, using both light and electron microscopy. We have characterized a number of interesting alterations occurring in both the cytoplasm and nucleus of cells following heat-shock treatment. For example, we have observed that incubation of rat fibroblasts at 42°C for 3 hours results in a fragmentation and vesicularization of intracellular membranous organelles, including the endoplasmic reticulum and Golgi complex. The disruption in these organelles is of interest since two of the mammalian stress proteins, the 80-kD and 100-kD proteins, are associated with the endoplasmic reticulum and Golgi complex, respectively (Welch et al., *J. Biol. Chem. 258*: 7102 [1983]). We have also observed changes that occur in the mitochondria of cells following heat-shock treatment, including a general swelling of the mitochondria, an increased electron density of the individual cristae, and an accompanying increase in the intramembrane space between individual cristae. These subtle changes in the structure of the mitochondria may turn out to be significant, since it has been suggested that changes in mitochondrial function per se may be involved in the triggering of the stress response. We are continuing to investigate this possibility by analyzing changes in mitochondrial activity using various biochemical techniques. Numerous changes with respect to various cytoskeletal components in stressed mammalian cells have also been found, including an increase in the number of actin-containing stress fibers that span the cytoplasm of the stressed cell, indicative of the growth-arrested state of the cells (Thomas et al., *Cold Spring Harbor Symp. Quant. Biol. 46:* 985 [1982]). In addition, there is a complete reorganization in the distribution of the vimentin-containing intermediate filaments following physiological stress. The filaments are observed to collapse around the nucleus, with large aggregated bundles of filaments very near the nuclear membrane. Removal of the stress agent and incubation of the cells under normal conditions result in a gradual restoration of normal intermediate filament distribution.

Considerable alterations also occur within the nucleus of the cells following heat-shock treatment. For example, we have observed unusual nuclear inclusion bodies that are composed of bundles of parallel filaments. Using biochemical and immunological techniques, we have determined that these structures consist of finely packed actin-containing filaments (see Fig. 1). These bundles of actin filaments were never observed to extend through the nuclear membrane into the cytoplasm. Suffice it to say, we as yet do not understand the significance of these structures with respect to the other changes occurring within the stressed cell. We have also found that dramatic changes occur within the nucleoli of cells following physiological stress, including a decondensation of the nucleoli and an accompanying swelling and/or unraveling of the nucleoli, a diminishment in the number of granular components, and changes in the organization of the fibrillar reticulum. These nucleolar alterations may be significant, since there is an accompanying inhibition of ribosomal RNA synthesis and ribosomal assembly following physiological stress. Moreover, these nucleolar alterations are of considerable interest since two of the major mammalian stress proteins, the 72-kD and 110-kD proteins, localize within the nucleolus in cells both during and after recovery from physiological stress (Welch and Suhan, *J. Cell Biol.* [1985] submitted).

Purification, Characterization, and Intracellular Localization of the Major Mammalian Stress Protein

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

Mammalian cells placed under stress synthesize (and accumulate) proteins with apparent molecular masses of 28, 72, 73, 80, 90, 100, and 110 kD. All of these proteins, with the exception of the 72-kD species, are synthesized at appreciable levels in cells grown under "normal" conditions. Their presence in normal cells suggests that many of the stress proteins probably serve some function(s) in

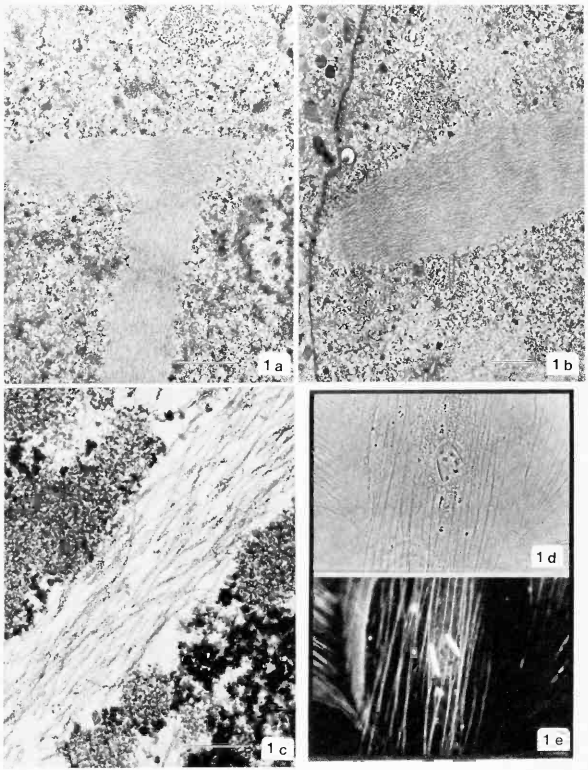

FIGURE 1 Intranuclear inclusions in heat-treated cells are composed of actin. Rat embryo fibroblasts were grown on plastic dishes at 42°C for 3 hr, and sections through the nucleus were analyzed by electron microscopy (*1a,1b*). Similarly heat-treated fibroblasts were fixed, extracted, and incubated with heavy meromyosin (*1c*). Note the typical arrowhead decoration of the actin filaments. The phase-contrast (*1d*) and corresponding fluorescent micrograph (*1e*) of a heat-treated cell analyzed by indirect immunofluorescence using a mouse monoclonal antibody specific for actin are shown. The phase-dense nuclear actin inclusion bodies are evident in *1d* and stained with the anti-actin antibody in *1e*. Bars: (*1a,1b*) 5 μM; (*1c*) 1 μm.

normal cells distinct from their role during stress. Indeed, we have found that the synthesis and/or posttranslational modifications of some of the stress proteins appear sensitive to extracellular levels of glucose, calcium, or even various growth factors. In the case of the most highly induced stress protein of 72 kD, its synthesis appears limited to cells experiencing stress; therefore, we have

paid considerable attention to the properties of this protein (Welch and Feramisco, *J. Biol. Chem. 257:* 14949 [1982]). We and others have found that the 72-kD protein appears to interact in vivo with nucleic acids, specifically RNA. In addition, we have recently observed that the 72-kD protein and its partially related counterpart (73 kD) interact with various nucleotides in vitro. We have taken advantage of their affinity for nucleotides and have now developed a rapid two-step purification of the 72-kD and 73-kD proteins, utilizing affinity chromatography on ATP agarose. In addition, this purification procedure has allowed us to purify one of the other major mammalian stress proteins, the 80-kD protein, as well as a noninduced protein, the 75-kD protein, which interestingly appears to be related to both the 72-kD and 73-kD proteins (Welch and Feramisco, *Mol. Cell. Biol.* [1985] in press).

We have raised both polyclonal and monoclonal antibodies to several of the mammalian stress proteins, including the major-induced 72-kD species. Using these antibodies, we have shown that the 72-kD protein localizes to the nucleolus in stressed cells and also during the recovery period following stress (Welch and Feramisco, *J. Biol. Chem. 259:* 4501 [1984]). More recently, we have found a number of significant differences with respect to the localization of the 72-kD protein in cells recovering from stress after prior exposure to either heat shock, amino acid analogs, or sodium arsenite (W.J. Welch, in prep.). We are continuing to examine the nuclear, nucleolar, and cytoplasmic distribution of the 72-kD protein and have defined some of the basic parameters governing its intracellular localization. First, we have found that the nucleolar deposition of the 72-kD protein is a stress-specific phenomenon. For example, microinjection of purified and fluorescently labeled 72-kD protein into the cytoplasm of cells incubated at 37°C resulted in the protein being distributed throughout the cytoplasm and the nucleus, but not the nucleolus. Following heat treatment of the cells, however, the fluorescently labeled 72-kD protein migrated into the nucleolus (see Fig. 2). This migration appears to be independent of the synthesis of the other stress proteins, since prior treatment with cyclohexamide and subsequent heat-shock treatment still resulted in the 72-kD protein being deposited within the nucleolus. We are currently assessing the effects of transcriptional inhibitors on the intranucleolar distribution of the 72-kD protein and are determining whether its distribution is affected by RNase and/or DNase treatment of the cells. In addition, using immunoelectron microscopy, we are very carefully examining where in the nucleolus the 72-kD protein localizes. Specifically, is the protein located within the granular or fibrillar regions? If in the granular region, this would be indicative of the protein migrating to the nucleolus to be assembled into some sort of ribonucleoprotein component. We find this possibility most plausible considering that the protein does show an affinity for RNA and that the nucleolus is the site of assembly of many different ribonucleoprotein complexes. Preliminary immunoelectron microscopy studies, as well as biochemical studies analyzing stabilized UV crosslinked ribonucleoprotein complexes, are consistent with this idea.

Changes in the Small 28-kD Stress Protein in Response to Mitogens and Growth Factors

W.J. Welch

We are paying considerable attention to the small 28-kD stress protein of mammalian cells. This protein, which contains no methionine, is composed of at least three highly related isoforms, two of which are heavily phosphorylated. During induction and recovery from stress, synthesis of the 28-kD protein increases approximately tenfold, yet there appears to be little change in the net phosphorylation of the protein. Interestingly, we have found that the phosphorylation of this protein, but not its synthesis, increases in quiescent cells following refeeding with fresh serum or after exposure of the cells to either phorbol esters or calcium ionophores (Welch, *J. Biol. Chem. 260*: 3058 [1985]). In addition, preliminary data in conjunction with J.R. Feramisco (this section) have shown an increased phosphorylation of the 28-kD protein in cells induced to proliferate following microinjection of the oncogenic form of the *ras* protein. Our excitement regarding these

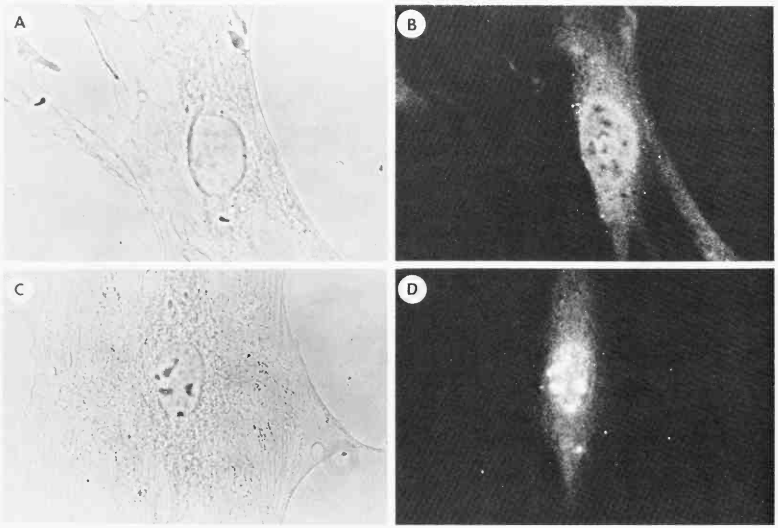

FIGURE 2 Microinjection of fluorescently labeled 72-kD and 73-kD stress proteins. The purified 72-kD and 73-kD stress proteins were conjugated with the chromophore tetramethyl rhodamine isothiocyanate, and the fluorescently labeled proteins were microinjected into the cytoplasm of rat embryo fibrobiasts (37°C). The cells were then incubated either at 37°C (*A,B*) or at 42°C (*C,D*). The proteins were visualized by fluorescent microscopy using the appropriate filters. (*A,C*) Phase-contrast micrographs; (*B,D*) corresponding fluorescent micrographs.

observations is twofold. First, we think it is interesting that synthesis or phosphorylation of the same protein is affected in growth-arrested cells (i.e., following heat shock) or in proliferating cells. Perhaps the 28-kD protein plays some central role in these seemingly opposed phenomena. Second, in *Drosophila melanogaster*, some of the analogous small stress proteins have been implicated in various development and/or differentiation processes. Hence, it will be interesting to see if the mammalian 28-kD stress protein similarly plays some role in growth processes, especially during development. To facilitate these types of studies, we are currently in the process of purifying the 28-kD protein and characterizing its various biochemical properties; we are also trying to prepare monoclonal antibodies specific for this protein.

Presence of the 90-kD Stress Protein in Steroid Hormone Receptor Complexes

W.J. Welch, J.R. Feramisco [in collaboration with E. Baulieu and M. Catelli, Inserum Lab, Bicetre, France]

Two forms of steroid hormone receptor appear to exist within cells: a nontransformed 8S form and a transformed or activated 4S form, which appears to be derived from the 8S form. SDS-PAGE of the 8S receptor shows a number of protein constituents, the major species having apparent molecular masses of 80, 90, and 110 kD. In the case of the 4S form of the receptor, only the 80-kD and 110-kD species are evident, with these two polypeptides showing affinity for various steroids. Using biochemical and immunological techniques, we have shown that the 90-kD protein

component present in the 8S progesterone receptor complex is in fact the 90-kD stress protein (Catelli et al., *Nature* [1985] submitted). It would appear that the 90-kD protein, which itself does not show an affinity for steroids, is lost from the 8S complex following binding of the steroid and the accompanying conversion to the 4S form of the receptor. Precedence for a transient association of the 90-kD stress protein with other macromolecules has been demonstrated. For example, investigators in many laboratories have shown that a number of retrovirus transforming proteins, all of which are tyrosine-specific kinases, are transiently associated with the 90-kD protein following their synthesis on free polysomes in the cytoplasm. This complex, containing the particular tyrosine kinase, the 90-kD stress protein, and another cellular protein of 50 kD, appears to be involved in the translocation of the oncogene protein from its site of synthesis to its final destination in the plasma membrane. Interestingly, when associated with the 90-kD and 50-kD proteins, the particular tyrosine kinase shows diminished activity. Thus, we now have a second example in which the 90-kD stress protein displays a transient association with other biologically important macromolecules, that being the steroid receptor. We are currently determining whether the 8S form of the steroid receptor also contains an associated tyrosine kinase activity.

Production of Monoclonal Antibodies to the Mammalian Stress Proteins

W.J. Welch, N. Kronenberg

To facilitate our studies on their structure, function, and intracellular location, we are preparing monoclonal antibodies to the various stress proteins. These efforts are now beginning to show success. Currently, we have obtained at least 20 different monoclonal antibodies to the major induced 72-kD stress protein. We are in the process of characterizing these antibodies with respect to their antigenic site within the molecule. In addition, we are in the process of making monoclonal antibodies specific to the 73-, 80-, and 90-kD stress proteins.

Comparison of the Stress Response in Normal and Transformed Cells

W.J. Welch, J.R. Feramisco

As we discussed in last year's report, transformed cells, in general, appear much more thermosensitive than do their nontransformed counterparts. However, the basis for this difference in thermal sensitivity is not at all clear. Athough synthesis of the stress proteins appears to occur in the transformed cells placed under stress, there do appear to be some differences with respect to the intracellular location of at least the 72-kD and 110-kD stress proteins. For example, whereas most all of the normal rat fibroblasts show a nucleolar locale of 72 kD during and after recovery from heat-shock treatment, only about 5% of either SV40- or adenovirus-transformed rat fibroblasts show a nucleolar distribution of the protein. Similar differences in the intranucleolar distribution of the 110-kD stress protein in normal and transformed cells have also been observed. Interestingly, the few transformed cells that did show the 72-kD nucleolar staining are always found in close association with one another, indicative perhaps of there being some correlation between the cell cycle and the ability of the cells to exhibit the nucleolar staining. Therefore, we are in the process of examining more carefully the possible correlation between induction and/or proper localization of the stress proteins as a function of the cell cycle in both normal and transformed cells. In addition, we should point out, with regard to cell cycle, that physiological stress itself seems to somehow arrest cells at a particular point in the cell cycle (W.J. Welch and J.R. Feramisco, in prep.).

We are also pursuing the possibility that basic differences in certain metabolic pathways of the transformed cells may be responsible for their observed high thermosensitivity. For example, it has been well established that transformed cells appear more dependent on glycolytic rather than respiratory metabolism, and therefore the cells utilize high levels of extracellular carbon sources such as glucose. Due to their high glycolytic activity (and consequent lactic acid production), the transformed cells tend to acidify their extracellular medium. Hence, we have been asking whether

these metabolic differences exhibited by the transformed cells could be at all responsible for their thermosensitivity. Therefore, we have manipulated the environment of the normal cells to mimic that found for the transformed cells and asked whether the cells will now exhibit thermosensitivities similar to that observed for the transformed cells. Indeed, we have found that inhibiting the ability of the normal cells to respire (by addition of agents that block mitochondrial function) or simply lowering the pH of the normal cells markedly increases the cells sensitivity to a

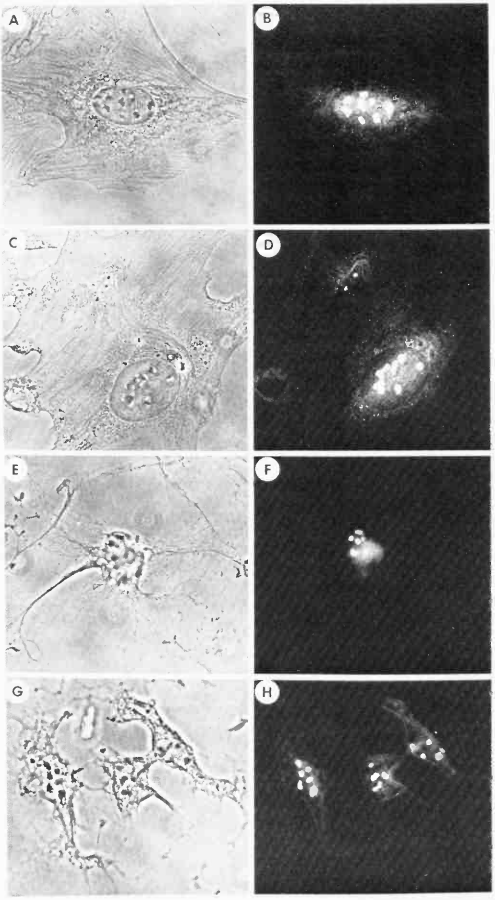

FIGURE 3 The 72-kD stress protein is translocated to the nucleus/nucleolus in heat-treated cells containing a disrupted cytoskeleton. REF52 cells growing on glass coverslips at 37°C were treated with colcemid (2 μM for 5 hr; i.e., no microtubules and collapsed intermediate filaments), cytochalasin E (2 μg/ml for 2 hr; i.e., no actin microfilaments), or with a mixture of colcemid and cytochalasin (2 μM for 5 hr and 2 μg/ml for 2 hr, respectively; i.e., disruption of all three cytoskeletal networks). The cells, in the presence of the drugs, were then incubated at 42°C for 3 hr. Following heat-shock treatment, the cells were fixed and processed for indirect immunofluorescence using a polyclonal antibody specific for the 72-kD heat-shock protein. (*A,C,E,G*) Phase-contrast micrographs; (*B,D,F,H*) fluorescence micrographs. (*A,B*) Control, untreated heat-shocked cells; (*C,D*) heat-shocked cells treated with 2 μM colcemid; (*E,F*) heat-shocked cells treated with 2 μg/ml of cytochalasin E; (*G,H*) heat-shocked cells treated with a mixture of colcemid (2 μM) and cytochalasin E (2 μg/ml).

heat-shock challenge. We are continuing these types of studies in hope of defining the basic parameters responsible for the high thermal sensitivities exhibited by the transformed cells.

Use of the Heat-shock Response to Study the Cytoskeleton

W.J. Welch, J.R. Feramisco

As mentioned above, physiological stress results in a collapse of the vimentin-containing intermediate filaments. We therefore asked whether other agents that similarly result in collapse of the intermediate filaments would also result in an induction of the stress response. Others have shown that treatment of the cells with various drugs that depolymerize microtubules (colcemid, colchicine, etc.) also cause a collapse of the intermediate filaments. However, we found that treatment of rat fibroblasts with these drugs did not result in an induction of the stress response. Moreover, there appeared to be little or no change in the overall pattern of protein synthesis in those cells treated with these cytoskeletal disrupting drugs. This was somewhat surprising since investigators in other laboratories have suggested that translational machinery in eukaryotic cells functions when in association with cytoskeletal elements. Using the heat-shock response to induce de novo transcription and translation events, we asked whether cells containing a disrupted cytoskeleton could faithfully make and translate new mRNAs even when the cells contain no integral and intact macrocytoskeleton. For these experiments, cells were treated with drugs that disrupt the actin-containing microfilaments, vimentin-containing intermediate filaments, and microtubules; they were placed under heat shock and then labeled with radioactive amino acids. Despite the absence of an intact cytoskeleton, the cells were still able to recognize and respond to the heat treatment as assayed by the vigorous production of the stress proteins. In addition, the drug-treated cells, after heat-shock treatment, were still able to translocate the 72-kD stress protein from the cytoplasm into the nucleus and nucleolus (see Fig. 3). These results thus raise doubts with regard to previous suggestions implicating a dependence of new translational events and the association of translational machinery with the cytoskeleton. They also demonstrate that some protein translocation events, specifically, migration of proteins from their site of synthesis in the cytoplasm into the nucleus, can occur in the absence of any integral cytoskeletal networks (Welch and Feramisco, *Mol. Cell. Biol.* [1985] in press).

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QUEST TWO-DIMENSIONAL GEL LABORATORY

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B.R. Franza, Jr. L. Cascio H. Wenzel

The Quest System for Two-dimensional Gel Analysis

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L. Cascio, H. Wenzel

DEVELOPMENT OF PROTEIN DATABASES

During 1984, our program for development of protein databases derived from two-dimensional gel analysis has turned from software development to problems of data management, distribution, and presentation. In our laboratory, the production of standardized two-dimensional gels has been routine, and the computer software for spot quantitation and matching has been highly refined. Although our computer system has been much too small for the size of the programs required and for the amount of data to be analyzed, the hardware limitations will be overcome in 1985 (see below). Even with the relatively small database that we now have, it has been clear that the next major area of technical development must be in the handling of the databases themselves. Here, we present several forms of analyses of our rat fibroblast protein database, illustrating some of the ways we have found to present large amounts of two-dimensional gel data reduced to an easily understood form. In addition, we discuss the hierarchical strategy we have developed for the management of much larger databases.

Most of the data in our rat fibroblast database come from an intensive study of the REF52 cell line and its transformed derivatives. The lines are described in our previous reports and in the following report. A number of experiments on these lines have been analyzed and some of the data are presented in Figure 1. This example points out the magnitude of the data we must manage. The output, of which only a small portion is shown, presents the quantitative changes for more than 900 proteins in 16 different experiments. Each small graph shows how the relative incorporation into a given protein changed throughout the course of an experiment. We find that the graphs, each scaled to put the highest point at full scale, are easily scanned visually for interesting regulatory patterns. The large array of graphs, with a different spot on each row and a different experiment on each column, can be quickly scanned in either dimension for changes of interest. Although the amount of data presented in this way is still large, it is vastly reduced from the original image data represented on hundreds of films. The fact that data in this form is usable and interesting can be seen by examination of the graphs for any particular spot.

The protein PCNA (proliferating cell nuclear antigen), spot 388, has been studied previously in our system. We find that its rate of synthesis decreases at confluence in normal cells (graph in column 1) but that its rate of synthesis does not decrease in proportion to density in transformed cells (next nine columns). The last two columns show the relative rates of synthesis of PCNA in normal and in nine transformed cells during growth phase on day 3 (column 15) or day 4 (column 16) after plating. These two graphs show almost identical patterns because little change in rates of protein synthesis occurs from one day to the next during the proliferative phase. The other experiments presented here are described in Figure 1.

When viewed on the graphics screen, the user can point to graphs of interest and have the computer display the appropriate regions of the gel images. Having found a protein of particular interest, the user can ask for more information about the protein, or having found an interesting pattern of change, the user can ask the computer to highlight other proteins that change in similar ways. The reader can see that other proteins in Figure 1 show interesting regulatory patterns, including the tropomyosins (spots 448, 350, 398, 328, 320, 420 for tropomyosins 1 through 6, respectively).

In another look at the data from REF52 and the nine viral transformants, we asked the computer to count the number of proteins that respond to fresh serum (after being deprived of serum) in normal REF52 cells and how many of

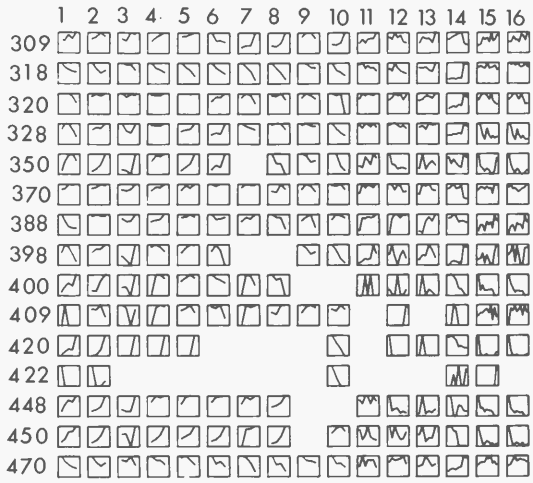

FIGURE 1 Spot graphs showing patterns of incorporation into 15 proteins throughout the course of 16 experiments. Numbers at the left are the standard spot number, and each column (1–16) represents a different experiment. In each experiment, six to ten samples were radiolabeled and analyzed by quantitative two-dimensional gel electrophoresis. The graphs are scaled to put the highest point at full scale. These small graphs are designed to present visually recognizable regulatory patterns that can be compared among experiments and among proteins. The experiments are as follows: (*1–10*) Growth curves for REF52, REF52-WT2, REF52-AG2, REF52-NU2, REF52-WT6, REF52-AG6, REF52-NU6, REF52-Ki-MSV, REF52-Ad5D.4A, and REF52-Ad5W.4A; (*11,12*) comparisons of REF52 with six SV40 transformants in normal and defined medium, respectively; (*13*) comparison of REF52 cells in normal and defined medium, followed by REF52-SV1A cells in normal and defined medium, followed by REF52-SV2A cells in normal and defined medium; (*14*) REF52 cells labeled for 30 min (two samples), 2 hr (two samples), 24 hr (two samples), and 24 hr followed by a 48-hr chase (two samples); (*15, 16*) comparisons of REF52 cells and the nine transformed lines mentioned above on days 3 and 4, respectively, after plating. For proteins not detected in a given sample, the data point is plotted at the base line. When a protein was not detected in any of the samples of the experiment, no graph is plotted.

these proteins are still serum-sensitive in each of the transformants. We find that analysis of dynamic differences (changes in the response of a protein to a stimulus) is often more rewarding than just the analysis of static differences (changes in the level of a protein). In the analysis of response to serum stimulation, we reduced the data from about 60 films to yield the results shown in Table 1. Of the 77 proteins that increase in rate of synthesis after addition of fresh serum to REF52 cells, only about 50% are induced in cells minimally transformed by SV40 (WT2 and WT6), only 35% are induced in SV40 cells selected for growth in soft agar (AG2 and AG6), and only 25–30% are induced in SV40-transformed cells selected for tumorigenicity (NU2 and NU6). The adenovirus-transformed REF52 cells are even less responsive to serum stimulation, and in one adenovirus transformant (a mutant lacking the 57K E1B protein), only 1 of

TABLE 1 Loss of Response to Fresh Serum in Transformants

	REF52 responses remaining in transformant			
	increases		decreases	
REF52	77	(100%)	59	(100%)
WT2	45	(58%)	26	(44%)
AG2	26	(34%)	8	(14%)
NU2	20	(26%)	24	(41%)
WT6	39	(51%)	7	(12%)
AG6	27	(35%)	6	(10%)
NU6	25	(32%)	11	(19%)
KIR	27	(35%)	7	(12%)
A5D	12	(16%)	10	(17%)
A5W (–E1B57K)	1	(1%)	10	(17%)

the 77 responses could be found. These changes follow in order of the apparent "degree of transformation," determined by overall relatedness of the protein pattern of each line to normal REF52.

Among the proteins of REF52 cells that decrease when serum is added, even fewer of the responses remained in the transformants, although the percentage of responses remaining was less well ranked. The proteins that increase in fresh serum must include many needed for return to rapid growth, whereas those that decrease in fresh serum include many of the possible indicators of the "differentiated" state, such as smooth-muscle actin, vinculin, and some of the tropomyosins. This type of analysis helps greatly to understand coordinate control and reveals which regulatory pathways may be affected by each form of transformation. The reproducibility of the regulatory changes found among several different lines transformed by the same virus indicates that these changes are fundamental to the transformed state.

In a third analysis, we compared the responses of normal and SV40-transformed REF52 cells to the effects of cell density. AG6 cells were refed every 48 hours to prevent media depletion and were labeled several times during growth to confluence. At confluence, REF52 cells became quiescent. The SV40-transformed cells did not stop dividing, but their rate was slowed considerably. Our hypothesis was that fewer changes would occur in the transformed cells because their growth rate is less affected by density. Accordingly, we expected to find many changes occurring in REF52 cells as a function of density that did not occur in AG6 cells.

The results in Table 2 show that SV40-transformed cells change by just as much as REF52 cells when they reach confluence but that the proteins involved are quite different. In both lines, more than 20% of the proteins show changes by more than twofold, but the difference between the two lines at confluence is much greater than it was during proliferation. The computer has revealed several interesting proteins that are detected at low levels in dividing or confluent REF52 cells and in dividing AG6 cells, but at dramatically higher levels in confluent AG6 cells. Analysis of other experiments being added to the database can shed more light on the regulation of these proteins.

A HIERARCHY OF DATABASES

The data presented above came from a series of studies of the REF52 cell line, and all experiments were matched to a single standard pattern. Ideally, all experiments with rat cells would be matched to the same standard pattern to form a comprehensive rat protein database. Unfortunately, a single standard map for rat cells is impractical, because (1) it becomes very complex and (2) its integrity is difficult to maintain in a multiuser environment if any user is allowed to add

TABLE 2 Relatedness of Normal and Transformed Cells during Proliferation and at Confluence

Comparison	Standard deviation[a]	Differences (>twofold)[b]	Qualitative differences[c]
REF52 dividing vs. AG6 dividing	0.97	18	9
REF52 dividing vs. REF52 confluent	1.11	22	4
AG6 dividing vs. AG6 confluent	1.01	22	7
REF52 confluent vs. AG6 confluent	1.26	29	13

[a]Based on logarithm (base 2) of spot ratios (~650 spots compared)
[b]Percentage of matched spots with ratios less than 0.5 or greater than 2.0 (~650 spots compared)
[c]Proteins prominent in one sample (~300 most abundant) but not detected in other sample.

new spots to it. Most users first need their own standard pattern so that they can compare the gels of one experiment among themselves, and only secondarily do they need to match their data to the standard patterns used for the higher-level databases. Therefore, we have developed our system to support an unlimited number of standard patterns. Using programs that allow standard patterns to be matched to one another, a hierarchical database structure becomes supportable.

The lowest level of the hierarchical database structure is the individual experiment. The typical experiment involves a related group of samples that can be easily compared with one another. A standard pattern representing all the spots detected in the experiment is automatically generated, and a mini-database is generated by matching each of the gels of the experiment to this standard. At this level, users can plot the changes of each protein throughout the course of the experiment and can find interesting differences. When users have highly related experiments and have used the same standard for each, they can plot the changes of each protein in several experiments. The procedures for analysis of a single experiment are simple enough that most users can build their own standards and analyze their own experiments with minimal help from the computer staff. Such a database should be private, accessible only to the scientists involved in the project.

At the second level, users may want to compare their data with other data in the system. These may be data from other experiments that were done using different standard patterns, as well as data made available from other investigators. To serve these needs, larger databases must be built up from individual experiments. These will be called public databases, because they contain data accessible to all investigators. Again, these databases must not be too general but should be focused in targeted areas, such as rat fibroblasts, mouse embryo cells, and human diploid fibroblasts. Microorganisms such as yeast or *Escherichia coli* can probably be handled as a single database, but a single database encompassing all proteins of a multicellular organism is still too complex. When data from a private database are made available to the public, the data can be entered into the public database by matching the standard patterns. This must be done by a trained staff person who can use special features of the system to check the quality of each spot and the correctness of the matches. Whenever possible, additional information about each spot, such as characteristic regulatory patterns, subcellular localization, or amino acid composition will be used to confirm the match to the appropriate spot in the higher database. Users of the private databases can perform preliminary matches of their standards to the public standards in order to compare their data to the public databases before releasing their data to the public.

At the third level, a species database is needed. This should encompass as many of the proteins as can be unambiguously identified in the diverse cell types and tissues studied within a single species. Most of the major proteins and many of the less abundant proteins will fall into this category. Proteins that are under intensive investigation often can be identified by antibodies, by composition, or by characteristic regulatory properties in many different cell types. The species database would contain the names of most identified proteins and would provide, as much as is possible, a cross-referencing between the lower-level public databases. Proteins in the species database would be identified on the species standard map as well as on the standard maps for one or more of the lower databases. Through the species database, a researcher working with NIH-3T3 cells could cross-reference from that database into the databases for mouse embryos, mouse erythroleukemia cells, or mouse muscle cells to ask how the proteins of interest are regulated in other cell types.

A NEW RESOURCE FACILITY

Our present computer facility has served well for program development, but falls far short of the capacity needed to analyze whole experiments routinely. Furthermore, scientists from other institutions have expressed strong interest in the use of our system and our databases. We felt that we were ready for an expanded program devoted primarily to database development and that if the two-dimensional gel databases were to find general utility, they would need to be made more readily available. During 1984, we made a proposal to become a Biotechnology Resource and were awarded 5 years of funding from the Na-

tional Institutes of Health for such a facility, beginning in January, 1985. The new facility will contain three computer workstation systems, each tenfold more powerful than our present computer system. A staff will be hired to operate the facility, to serve the outside users, and to manage the public databases. Core research funds will be used to build up the public databases and to put many more identified proteins on the maps. The planning for this facility and the work we have done on database methods during this year should help us to build large multiuser databases in several areas of key scientific interest during the next year.

Response of Cellular Proteins to Oncogene Expression

B.R. Franza, Jr., H.E. Ruley, J. Moomaw, C. Chang, H. Wenzel, J.I. Garrels

One of the major goals of our research is to study the effects of expression of different oncogenes on cellular proteins. The REF52 cell line has been used in much of this work because of its stability in tissue culture, because individual oncogenes such as T24 Ha-*ras*-1 or Ad5E1A will not oncogenically transform these cells, and because combinations of oncogenes result in a variety of transformed phenotypes, some of which are oncogenic. We have now isolated single cell clones of REF52 that contain either the T24 Ha-*ras*-1 or Ad5E1A oncogene. We have also isolated a number of transformants that result from coexpression of these two oncogenes. For the first time, we are able to compare systematically the effects of each oncogene, expressed alone or together, on cellular proteins. Here, we present a brief description of a few of the interesting observations we have already been able to make.

The motivation for comparing the cells under normal as well as heat-shock conditions primarily was the previously reported adenovirus effect on expression of the "HSP70K" gene. Work done in the past in collaboration with W.J. Welch (this section) had already revealed that one protein ("73K") in REF52 cells, though expressed at 37°C, is substantially increased in response to heat shock, and another protein("72K") is synthesized at detectable levels only when the cells are stressed. We therefore wanted to know if either of these two proteins would respond to constitutive expression of Ad5E1A, and if so, would this in any way alter their synthesis during heat shock.

Analysis of the experiment described in Figure 2 revealed that expression of Ad5E1A alone resulted in changes in protein synthesis that were in some cases exactly the opposite of those induced

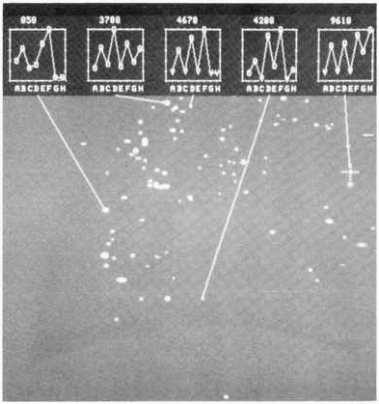

FIGURE 2 Graphic analysis of certain cellular proteins altered by oncogene expression. A display of database information for five cellular proteins is presented. A region of the standard REF52 protein map was displayed on the graphics monitor, the five spots were individually selected, and their corresponding graphs were displayed. After displaying all five graphs the photograph shown in this figure was made. Each graph shows the relative intensity for that spot in each of the samples analyzed, with the highest intensity set at full scale. The experiment reported herein involves metabolically labeling of 70% confluent, dividing cells with [^{3}H]-leucine for 2 hr. Cells exposed to elevated temperature for 4 hr were labeled for the final 2 hr of the heat shock. (*A*) Normal REF52 cells, 37°C; (*B*) normal REF52 cells, 42°C; (*C*) REF52 cells expressing Ad5E1A, 37°C; (*D*) REF52 cells expressing Ad5E1A, 42°C; (*E*) REF52 cells expressing T24 Ha-*ras*-1, 37°C; (*F*) REF52 cells expressing T24 Ha-*ras*-1, 42°C; (*G*) REF52-transformed cells expressing both Ad5E1A and T24 Ha-*ras*-1, 37°C; (*H*) REF52-transformed cells expressing both Ad5E1A and T24 Ha-*ras*-1, 42°C. The response of the following proteins are shown in the graphs: tropomyosin 1 (858), stress-response protein, 73K (3708), stress-response protein, 72K (4678), stress-response protein, 28K (4208), and previously unidentified transformation-sensitive protein, 50K (9618).

by T24 Ha-*ras*-1 expression. Spot 858 (shown in Fig. 2) demonstrates the reduction in synthesis of the cytoskeleton protein, tropomyosin 1, in cells expressing Ad5E1A, and an increase in synthesis in cells expressing T24 Ha-*ras*-1. The same result was observed with the sm-Actin (*s*mooth *m*uscle form) synthesized in REF52 cells. We have already demonstrated that these two proteins are coordinately regulated in growth-curve experiments, in serum-stimulation experiments, and in SV40 and adenovirus DNA transformants of REF52. Interestingly, in the AD5E1A + T24 Ha-*ras*-1 transformant, the prevailing effect on tropomyosin 1 is that seen in the Ad5E1A-expressing cells, i.e., decreased synthesis. In the case of sm-Actin, no detectable synthesis occurs in the transformant. Therefore, expression of each oncogene alone can exert effects that are exactly the opposite of the other. In addition, their coexpression is not the simple summation of effects. The interaction of two oncogenes in a transformed cell should therefore be thought of in terms of comodulation of cellular protein synthesis. The predominant effect, of one oncogene or the other when coexpressed in the transformed cell, is discernible as a result of having each oncogene individually expressed in cell lines derived from the same parent as the transformant.

Spot 3708 presents data on the synthesis of 73K in each of the cell lines under study. As shown in Figure 2, cells expressing Ad5E1A synthesize slightly more 73K than do normal cells at 37°C. In addition, the induced expression at 42°C is higher than in normal cells. The transformant (expressing both oncogenes) shows even greater synthesis of 73K at 37°C, but coexpression of T24 Ha-*ras*-1 may account for the reduction in the level synthesized during heat shock. Comparison of the results shown in the graph of spot 4678 for 72K clearly shows that these two proteins are regulated differently. Ad5E1A-containing cells show higher induction of 72K than normal cells when both are exposed to 42°C, and T24 Ha-*ras*-1 cells show an even greater induction of 72K when heat-shocked. In all the T24 Ha-*ras*-1 + Ad5E1A-transformed REF52 clones, the level of 72K induced by heat shock is less than that seen in clones containing one or the other gene, and in the case of this particular transformant, it is not detectable. In this particular transformant, the level of the heat-shock 110K proteins (graph not shown) is substantial even at 37°C, although it is not detectable in the other lines shown in this study unless they are heat-shocked. It may be that constitutive production of 110K at 37°C is the result of coexpression of the two oncogenes and affects the inducibility of 72K in these cells.

The graph of spot 9618 represents a previously unidentified transformation-sensitive protein, "50K." Interestingly, this protein is synthesized at 37°C in the transformed cell line (much like 110K mentioned above) but not in other lines. In the transformant, 50K is also inducible by elevated temperature to even higher levels of synthesis. 50K and 110K therefore represent proteins whose regulation is sensitive to both stress and coexpression of two oncogenes.

The final protein presented here is known as "28K" and its response is shown in graph 4208. The two spots immediately to the left of 28K (see Fig. 2) are its phosphorylated forms. The identification of these phosphate-modified forms was done in collaboration with W.J. Welch (this section). He has shown that serum, stress, and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) can induce increased phosphorylation of 28K. Indeed, in experiments in which we have compared the effects of serum, platelet-derived growth factor (PDGF), and TPA, within 3 minutes of exposure to any of these agents, a significant increase in the density of the spots representing the phosphorylated forms of 28K occurs. It was therefore very interesting to see that the synthesis of the nonphosophorylated 28K was substantially reduced in cells expressing Ad5E1A but was induced in the cells expressing T24 Ha-*ras*-1 alone. The prevailing influence in the transformant seems to be Ad5E1A. The response of this protein to 42°C in each line is also shown and indicates again the complexity of coexpression versus individual expression of the two oncogenes under study.

Normal REF52 cells require fetal calf serum for growth. W. Topp (Otisville Biotech, Inc.) and D. McClure (W. Alton Jones Cell Science Center) have shown that in defined media, PDGF will not induce REF52 cells to leave density-arrest. They have also shown that in defined media, dividing REF52 cells do not require PDGF for continued growth and cell division. Cells expressing the T24 Ha-*ras*-1 oncogene, either stably incorporated or after microinjection, demonstrate a substantial

increase in serum dependence. Serum deprivation causes the cells to flatten out, to develop significant cytoplasmic vacuolization, and to accumulate multiple nuclei in individual cells. The serum deprivation response is induced if the media containing 10% fetal calf serum is not changed every other day. Cells expressing the Ad5E1A gene alone also show increased serum dependence, but their response to fresh serum as well as serum deprivation is different. When plated in fresh serum, these cells are often small, more refractile, and show a greater number of mitotic forms. One to two days after serum is added they begin to flatten out and divide more slowly. Unless the cells are refed with fresh serum they become round, detach from the plastic, and die. Thus, the effects of the Ad5E1A gene and the T24 Ha-*ras*-1 gene are distinctly different with respect to morphology and survival in serum-depleted medium. Cells coexpressing both genes are fully transformed and serum-independent.

Analysis of proteins such as "28K" may help us sort out the differences in growth control exhibited by cells expressing either Ad5E1A or T24 Ha-*ras*-1. We are currently studying the response of 28K in experiments in which PDGF is added to the cells to see if the phosphorylation that occurs in the cells expressing Ad5E1A is substantially different from that seen in the cells expressing T24 Ha-*ras*-1. When these results are placed in the context of the other induced changes in phosphorylation/dephosphorylation of cellular proteins acutely exposed to different growth-promoting or retarding agents, we expect that sets of proteins specifically altered in cells expressing each oncogene will be identified. Such knowledge will allow us to begin defining the proteins involved in the growth-control pathways of the cell.

Among the applications available once the above analysis is complete is a strategy for more efficient and directed isolation of the genes encoding proteins involved in cellular growth control. It will employ two-dimensional analysis of in vitro translates of cellular mRNA prepared from each of the different cell types exposed to various conditions to determine quantitatively when the message for a particular protein is most abundant. From this information, protocols for differential hybridization can be optimized, and the resultant hybrid-selected mRNA can be translated and analyzed on two-dimensional gels to ensure that the proteins of interest are indeed encoded by the selected cDNA.

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NEUROBIOLOGY

During mammalian neurogenesis, a plate of approximately 10^5 morphologically homogeneous cells gives rise to 10^{12} morphologically and physiologically heterogeneous neurons in the adult brain. Estimates of mRNA complexity indicate that the adult mammalian brain contains more than 30,000 different brain-specific proteins. The studies of the Neurobiology Group continue to explore several features of this enormous molecular diversity using both vertebrate and, simpler, invertebrate nervous systems. Our previous studies showed that in contrast to the immune system, where molecular diversity among lymphocytes is expressed within a single class of molecule (immunoglobulin), diversity in the nervous system is not confined to one class of molecule (such as neurotransmitter), as antigenic heterogeneity is manifest in many different subcellular structures.

One of the potential uses of cell-specific markers is to trace lineage relationships through development. Somewhat surprising was our finding that all of our markers that differentiate between adult mammalian neurons recognize antigens that are first expressed relatively late in development. Some of these antigens first appear long after the apparent establishment of adult structure. In the last year, we have focused increasingly on developmental issues. Our developmental studies suggest that the temporal control of antigen expression and the transient presence of some classes of cells are importantly related to the normal development of the adult nervous system. One of the most exciting observations in the last year was the demonstration that the expression of an antigen recognized by one of our monoclonal antibodies is controlled by early visual experience. This suggests that the mechanisms of experience-induced neuronal development may be accessible to molecular analysis.

Our studies using invertebrate nervous systems have addressed issues that are not easily accessible in the mammalian brain. Using the moth, we are exploring the basis of sexual dimorphism in the central nervous system (CNS). Our studies on the leech CNS continue to examine features of the segmental organization of nervous systems.

VERTEBRATES

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A MARKER FOR EARLY AXONS ALLOWS US TO VISUALIZE THE FIRST EVENTS IN AXON OUTGROWTH

One of the great difficulties in studying how connections are made in the developing mammalian nervous system has been the inability to identify the earliest axons reproducibly and unambiguously. Available methods have severe limitations. Using electron microscopy at very early stages of development, one cannot reliably discriminate between axons and other profiles. Neither neurofibrillary stains nor intra-axonal tracers can be used to detect axons at their earliest stages of growth. We have generated a monoclonal antibody, Rat-202, that identifies axons at the earliest stages of axon growth. The antibody binds to an

antigen distributed throughout growing axons, including the growth cone and filopodia, the structures believed to be responsible for axon navigation.

Studies in tissue culture of growing axons have described the morphology and behavior of growth cones and filopodia. These studies have demonstrated that growing axons show a preference for particular substrates. Due to the vastly greater complexity of the intact nervous system, similar studies in tissue sections were not possible. Using antibody Rat-202, we have been able to identify and study growth cones and filopodia in sections. One of the advantages of antibody identification is that histological analyses can be carried to the ultrastructural level. Electron microscopic examination of Rat-202-stained material shows that the filopodia of growth cones in the developing spinal cord contact nonneuronal profiles. Using double-labeling techniques, we are now trying to identify simultaneously these nonneuronal profiles and early axons to determine the structures filopodia contact to guide axon growth in the CNS.

A NONNEURONAL CELL MAY SUPPORT INITIAL AXON GROWTH AND AXON REGENERATION IN THE PERIPHERY

Axon regrowth after injury occurs in the peripheral nervous system, but not in the CNS, of adult mammals. Accumulating evidence indicates that axon growth depends on environmental factors such as nonneuronal cells and extracellular matrix present in peripheral nerve. However, the mechanism of axon interaction with the peripheral nerve environment is not well understood. One unsettled question is whether axons contact one specific class of nonneuronal cells during growth in immature or regenerating peripheral nerves and/or whether they preferentially contact noncellular, extracellular matrix components secreted by these nonneuronal cells. In the past, these questions have been inaccessible to study because early nonneuronal cell types cannot be reliably distinguished on morphological grounds and there was no ready means to generate markers for peripheral nerve nonneuronal cells or extracellular matrix components. The development of hybridoma technology has provided a useful tool to generate anatomical probes for the study of early events during peripheral nerve development and regeneration.

Monoclonal antibody Rat-401, generated in our laboratory by immunizing mice with paraformaldehyde-fixed spinal cord from rats at day 15 of gestation, recognizes nonneuronal cells in both the developing peripheral nervous system and the CNS. Rat-401-stained cells are present in peripheral nerve roots as early as day 11 of gestation. Rat-401-positive nonneuronal cells are present before axons grow into the peripheral nerve. Interestingly, as the early axons enter the nerve roots, they contact Rat-401-stained cells. Thus, these nonneuronal cells are in a position where they may guide or support initial peripheral axonal growth. Embryonic optic nerve and other CNS axon tracts contain Rat-401-stained cells, an indication that Rat-401-positive cells may also assist axon growth in the CNS.

Rat-401-stained cells in the developing CNS are present in the early neural tube and are morphologically similar to the radial glial cells. The radial glial cells act as guides for neuronal migration in the CNS, suggesting that the cells that guide axon outgrowth and neuronal migration are related. Our earlier studies in the leech suggested that axons may selectively fasciculate with one another through diverse surface-recognition molecules. The observations with Rat-401 raise the possibility that initial axon outgrowth and navigation occur in relation to a nonneuronal cell, and subsequent navigation may be mediated by interaxonal interactions. We have now established cultures of Rat-401 cells from the embryonic CNS so that the role of these cells in cell migration and neurite outgrowth can be studied and manipulated in vitro.

The adult CNS lacks Rat-401-stained cells, which is consistent with the absence of neuronal cell migration and axon regeneration following injury in the mature CNS. In contrast, the mature peripheral nerve (where axon regeneration does occur) contains Rat-401-positive process. Rat-401-stained cells in the sciatic nerve are located external to myelin sheathes in a configuration similar to that of Schwann cells, a nonneuronal cell type that ensheathes axons. This suggests that a set of the embryonic Rat-401 cells develop into myelin-related Schwann cells. We have now shown that cultured Schwann cells, but not fibroblasts, are Rat-401-positive, increasing

our confidence that Rat-401 is a Schwann cell marker. We are now determining if Rat-401 cross-reacts with human Schwann cells. A well-characterized Schwann cell marker could be useful to clinicians for the rapid diagnosis of Schwann cell tumors at the light microscopic levels, thus avoiding the expensive, time-consuming electron microscopic methods currently used to identify such tumors. In addition, we are examining Rat-401-stained cells in relation to axons in injured peripheral and central nerve tissue. By continuing to generate new monoclonal antibodies to embryonic peripheral nerve tissue and to regenerating the adult peripheral nerve, we hope to obtain additional markers for nonneuronal cells and for growing axons.

EXPRESSION OF THE ANTIGEN RECOGNIZED BY MONOCLONAL ANTIBODY CAT-301 REQUIRES NORMAL VISUAL EXPERIENCE

Once growing axons have reached their targets, they make synapses. In most areas that have been studied, the number of synapses initially made far exceeds the number maintained into adulthood. One of the most philosophically compelling discoveries of modern neuroscience is that the early experience of an organism plays a critical role in determining the adult synaptic structure. Experience-induced developmental processes have been well documented in the mammalian visual system.

Monoclonal antibody Cat-301 recognizes a morphologically distinct population of neurons in the cat and primate lateral geniculate nucleus (LGN), a central relay nucleus in visual processing. During the past year, we have identified Cat-301-positive neurons in the cat LGN as the (physiologically defined) Y cells, using morphometric and tract-tracing techniques. The size, shape, location, and projection to specific cortical areas of antibody-positive neurons correlate well with described features of Y cells. We also have now shown that the onset of Cat-301 staining parallels the development of Y cells.

Detailed physiological and morphological studies have shown that normal Y-cell development is closely correlated with exposure to normal visual environments. Y-cell development is perturbed in cats that have been reared from birth in the dark or with one or both eyes sutured closed. We have now demonstrated that the characteristic Cat-301-staining pattern does not develop in animals reared in visually deprived settings. In animals reared in the dark from birth, Cat-301 staining is virtually absent throughout the LGN. In animals reared in the light with one eye sutured at birth, the cell layers in the LGN that receive input from the closed eye fail to develop Cat-301 staining (Fig. 1). The expression of the antigen recognized by antibody Cat-301 correlates with the development of mature Y-cell characteristics, perhaps reflecting the maintenance of

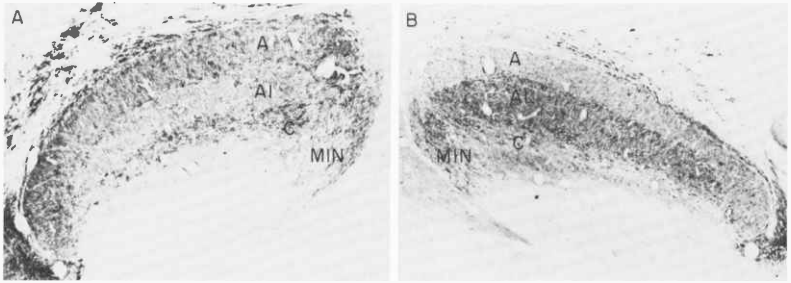

FIGURE 1 Monocular deprivation at birth results in a depletion of Cat-301 immunoreactivity in deprived LGN laminae. (*A*) LGN ipsilateral to the deprived eye (at 1 year of age) shows decreased Cat-301 staining in lamina A1. The reduction in staining in A1 allows the staining of lamina C to be clearly seen. (*B*) LGN contralateral to the deprived eye shows a decrease in Cat-301 immunoreactivity in laminae A and C relative to A1. Both contralaterally and ipsilaterally immunoreactivities are also lost in the MIN.

the mature set of synaptic connections. This finding has important implications for the elucidation of the mechanisms of neuronal development. It shows that antigen expression correlates with events at critical periods of development and opens the possibility that the molecular biology of experience-determined neuronal development may be accessible to study.

Experiments are in progress to determine whether normal synaptic development in vivo can be retarded or disrupted by administration of Cat-301 or its antigen. Our preliminary studies have shown that Cat-301 specifically binds to subsets of neurons when administered in vivo. Antibody-mediated cell killing will be attempted by injection of antibody coupled to toxins to determine the role of the affected cells in neural development and in visual processing.

Efforts are now being directed toward purification of the antigen recognized by Cat-301. Histochemical examination indicates that the antigen is relatively rare within whole brain. Direct Western blot analysis of unfractionated tissue thus far has not identified the antigen. However, detergent extracts of guinea pig brain stem, when concentrated 10–20-fold by ultrafiltration, demonstrate Cat-301 immunoreactivity on nitrocellulose dot blots. Such an assay is being used to monitor antigen purification by immunoaffinity chromatography. The purpose of this purification effort is to generate monospecific polyclonal sera that in turn will enable us to identify the gene responsible for Cat-301 antigen expression in a brain cDNA library. In addition, we will be asking whether structurally homologous antigens exist in other brain areas and whether they are involved in the development of normal adult synaptic connections.

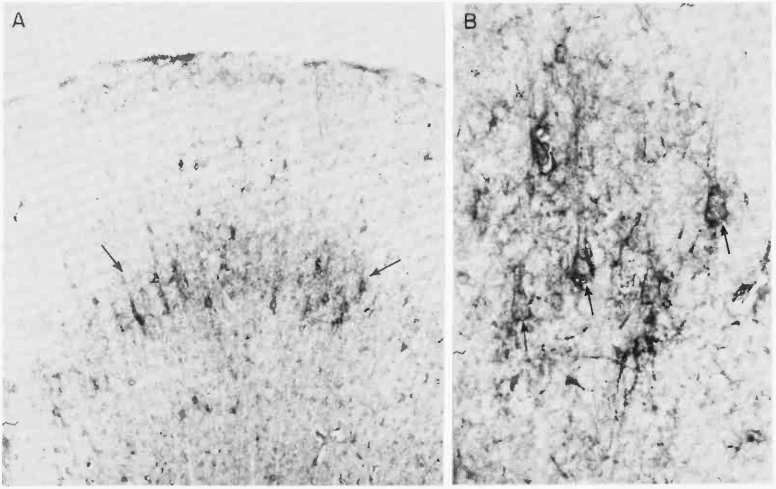

FIGURE 2 Human visual cortex contains Cat-301-positive neurons. (*A*) Sections of human visual cortex stained with Cat-301 contain patches of antibody-positive neurons. The high level of background staining results from fixation by immersion rather than by perfusion. (*B*) At higher magnification, Cat-301-positive neurons (arrows) display the same surface staining characteristics that have been demonstrated in cat and monkey CNS. This conservation of subcellular localization suggests that Cat-301 recognizes the same, or closely related, antigens in all three species.

MARKING AREAL BOUNDARIES WITH MONOCLONAL ANTIBODIES

Classical neurobiology has provided maps of the vertebrate cerebral cortex that divide the brain into areas with specific functions and body representations. Nonspecific cell stains have illustrated different neuronal cell types in the cortex and their organization. Hybridoma technology provides a means of selectively staining identified neuronal types and establishing a molecular basis for their anatomical and physiological diversity. Monoclonal antibodies have shown differences among cell populations that were heretofore unrecognized.

Previously, we have reported that in the primate visual cortex, Cat-301 identifies neurons in a pattern that reflects the ocular dominance organization of the primary visual cortical area (V1). A dramatic shift in staining patterns marks the boundary between V1 and the adjacent second visual cortical area (V2). This boundary can be resolved using more classical techniques, but elsewhere in the primate cortex, Cat-301 staining marks boundaries between less well resolved areas. For example, the boundaries between areas MT and MST and between V3 and V4 appear to be marked by changes in Cat-301-staining patterns. Currently, electrophysiological and tract-tracing techniques are being used in conjunction with Cat-301 immunohistochemistry to confirm these areal borders.

One of the difficulties in understanding human cortical function has been the inability to set normal ranges for the size and location of cortical areas. In experimental animals, areal boundaries have been determined by invasive methods, such as electrophysiological recording and tract-tracing studies. The conservation of antigens through evolution may allow us to use markers like Cat-301 to study the organization of the human cortex in postmortem material. We have already shown that Cat-301 recognizes an antigen in human cortex (Fig. 2) that is related to the antigen recognized in cat and monkey. The cells that express the Cat-301 antigen in human area V1 have a patchy distribution similar to that seen in the macaque monkey. Our current studies are aimed at establishing rapid, reproducible criteria for mapping areal boundaries in the human cortex.

Another recently generated monoclonal antibody, Rat-203, recognizes subsets of neurons in the rat CNS and shows sharply defined cortical boundaries in rat cortex. The antibody, which identifies a 180,000-molecular-weight polypeptide in Western blot experiments, recognizes projection neurons in rat neocortex, but not in paleocortical areas such as the olfactory cortex and dentate gyrus. In the cerebellar cortex, Rat-203 recognizes a small group of interneurons in the paleocerebellar area, but not in the archicerebellar area, whereas projection neurons are Rat-203-positive throughout the cerebellum.

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INVERTEBRATES

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Monoclonal Antibodies Demonstrate Sexually Dimorphic Structures in the Moth CNS

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The relative simplicity of the invertebrate CNS compared with the vertebrate CNS often makes basic problems in neurobiology more accessible to study. The existence of sexual dimorphism in the CNS is a highly charged issue, but several studies have shown differences between sexes in both invertebrate and vertebrate systems. In the moth, *Manduca sexta,* male-specific sensory neurons in the antennae, which respond to the female sex pheromone, connect to male-specific neurons in the antennal lobes of the CNS at the male-specific synaptic region, the macroglomerular complex (MGC). Previous studies showed that surgical transplantation of the imaginal disks of the male antennae into female hosts gives rise to MGC-like structures in the gynandromorphic female antennal lobe. To obtain reagents with which to study the mechanisms that govern the development of sexually dimorphic structures, monoclonal antibodies were generated to the male moth CNS.

The olfactory-specific antibody (OSA) stains the primary olfactory neurons both in males and females. Cell bodies, proximal parts of dendrites, and axons are recognized by this antibody. The intracranial portion of the antennal nerve contains sensory axons of two different modalities. Here, the olfactory axons are stained by the OSA but the mechanosensory axons are not. In the antennal lobe, the olfactory axons make synaptic contacts in structures called glomeruli. The OSA recognizes every glomerulus (Fig. 1A). Cell bodies of another set of primary olfactory neurons are located in a part of the mouth, the labial pulp organ, which also sends axons into the antennal lobe. The tracts from the labial pulp organ to the antennal lobes are also stained by the OSA antibody. To confirm the specificity of OSA for olfactory structures, the whole brain and the thoracic ganglion were tested. OSA staining was confined to the primary olfactory neurons. At the electron microscopic level, OSA staining is associated with the external side of the plasma membrane of cell bodies and axons. As in our previous studies in the leech, axons with surface OSA staining travel together in fascicles. We have previously suggested that such axonal surface molecules may be chemoaffinity molecules by which axons navigate along specific routes to their targets. The synaptic terminals of the primary olfactory neurons in the glomeruli of the antennal lobe, which cannot be resolved at the light microscopic level, can be identified by the presence of antibody staining at the electron microscopic level.

The male-specific antibody (MSA) stains the primary olfactory neurons in males but *not* in females. The MSA recognizes cell bodies, dendrites, and axons. There are two types of hair-like sensillae in the antennae; longer ones are found only in males and shorter ones in both sexes. The dendrites of the primary olfactory neurons that lie in long sensillae are stained by MSA. In females, primary olfactory neurons are not stained by MSA. In the antennal lobe, only the male-specific glomerulus is stained by MSA (Fig. 1B). The ordinary glomeruli, which are common to males and females, are unstained. Another cell type, associated with the limiting membrane of the antennae, is stained in both males and females. The position and developmental appearance of this second type of MSA-positive cell suggest that these cells are developmentally related to the primary olfactory neurons, perhaps being an undifferentiated progenitor cell.

At present, we are characterizing the antigens recognized by these antibodies by Western blot analysis and studying the expression of their antigens in the development of the moth. We hope to be able to describe developmental events that give rise to male-specific structures.

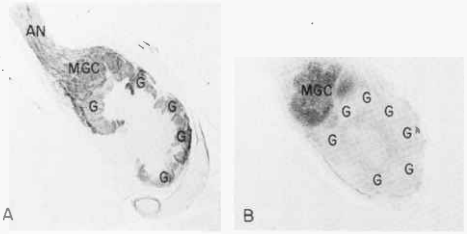


FIGURE 1 Monoclonal antibodies recognize olfactory structures in male and female (*A*) and male-specific structures (*B*) in mature CNS. (*A*) Olfactory-specific antibody recognizes primary olfactory neurons, their axons in the antennal nerve (AN), and their terminals in the male-specific glomerulus (MGC) and in the glomeruli common to both males and females (G). (*B*) In contrast, the male-specific antibody (MSA) only recognizes profiles in the male-specific MGC and not in the common glomeruli.

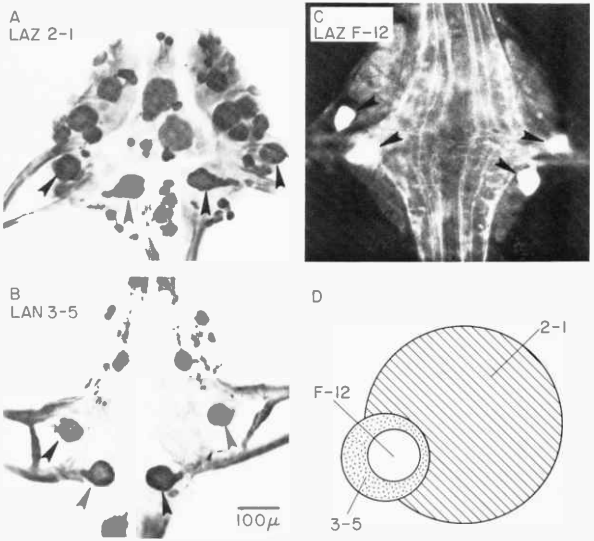


FIGURE 2 A variety of monoclonal antibodies immunocytologically label sets of antigenically homologous neurons in the leech CNS. Three such neuron sets are indicated in this plate. Pressure-sensitive mechanosensory neurons are contained in cell sets labeled with Laz2-1 (*A*) and Lan3-5 (*B*) and are exclusively labeled by LazF-12 (*C*). Cell sets Laz2-1 and Lan3-5 intersect, and their intersection contains cell set LazF-12 (*D*).

Cellular Organizing Systems in the Leech: Are Nervous Systems Built by Interactions among Antigenically Related, Functionally Heterogeneous Sets of Neurons?

G. Bablanian, T. Flanagan, B. Zipser

An extension of classic approaches to the study of nervous system networks has been the application of immunocytological probes to define sets of immunochemically related cells. In the leech, we have identified 11 different antigenically related neuron sets. Such sets vary in size, containing from 2 to 50 neurons. We have identified individual neurons within these sets and we realize that antigenically related neurons can serve diverse physiological functions. Some identified neurons belong to more than one set of antigenically related cells. In the leech, antigenically related cell sets are thus either intersected by or contained within other antigenically related cell sets (see Fig. 2). We have recently identified a pair of neurons that are common to a disproportionately large number of antigenically related cell sets. Why do these cells (which we call α cells) carry such a rich array of set-specific antigens? Do these antigens correlate with or specify synaptic connections? If such a correlation exists, then these α cells function in a variety of neuronal networks. Perhaps they play a pivotal role in activating one of several mutually exclusive behavioral networks. Electrophysiological analysis will be able to address this hypothesis in future studies of the leech nervous system. At present, the broader question of the significance of diverse and intersecting antigenically related cell sets remains unanswered. As we expand our catalog of antigenically related cell sets, we are continually searching for clues (such as α cells) that might suggest the biological basis for this phenomenon.

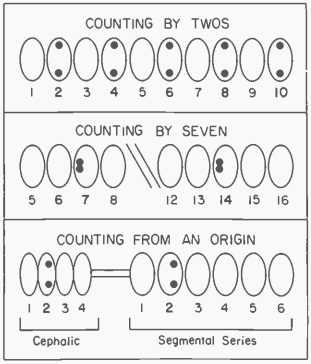


FIGURE 3 Immunocytologically labeled neurons that appear in nonadjacent ganglia along the axis of the leech CNS suggest the existence of segmental counting mechanisms. One such mechanism assigns immunoreactive neurons to even-numbered ganglia, counting through the CNS in two-segment units (*top*). Another such mechanism assigns immunoreactive neurons to seven-segment units within the midbody ganglionic chain (*middle*). A third mechanism counts off two-segment units from the origin of the cephalic and midbody ganglionic series (*bottom*).

Supersegmental Organizing Systems in the Leech: Immunocytologically Identified Neurons That Appear in Serially Repeated Multiganglion Units Along the Segmental Axis of the Leech CNS Suggest the Existence of Supersegmental Counting Mechanisms

T. Flanagan, C. Schley, B. Zipser

The leech central nervous system (CNS) is composed of a series of 32 homologous ganglia that are each derived from one of the 32 embryonic segments that form the leech body axis. Each ganglion consists of a core population of about 400 individually identifiable neurons and an additional set of specialized cells restricted to selected ganglia. We have used immunocytological methods to identify three sets of specialized cells that are serially expressed in nonadjacent ganglia throughout the leech CNS. The distribution of these cells define multisegmental units within the leech CNS and suggest the existence of supersegmentation counting mechanisms that individually label each leech ganglion (Fig. 3). We have begun electrophysiological analysis of these specialized counting neurons, and we now realize that they are interganglionic interneurons. Some of these

cells project pervasively throughout the axis of the CNS. We suspect that such cells contact and repress the expression of their segmental homologs in adjacent ganglia and thus sculpture repeating patterns in nonadjacent ganglia throughout the CNS. Our hypothesis predicts that transection of interganglionic connectives in embryonic leech will prevent cellular interactions among interganglionic homologs and will alter the supersegmental structure of the leech CNS.

Vertebrate Neurotransmitter Substances in the Leech: A Substance-P-like Peptide in Leech Interneurons

T. Flanagan [in collaboration with R. Stewart and E. Macagno, Columbia University, and E. Floor and S. Leeman, University of Massachusetts Medical Center]

Polyclonal antisera raised against substance P (RD2-C1) was screened immunocytologically on leech ganglia to identify neurons that contain a substance-P-like antigen. This immunoreactivity is confined to four identifiable cephalic interneurons and is fully blocked when antisera is preadsorbed with substance P. Homogenates of leech CNS were analyzed by radioimmunoassay to corroborate immunocytological observations, and, subsequently, 150 leech nerve cords were pooled and fractionated on Sephadex G-25 to determine the size of the leech substance-P-like antigen (Fig. 4). These studies indicate that the leech can now be added to the list of invertebrates that contain a neuronal peptide antigenically homologous to vertebrate substance P. Future studies include plans first to identify leech substance-P target cells and then to characterize their receptors pharmacologically. The precision with which cellular circuits can be analyzed in the leech promises that these studies will contribute a solid reference for the biology and evolution of phylogenically conserved peptide transmitter systems.

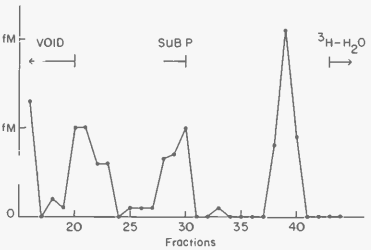


FIGURE 4 Polyclonal antiserum raised against substance P (RD2-C1) was used in a radioimmunoassay to identify a peptide antigen from homogenates of a leech CNS that comigrates with vetebrate substance P. This antiserum also recognizes a high-molecular-weight antigen that may be a precursor to the leech substance-P-like peptide.

PUBLICATIONS

Stewart, R.R., E.R. Macagno, and B. Zipser. 1985. The embryonic development of peripheral neurons in the body wall of the leech *Haemopis marmorata*. *Brain Res.* **332:** 150–157.

Zipser, B. 1984. Preparation of monoclonal antibodies and their advantages in identifying specific neurons. *Adv. Cell. Neurobiol.* **5:** 271–305.

———. 1985. A monoclonal antibody to an internal molecule differentiates three electrically coupled leech neurons. In *Model neural networks and behavior* (ed. A. Selverston), pp. 479–490. Plenum Press, New York.

In Press, Submitted, and In Preparation

Bablanian, G., T. Flanagan, and B. Zipser. Three monoclonal antibodies which each label sets of functionally diversified neurons: A generalized phenomenon in the leech CNS. (In preparation.)

Flanagan, T. and B. Zipser. 1985. Families of opioid and substance P-like peptides in the leech central nervous system. In *Handbook of comparative aspects of opioid and related neuropeptide mechanisms* (ed. G. Stefano). CRC Press, Boca Raton, Florida. (In press.)

———. Varicosity-associated antigens define neuropile subfields in the leech nervous system. *J. Neurocytol.* (in press).

Flanagan, T., C. Schley, and B. Zipser. Antibody staining reveals novel aspects of segmentation in the leech CNS. *Brain Res.* (in press).

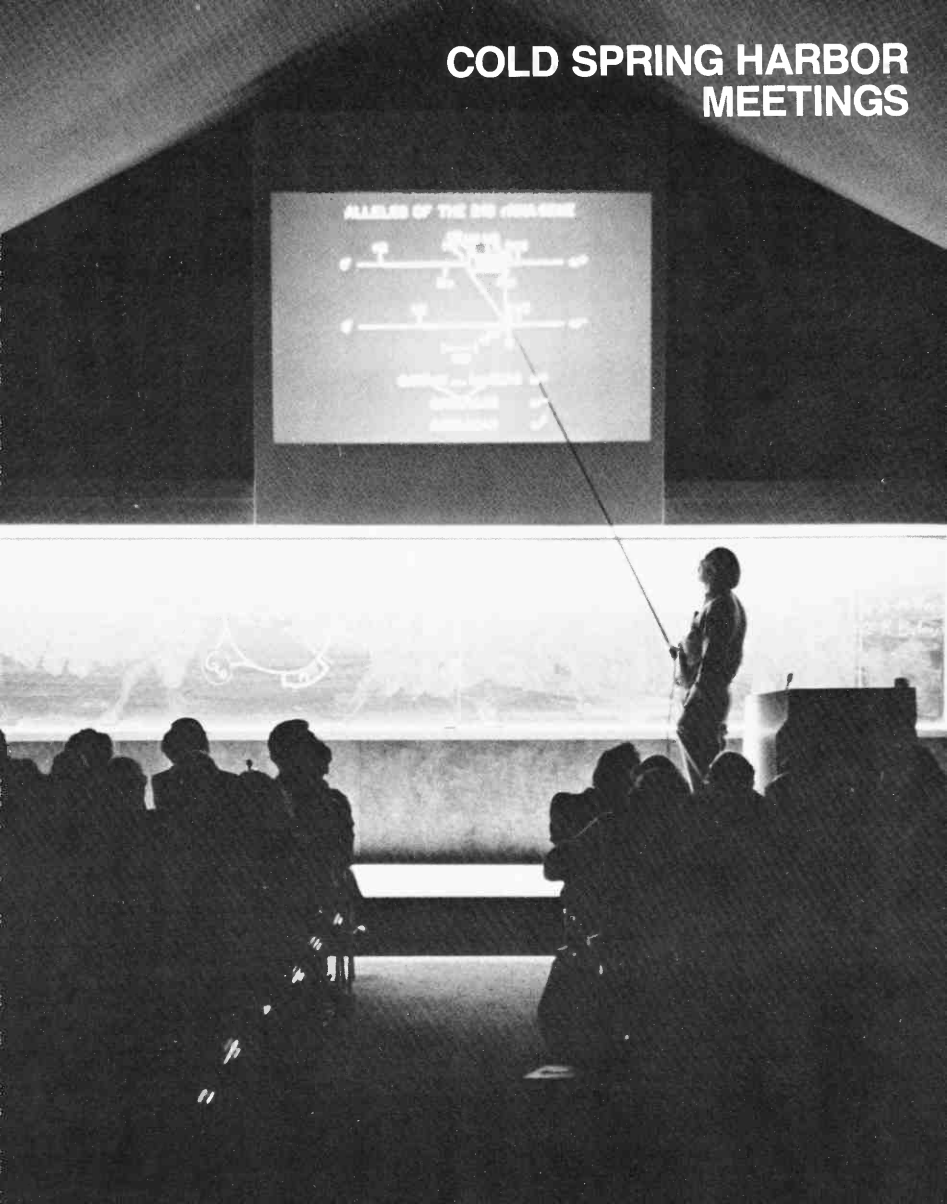
Flanagan, T., J. McInnes, C. Schley, and B. Zipser. Immunochemical analysis of a set of 130 kD antigens expressed by three identified cell types in the leech central nervous system. (In preparation).

Flaster, M. and B. Zipser. Macroglial cells of the leech are molecularly heterogeneous. (In preparation.)

Hishinuma, A., S. Hockfield, R. McKay, and J. Hildebrand. Monoclonal antibodies reveal cell-type specific antigens in the sexually dimorphic olfactory system of *Manduca sexta*. (In preparation.)

Zipser, B., G. Bablanian, T. Flanagan, and S. Benzer. The leech CNS is divided into overlapping sets of neurons which are defined by chemical labels. (In preparation.)

COLD SPRING HARBOR MEETINGS



Recto: Vannevar Bush Lecture Hall, since its construction in 1953, has been the site of the annual Symposium and other meetings. Vannevar Bush was president of the Carnegie Institution of Washington when this building was erected.

49TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Recombination at the DNA Level

May 30–June 6

248 participants

The principle of genetic linkage in Mendelian inheritance was established by T.H. Morgan in 1911. That genetic recombination occurs by chromosomal crossing-over events was first shown in two independent studies, both published in 1931—one by H.S. Creighton and B. McClintock working with maize and the other by C. Stern working with *Drosophilia*. Since that time a small, elite group of geneticists have thought about and experimentally analyzed genetic recombination with such profound consequences that introductory genetics was, and still is, learned by studying chromosome recombination.

Publication by R. Holliday in 1964 of the first conceptual framework to explain genetic recombination in molecular terms spurred an ever-increasing number of scientists to work in this area. However, the main tool of investigation remained strictly transmission genetics until the dawn of recombination in molecular detail. Our choice of the topic "Recombination at the DNA Level" for the 49th annual Symposium underscores these recent achievements as well as the future prospects in this field. In planning this Symposium, we emphasized primarily the biochemical aspects of recombination, including systems ranging from bacteriophage to mammalian cells.

To make this meeting most exciting and informative, we called upon the advice of our colleagues in the field. In particular we wish to acknowledge the wonderful counsel given by Charles Radding, Nick Cozzarelli, Barbara McClintock, and Bob Weisberg. The formal meeting program contained 94 excellent presentations, with a high proportion of attendees staying through to the very end of this rather lengthy meeting. The introductory remarks by Bruce Alberts and the summary delivered by Allan Campbell were most appropriate; we thank them for their efforts.

Financial support for this year's meeting was provided by the National Cancer Institute, Fogarty International Center, the Department of Energy, and the National Science Foundation.

Introduction: **B. Alberts,** University of California, San Francisco

SESSION 1 CHROMOSOME MECHANICS

Chairperson: F. Stahl, University of Oregon, Eugene, Oregon

Rossignol, J.-L., Nicolas, A., Hamza, H., Kalogeropoulos, A., Langin, T., Laboratoire d'Etude des Interactions Moléculaires Génomiques, Université Paris, France: Origins of gene conversion and reciprocal exchange in *Ascobolus*.

Kohli, J., Munz, P., Aebi, R., Amstutz, H., Gysler, C., Heyer, W., Schuchert, P., Szankasi, P., Leupold, U., Institute of General Microbiology, University of Bern, Switzerland: Intergenic conversion between tRNA genes and the influence of specific mutations on allelic recombination.

Carpenter, A.T.C., Dept. of Biology, University of California, San Diego, La Jolla: Meiotic coconversion length in *Drosophila* recombination-defective mutants.

A. Klar, B. McClintock

J. Strathern

Esposito, M.S.,[1] Hosoda, J.,[1] Golin, J.,[2] Moise, H.,[1] Bjornstad, K.,[1] Maleas, D.,[1] [1]Lawrence Berkeley Laboratory, University of California, Berkeley; [2]Central Research and Development, E.I. Dupont de Nemours, Wilmington, Delaware: Recombination in *S. cerevisiae*–*rec*-gene mutants and ssDNA-binding proteins.

Hastings, P.J., Dept. of Genetics, University of Alberta, Edmonton, Canada: Conversion by mismatch repair in yeast.

SESSION 2 YEAST SYSTEMS

Chairperson: J. Strathern, Cold Spring Harbor Laboratory, New York

Fogel, S., Welch, J.W., Louis, E., Dept. of Genetics, University of California, Berkeley: Meiotic gene conversion mediates gene amplification in yeast.

Haber, J., Borts, R., Lichten, M., Hearn, M., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Initiation and timing of meiotic recombination in yeast.

Klar, A., Strathern, J., Hicks, J., Cold Spring Harbor Laboratory, New York: Initiation and resolution steps of yeast mating-type gene transposition.

Kostriken, R.,[1] Heffron, F.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Molecular Biology, Salk Institute, La Jolla, California: The product of the HO gene is a site-specific, double-strand endonuclease that initiates mating-type switching.

Jensen, R., Stern, M., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Multifaceted control of *HO*-gene expression—Pattern of mating-type interconversion.

Miller, A.M., Nasmyth, K.A., MRC Laboratory of Molecular Biology, Cambridge, England: Role for DNA replication in the control of gene expression at a distance.

Butow, R.A., Hudson, A.P., Zinn, A., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Gene conversion at the yeast mitochondrial 21S rDNA locus.

A. Campbell, N. Cozzarelli

SESSION 3 MAMMALIAN HOMOLOGUS RECOMBINATION

Chairperson: O. Smithies, University of Wisconsin, Madison, Wisconsin

Folger, K., Thomas, K., Capecchi, M.R., Dept. of Biology, University of Utah, Salt Lake City: Analysis of homologous recombination in cultured mammalian cells.

Lin, F.-L., Sperle, K., Sternberg, N., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Homologous recombination in cultured mouse L cells.

Brenner, D.A., Smigocki, A.C., Camerini-Otero, R.D., NIADDK, National Institutes of Health, Bethesda, Maryland: Effect of heterology on homologous recombination between mutant *tk* genes in mouse Ltk⁻ cells.

Smithies, O.,[1] Koralewski, M.A.,[1] Song, K.-Y.,[2] Kucherlapati, R.S.,[2] [1]Laboratory of Genetics, University of Wisconsin, Madison; [2]Center for Genetics, University of Illinois College of Medicine, Chicago: Homologous recombination with DNA introduced into mammalian cells.

Smith, A.J.H., Berg, P., Dept. of Biochemistry, Stanford University, California: Measurements of the frequencies of homologous recombination events between defective *neo* genes in mouse 3T6 cells.

Liskay, R.M., Stachelek, J.L., Letsou, A., Depts. of Therapeutic Radiology and Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Homologous recombination between tandemly duplicated genes in cultured mouse cells.

Kucherlapati, R., Hannaken, A., Spenser, J., Moore, P., Dept. of Microbiology and Immunology, University of Illinois College of Medicine, Chicago: Study of homologous recombination in monkey cells and human cell-free extracts.

B. Alberts, O. Smithies, P. Starlinger

SESSION 4 TRANSPOSONS

Chairperson: N. Kleckner, Harvard University, Cambridge, Massachusetts

Redfield, R., Campbell, A., Dept. of Biological Sciences, Stanford University, California: Generation of nontandem duplications by homol ogous recombination with a λ prophage.

Ohtsubo, E.,[1] Ishizaki, K.,[2] [1]Institute of Applied Microbiology, University of Tokyo; [2]Radiation Biology Center, Kyoto University, Japan: Functional difference of the two ends of insertion element IS*1* in transposition and cointegration.

Berg, D.E., Lodge, J., Weston-Hafer, K., Lowe, J.B., Carle, G.F., Sasakawa, C., Depts. of Microbiology and Immunology, and Genetics, Washington University Medical School, St. Louis, Missouri: Specificity determinants in Tn*5* transposition.

Sherratt, D.,[1] Dyson, P.,[2] Brown, L.,[1] Summers, D.,[1] Stewart, G.,[1] [1]Dept. of Genetics, University of Glasgow, Scotland; [2]Dept. of Microbiology, Dalhousie University, Halifax, Canada: Site-specific recombination in transposition and plasmid stability.

Morisato, D., Bender, J., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Physical and genetic analysis of the Tn*10* transposition.

Reed, R.R., Moser, C.D., Dept. of Molecular Biology and Genetics, Johns Hopkins School of Medicine, Baltimore, Maryland: Mapping regions of the transposase important in the recognition of Tn*3*-family terminal inverted repeats.

Weinert, T.A., Grindley, N.D.F., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Transposition of Tn*903* – Evidence for both replicative and conservative modes of transpositional recombination.

Newman, B., Falvey, E., Wiater, L., Grindley, N., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Site-specific recombination by the γ δ resolvase – Analysis of the protein and its interaction with the *res* site.

SESSION 5 Mu

Chairperson: N. Symonds, University of Sussex, Sussex, England

Akroyd, J., Barton, B., Lund, P., Smith, S.M., Sultana, K., Symonds, N., University of Sussex, School of Biological Sciences, England: Two aspects of Mu recombination.

Patterson, T.A., Martin, K.A., Weiss, R., Gould, J., Bukhari, A.I., Cold Spring Harbor Laboratory, New York: Studies of the expression and regulation of bacteriophage Mu genes using Mu-*lacZ* fusions.

Harshey, R.M., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: One mode of bacteriophage Mu transposition is not coupled to replication.

Chaconas, G., Gloor, G., Miller, J.L., Giddens, E.B., Depts. of Biochemistry and Microbiology, and Immunology, University of Western Ontario, London, Canada: Studies on bacteriophage Mu DNA transposition.

Kahmann, R., Mertens, G., Max-Planck-Institut, Berlin, Federal Republic of Germany: Substrate and enzyme requirements for in vitro site-specific recombination in bacteriophage Mu.

Plasterk, R.H.A., van de Putte, P., Laboratory of Molecular Genetics, State University of Leiden, The Netherlands: Inversion of the G-DNA segment in bacteriophage Mu and related systems in prokaryotes.

Kamp, D., Kardas, E., Ritthaler, W., Max-Planck-Institut, Martinsried, Federal Republic of Germany: Comparative analysis of invertible DNA in phage genomes.

Shapiro, J.A., Dept. of Microbiology, University of Chicago, Illinois: Programming of DNA rearrangements involving Mu prophages.

SESSION 6 PLANT TRANSPOSONS/T4 RECOMBINATION

Chairperson: P. Starlinger, University of Cologne, Cologne, Federal Republic of Germany

Dellaporta, S.L.,[1] Chomet, P.S.,[1] Mottinger, J.P.,[2] Hicks, J.B.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]University of Rhode Island, Kingston: Spontaneous mutation caused by insertion elements in maize.

Courage, U., Döring, H.-P., Kunze, R., Laird, A., Merckelbach, A., Müller-Neumann, M., Starlinger, P., Tillmann, E., Weck, E., Werr, W., Yoder, J., Institut für Genetik, Universität Köln, Federal Republic of Germany: Transposable elements *Ac* and *Ds* in *Z. mays*.

Fedoroff, N.,[1] Kelly, S.,[1] Pohlman, R.,[2] Messing, J.,[2] Furtek, D.,[3] Nelson, O.,[1] [1]Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland; [2]Dept. of Biochemistry, University of Minnesota, St. Paul; [3]Dept. of Genetics, University of Wisconsin, Madison: Molecular analysis of transposable controlling elements in maize.

Peacock, W.J.,[1] Dennis, E.S.,[1] Gerlack, W.L.,[1] Schwartz, D.,[2] [1]Division of Plant Industry, CSIRO, Canberra, Australia; [2]Dept. of Biology, Indiana University, Bloomington: Insertion and excision of *Ds*-elements in maize.

Saedler, H., Bonas, U., Harrison, B., Krebbers, E., Nevers, P., Piotrowiak, R., Sommer, H., Upadhyaya, K., Gierl, A., Klösgen, R.B., Peterson, P.A., Schwartz-Sommer, Z., Wienand, U., Max-Planck-Institute, Cologne, Federal Republic of Germany: Transposable elements in *A. majus* and *Z. mays*.

Formosa, T., Alberts, B.M., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Studies on genetic recombination proteins of bacteriophage T4.

Mosig, G., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Processing of recombinational intermediates in bacteriophage T4.

SESSION 7 TOPOISOMERASE, RESOLVASE, AND GYRASE

Chairperson: N. Cozzarelli, University of California, Berkeley

Benjamin, H., Wasserman, S., Gerrard, S., Dungan, J., Matzuk, M., Krasnow, M., Cozzarelli, N., Dept. of Molecular Biology, University of California, Berkeley: Topological and structural analysis of the recombination by Tn3 resolvase.

Ikeda, H., Miura, A., Shiozaki, M., Institute of Medical Science, University of Tokyo, Japan: Nonhomologous recombination mediated by *E. coli* DNA gyrase – Possible involvement of DNA replication.

Wang, J., Dept. of Biochemistry/Molecular Biology, Harvard University, Cambridge, Massachusetts: Breakage and rejoining of DNA strands by DNA topoisomerases.

O'Connor, M.B., Malamy, M.H., Tufts University School of Medicine, Boston, Massachusetts: Mapping of in vivo DNA-gyrase cleavage sites in pBR322 and their role in site-specific recombination.

Menzel, R., Gellert, M., NIADDK, National Institutes of Health, Bethesda, Maryland: Control of the expression of DNA gyrase in *E. coli*.

Champoux, J.J., McCoubrey, W.K., Been, M.D., Dept. of Microbiology and Immunology, University of Washington School of Medicine, Seattle: Specificity of eukaryotic type-1 DNA topoisomerase.

SESSION 8 *E. coli* GENERAL RECOMBINATION

Chairperson: **A. Clark,** University of California, Berkeley

Mahajan, S.K., Pandit, N.N., Sarkari, J.F., Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay, India: Host functions in TN9 amplification – A new model for DNA amplification.

Clark, A.J., Sandler, S.J., Blanar, M.A., Willis, D.K., Chu, C., University of California, Berkeley: Genes of the RecF pathway of recombination in *E. coli*.

Kushner, S.R., Dykstra, C.C., Dept. of Genetics, University of Georgia, Athens: Purification and characterization of exonuclease V from *E. coli* K-12.

Lloyd, R., Picksley, S., Attfield, P., Benson, F., Buckman, C., Dept. of Genetics, University of Nottingham, England: Genetic analysis and regulation of inducible recombination in *E. coli*.

Hays, J.B., Smith, A.T., Friedman, S.F., Lee, E., Coffman, G.L., Dept. of Chemistry, University of Maryland-Baltimore County, Catonsville, Maryland: Recombination of nonreplicating UV-irradiated bacteriophage λ DNA.

Smith, G.R., Amundsen, S.K., Chaudhury, A.M., Cheng, K.C., Ponticelli, A.S., Roberts, C.M., Schultz, D.W., Taylor, A.F., Fred Hutchinson Cancer Research Center, Seattle, Washington: Roles of RecBC enzyme and Chi sites in homologous recombination.

Kobayashi, I., Stahl, M.M., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: Special sites in homologous recombination – Mechanism of the Chi-*cos* interaction in RecA-RecBC-mediated recombination in bacteriophage λ.

Top: S. Shaner, R. Kahn, S. Honigberg, R.P. Cunningham, D. Gonda, J. Flory, C.M. Radding, T. Shibata, M. Leahy
Bottom: M. Bianchi, C. DasGupta, S. Chow

SESSION 9 *recA*

Chairperson: **C. Radding,** Yale University, New Haven, Connecticut

Muniyappa, K., Tsang, S.S., Shaner, S., Radding, C., Depts. of Human Genetics and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Relationship of secondary structure, aggregation, and conjunction of DNA molecules to the mechanism of homologous pairing promoted by *recA* protein.

Morrical, S., Neuendorf, S., Cox, M., Dept. of Biochemistry, University of Wisconsin, Madison: Light-scattering studies of *recA* protein filaments in the presence and absence of ssDNA.

Bryant, F.R., Riddles, P.W., Lehman, I.R., Dept. of Biochemistry, Stanford University School of Medicine, California: Studies of the mechanism of strand exchange promoted by the *recA* protein of *E. coli*.

Shibata, T.,[1] Makino, O.,[1] Ohtani, T.,[1] Iwabuchi, M.,[1] Ikawa, S.,[1] Ando, T.,[1] Shibata, Y.,[2] Maeda, H.,[2] [1]Dept. of Microbiology, Riken Institute, Saitama; [2]Blood Transfusion Service, Tokyo University Hospital, Japan: Roles of processive unwinding by *E. coli recA* protein in homologous recombination.

Griffith, J., Chrysogelos, S., Register, J. III., Welsh, G., Lineberger Cancer Research Center, University of North Carolina, Chapel Hill: A hybrid complex involving SSB and RecA proteins bound to ssDNA in-

R. Sager

A. Skalka, A. Hershey

itiates the proper assembly of RecA-DNA filaments.

Stasiak, A., Stasiak, A.Z., Koller, T., Institute for Cell Biology, Zurich, Switzerland: Complexes of *recA* with DNA under condition of in vitro recombination.

Howard-Flanders, P., West, S.C., Rusche, J.R., Dept. of Molecular Biophysics and Biochemistry, and Dept. of Therapeutic Radiology, Yale University, New Haven, Connecticut: *recA* protein can promote strand-exchange reactions past double-strand breaks in certain positions, indicating that each *recA* monomer may have two non-equivalent DNA-binding sites.

SESSION 10 REPAIR

Chairperson: **S. Linn,** University of California, Berkeley

Linn, S., Samson, L., Brooks, P., Mosbaugh, D., Evans, D., Dept. of Biochemistry, University of California, Berkeley: Enzymology of DNA excision repair in mammalian cells.

Lu, A.-L., Welsh, K., Su, M., Clark, S., Modrich, P., Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Repair of DNA base-pair mismatches in vitro.

Walker, G.C., Pang, P.P., Tsen, S.D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mismatch repair genes of *S. typhimurium*.

Fishel, R.A., Kolodner, R., Laboratory of Molecular Genetics, Dana Farber Cancer Institute, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: An *E. coli* cell-free system that catalyzes the repair of symmetrically methylated heteroduplex DNA.

Radman, M., Mutagenes Group, Institut Jacques Nomod, RBM, Paris, France: Genetic consequences of mismatch repair in *E. coli*.

Kunes, S., Botstein, D., Fox, M.S., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Inverted dimer formation and interplasmid recombination in the repair of linearized plasmids during yeast transformation.

Rothstein, R., Dept. of Microbiology, New Jersey Medical School, Newark: Double-strand-break repair and recombination.

SESSION 11 EUCARYOTIC ENZYMES

Chairperson: **R. Holliday,** National Institute for Medical Research, London, England

Resnick, M., Chow, T., Nitiss, J., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Changes in chromosomal DNA during meiosis in repair mutants and the possible role of a deoxyribonuclease.

Skalka, A.M.,[1] Duyk, G.,[2] Longiaru, M.,[1] DeHaseth, P.,[2] Terry, R.,[1] Leis, J.,[2] [1]Dept. of Molecular Genetics, Hoffmann-La Roche Inc., Roche Research Center, Nutley, New Jersey; [2]Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio: Integrative recombination—A role for the retroviral reverse transcriptase.

Honjo, T., Kataoka, T., Konda, S., Nishi, M., Kodaira, M., Dept. of Genetics, Osaka University Medical School, Japan: In vitro studies on immunoglobulin gene rearrangement.

Holliday, R.,[1] Taylor, S.Y.,[1] Kmiec, E.,[2] Holloman, W.K.,[2] [1]National Institute for Medical Research, London, England; [2]Dept. of Immunology and Medical Microbiology, University of Florida, Gainesville: Characterization of *rec1* mutants and *rec1* protein of *U. maydis*.

Kmiec, E., Kroeger, P., Holloman, W., University of Florida, Gainesville: Interaction between *Ustilago rec1* protein and Z-DNA.

Ferro, A.M., McElwain, M.C., Reid, E.J., Olivera, B.M., Dept. of Biology, University of Utah, Salt Lake City: Poly ADP-ribose synthetase, DNA topoisomerase I and the suppression of strand-exchange events in somatic cells.

SESSION 12 INTEGRATION AND EXCISION OF BACTERIOPHAGE

Chairperson: H. Nash, National Institutes of Health, Bethesda, Maryland

Miller, H.I., Dept. of Molecular Biology, Genentech Inc., South San Francisco, California: *himA* gene of *E. coli*—Gene structure and regulation, homology with DNA-binding proteins, and role in site-specific recombination and gene regulation.

Leong, J.,[1] Nunes-Duby, S.,[1] Oser, A.,[1] Lesser, C.,[1] Youderian, P.,[2] Susskind, M.,[2] Landy, A.,[1] [1]Dept. of Molecular and Cell Biology, Brown University, Providence, Rhode Island; [2]Dept. of Microbiology, University of Massachusetts, Worcester: Site-specific recombination systems of φ80, P22, and λ.

de Massy, B.,[1] Studier, F.W.,[2] Dorgai, L.,[1] Appelbaum, E.,[1] Weisberg, R.,[1] [1]NICHD, National Institutes of Health, Bethesda, Maryland; [1]Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Role of Holliday structures in recombination.

Echols, H.,[1] Dodson, M.,[1] Roberts, J.D.,[2] McMacken, R.,[2] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland: Role of specialized nucleoprotein structures in site-specific recombination and initiation of DNA replication.

Nash, H.A., Kitts, P., Richet, E., National Institute of Mental Health, Bethesda, Maryland: λ Integrative recombination—Approaches to the synaptic mechanism.

Spengler, S.,[1] Stasiak, A.,[2] Benjamin, H.,[1] Wasserman, S.,[1] Gerrard, S.,[1] Dungan, J.,[1] Matzuk, M.,[1] Krasnow, M.,[1] Cozzarelli, N.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Institute for Cell Biology, Zurich, Switzerland: Topological tests of the mechanism of recombination by λ Int and Tn*3* resolvase.

Spengler, S.,[1] Stasiak, A.,[2] Cozzarelli, N.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Institute for Cell Biology, Zurich, Switzerland: Structure of knots and catenanes produced by λ Int—implications for mechanism and DNA structure.

Wine and Cheese Party

SESSION 13 SITE-SPECIFIC RECOMBINATION

Chairperson: J. Broach, State University of New York, Stony Brook, New York

Johnson, R., Bruist, M., Glaccum, M., Simon, M., Biology Division, California Institute of Technology, Pasadena: Molecular mechanism of phase variation.

Hoess, R., Abremski, K., Sternberg, N., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Studies on the Cre-*lox* site-

specific recombination system of bacteriophage P1.

Iida, S., Huber, H., Hiestand-Nauer, R., Meyer, J., Bickle, T., Arber, W., Biozentrum, University of Basel, Switzerland: Sequences used in crossover by the site-specific *cin* recombinase of bacteriophage P1.

McLeod, M., Volkert, F., Broach, J., Dept. of Microbiology, State University of New York, Stony Brook: Analysis of the *FLP* recombination system of the yeast plasmid 2-μm circle.

Sadowski, P., Lee, D., Beatty, L., Andrews, B., Babineau, D., Proteau, G., Vetter, D., Dept. of Medical Genetics, University of Toronto, Canada: Genetic recombination of bacteriophage T7 and yeast 2-μm circle DNA in vitro.

Cox, M., Bruckner, R., Meyer-Leon, L., Senecoff, J., Dept. of Biochemistry, University of Wisconsin, Madison: Purification and properties of the *FLP* protein of the yeast 2-μm plasmid.

SESSION 14 RECOMBINATION IN VITRO

Chairperson: M. Gellert, National Institutes of Health, Bethesda, Maryland

Kolodner, R., Symington, L., Howard, M., Muster-Nassal, C., Dana Farber Cancer Institute, Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Plasmid recombination catalyzed by cell-free extracts of yeast.

Kemper, B., Depka, M.V., Hoffmann, H., Jensch, F., Institute of Genetics, University of Cologne, Federal Republic of Germany: Resolution of Holliday structures in cruciform DNA by endonuclease VII.

Higgins, N.P.,[1] Krause, H.M.,[1] Olivera, B.M.,[2] [1]Dept.of Biochemistry, University of Alabama, Birmingham; [2]Dept. of Biology, University of Utah, Salt Lake City: Definition of the Mu operator and the use of repressor in analyzing in vivo and in vitro intermediates of transposition.

Mizuuchi, K., Mizuuchi, M., Craigie, R., NIADDK, National Institutes of Health, Bethesda, Maryland: Mechanism of transposition of bacteriophage Mu – Site-specific recognition of Mu ends by the Mu *A*-gene product and polarity of the strand-transfer reaction.

Rosenberg, J., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Kinked DNA in crystalline complex with *Eco*RI endonuclease.

Summary: A. Campbell, Stanford University, Stanford, California

Molecular Biology of the Cytoskeleton

April 25–April 29

ARRANGED BY
Gary Borisy, University of Wisconsin
Don Cleveland, Johns Hopkins Medical School
Douglas Murphy, Johns Hopkins Medical School

122 participants

The meeting Molecular Biology of the Cytoskeleton sought to bring together a diverse group of researchers whose principal interests center on some molecular aspect of cytoskeletal elements, but who by virtue of the particular experimental approach or organism employed do not have an opportunity for scientific exchange at other meetings. To this end, 122 investigators were assembled whose efforts included: genetic approaches to determination of function of cytoskeletal proteins in a diverse number of genetic systems, determination of the dynamics of assembly of cytoskeletal components both in vivo and in vitro, biochemical analysis of various cytoskeletal proteins and their variants, and finally, dissection of the regulatory pathways that control cytoskeletal gene expression. Overall, the congregation of investigators using diverse approaches to study cytoskeletal function and regulation achieved the original goal of introducing workers in each area of cytoskeletal investigation to alternative approaches and concepts.

This meeting was supported in part by the National Science Foundation and the following divisions of the National Institutes of Health: Fogarty International Center, National Cancer Institute, and the National Institute of General Medical Sciences.

Welcoming Remarks: **D. Murphy,** Johns Hopkins Medical School, Baltimore, Maryland

SESSION 1 STRUCTURE AND DYNAMICS OF THE CYTOSKELETON

Chairperson: G. Borisy, University of Wisconsin, Madison, Wisconsin

Borisy, G., Kronebusch, P., Laboratory of Molecular Biology, University of Wisconsin, Madison: Microtubule dynamics in vivo.

Kirschner, M., Mitchison, T., Dept. of Biochemistry, University of California, San Francisco: Microtubule dynamics in vitro.

Weber, K., Osborn, M., Geisler, N., Max-Planck-Institute, Goettingen, Federal Republic of Germany: Intermediate filaments—Structural and immunological analysis.

Kreis, T.E.,[1] Schlessinger, J.,[2] Geiger, B.,[2] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: Dynamic properties of actin, α-actinin, and vinculin in focal contact areas.

Salmon, E.D.,[1] Saxton, W.M.,[2] Leslie, R.J.,[2] Karow, M.L.,[2] Neighbors, B.,[2] McIntosh, J.R.,[2] [1]Dept. of Biology, University of North Carolina, Chapel Hill; [2]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Measurements of spindle microtubule dynamics by fluorescence redistribution after photobleaching.

S. Ward, P. Anderson

SESSION 2 MOSTLY MOLDS: MOLECULAR GENETICS

Chairperson: D. Botstein, Massachusetts Institute of Technology, Cambridge, Massachusetts

Botstein, D., Shortle, D., Novick, P., Thomas, J.H., Huffaker, T., Schatz, P., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Applying old and new genetic technology toward understanding the cytoskeleton of yeast.

Kilmartin, J.V., MRC Laboratory of Molecular Biology, Cambridge, England: Microtubules and actin filaments during the yeast cell cycle.

Pringle, J.R., Coleman, K., Lillie, S., Haarer, B., Adams, A., Jacobs, C., Robinson, J., Evans, C., Stapleton, A., Division of Biological Sciences, University of Michigan, Ann Arbor: Cellular morphogenesis and the cytoskeleton in yeast.

Thomas, J.H., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Yeast mutants affecting spindle function.

Novick, P.J., Osmond, B., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic analysis of actin and interacting proteins.

Rose, M., Fink, G.R., Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge: Mutations affecting nuclear fusion in yeast.

Birkett, C.R.,[1] Boston, R.S.,[2] Burland, T.G.,[2] Dove, W.F.,[2] Foster, K.E.,[1] Green, L.,[2] Gull, K.,[1] Johnston, L.,[2] Paul, E.C.A.,[1] Roobol, A.,[1] Schedl, T.,[2] Wilcox, M.,[1] [1]Biological Laboratory, University of Kent, Canterbury, England; [2]McArdle Laboratory, University of Wisconsin, Madison: Differential expression of the tubulin gene family in the life cycle of *Physarum polycephalum*.

Burland, T.G.,[1] Schedl, T.,[1] Gull, K.,[2] Dove, W.F.,[1] [1]McArdle Laboratory, University of Wisconsin, Madison; [2]Biological Laboratory, University of Kent, Canterbury, England: Genetics of the tubulin gene families in *Physarum*.

Morris, N.R., Weatherbee, J.A., Gambino, J., Bergen, L.G., Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Tubulins of *Aspergillus nidulans* – Genetics, biochemistry, and function.

Lai, E.Y., Remillard, S.P., Fulton, C., Dept. of Biology, Brandeis University, Waltham, Massachusetts: The α-tubulin gene family of *Naegleria* and its expression during differentiation.

SESSION 3 MOSTLY MITOSIS: CENTROMERES AND KINETOCHORES

Chairperson: M. Kirschner, University of California, San Francisco, California

Amaya, E., Bloom, K., Dept. of Biology, University of North Carolina, Chapel Hill: Molecular architecture of a yeast centromere.

Valdivia, M.M.,[1] Sykes, R.C.,[2] Chinault, A.C.,[2,3] Brinkley, B.R.,[1] Depts. of [1]Cell Biology, [2]Biochemistry, [3]Medicine, Baylor College of Medicine, Houston, Texas: Biochemical studies of the kinetochore/centromere of mammalian chromosomes.

Cox, J.V., Olmsted, J.B., Dept. of Biology, University of Rochester, New York: Kinetochore antigens – Synthesis, phosphorylation, and evolutionary conservation.

Vandre, D.D.,[1] Davis, F.M.,[2] Borisy, G.G.,[1] [1]Laboratory of Molecular Biology, University of Wisconsin, Madison; [2]University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston: Phosphoproteins are components of microtubule organizing centers of the mitotic spindle in Chinese hamster ovary cells.

Mitchison, T.J., Kirschner, M., Dept. of Biochemistry, University of California, San Francisco: Kinetochores as in vitro microtubule organizing centers.

SESSION 4 MOSTLY WORMS: MOLECULAR GENETICS IN WORMS AND HIGHER EUKARYOTES

Chairperson: E. Raff, Indiana University, Bloomington, Indiana

Raff, E.C., Fuller, M.T., Caulton, J.H., Rudolph, J.E., Kimble, M., Hutchens, J.A., Dept. of Biology, Indiana University, Bloomington: Tubulin gene expression and microtubule function in *Drosophila*.

Cox, K.H., Angerer, L.M., Angerer, R.C., Dept. of Biology, University of Rochester, New York: Individual actin gene expression – Markers for early differentiation of cell lineages in developing sea urchin embryos.

Bejsovec, A., Eide, D., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: Genetic techniques for analysis of nematode muscle.

Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Acquisition of cellular asymmetry during *C. elegans* spermatogenesis.

Epstein, H.F., Ortiz, I., Berliner, G.C., Miller, D.M. III, Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Functionally distinct

myosins in *C. elegans* analyzed through mutants.

Honda, S., Berman, S.A., Epstein, H.F., Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Regulation of myosin heavy-chain expression in *C. elegans*.

Krause, M.,[1] Wild, M.,[1] Hirsh, D.,[1] Waterston, R.,[2] [1]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder; [2]Dept. of Genetics, Washington School of Medicine, St. Louis, Missouri: *C. elegans* actin genes.

SESSION 5 MOSTLY CHO AND CHLAMY: MOLECULAR GENETICS OF CYTOSKELETAL PROTEINS

Chairperson: J. Rosenbaum, Yale University, New Haven, Connecticut

Cabral, F., Schibler, M., Division of Endocrinology, University of Texas Medical School, Houston: Analysis of the role of microtubules in mitosis using CHO mutants requiring taxol for cell growth.

Gottesman, M.M.,[1] Abraham, I.,[1] Kuriyama, R.,[2] Mackensen, S.,[1] Whitfield, C.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Molecular Biology, University of Wisconsin, Madison: Genetic analysis of microtubule function in CHO cells.

Abraham, I., McClurkin, C., Gottesman, M.M., NCI, National Institutes of Health, Bethesda, Maryland: Cross-resistance of CHO cells with tubulin mutations.

Whitfield, C., Doherty, P., Gottesman, M.M., NCI, National Institutes of Health, Bethesda, Maryland: Cloning wild-type and mutant tubulins from CHO cells.

Elliott, E.M.,[1] Okayama, H.,[2] Sarangi, F.,[1] Henderson, G.,[1] Ling, V.,[1] [1]Dept. of Medical Biophysics, University of Toronto and Ontario Cancer Institute, Canada; [2]National Institutes of Health, Bethesda, Maryland: Expression of multiple α-tubulin genes in CHO cells.

Dutcher, S.K.,[1] Ramanis, Z.,[2] Luck, D.J.L.,[2] [1]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder; [2]Rockefeller University, New York: Flagellar mutants of *C. reinhardtii* that show altered molecular phenotypes in isolated basal body preparations.

Youngblom, J.,[1] Schloss, J.,[2] Silflow, C.,[1] [1]Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul; [2]Dept. of Biology, Yale University, New Haven, Connecticut: Comparison of the two β-tubulin genes in *Chlamydomonas*.

Brunke, K.,[1,2] Anthony, J.,[1,2] Sternberg, E.,[1,3] Weeks, D.,[2] [1]Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania; [2]Zoecon Corp., Palo Alto, California; [3]Smith-Kline and French Laboratories, Swedeland, Pennsylvania: Coordinate expression of the four tubulin genes in *Chlamydomonas*.

Lefebvre, P., Barsel, S., Stuckey, M., Swartz, L., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Isolation and characterization of mutants of *Chlamydomonas* defective in the regulation of flagellar gene expression.

Kuchka, M., Jarvik, J., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Genetic analysis of flagellar size control in *C. reinhardtii*.

Baldwin, D., Chojnacki, B., Jarvik, J., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Defective flagellar assembly in stumpy flagella mutants of *C. reinhardtii*.

SESSION 6 PROTEIN ISOFORMS

Chairperson: D. Murphy, Johns Hopkins Medical School, Baltimore, Maryland

Murphy, D.B., Grasser, W.A., Wallis, K.T., Dept. of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland: Expression of a unique β-tubulin variant in chicken-red-cell development.

Craig, S.W.,[1] Pardo, J.V.,[2] Pittenger, M.F.,[1] [1]Johns Hopkins Medical School, Baltimore, Maryland; [2]Duke Medical School, Durham, North Carolina: Suellular sorting of isoactins.

Blose, S.H., Matsumura, F., Cold Spring Harbor Laboratory, New York: Molecular analysis of microtubules rapidly purified from cells using monoclonal-antibody-induced aggregation.

Vallee, R., Bloom, G., Luca, F., Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Differential cellular and suellular distribution of microtubule-associated proteins.

Lazarides, E., Nelson, W.J., Kasamatsu, T., Division of Biology, California Institute of Technology, Pasadena: Expression, assembly, and topogenesis of membrane-cytoskeletal domains in neurons.

Piperno, G., Rockefeller University, New York: Analysis of the mitotic apparatus by monoclonal antibodies to components of flagellar axonemes.

Wiche, G.,[1] Briones, E.,[1] Koszka, C.,[1] Leichtfried, F.E.,[1] Krepler, R.,[2] Artlieb, U.,[2] [1]Institute of Biochemistry; [2]Dept. of Pathology, University of Vienna, Austria: Widespread occurrence of microtubule-associated proteins MAP 1 and MAP 2 in nonneuronal cells/tissues and association of both polypeptides with microtubules polymerized in vitro.

Aebi, U.,[1] Salvo, H.,[1] Leavitt, J.,[2] [1]Dept. of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland;

[2]Linus Pauling Institute of Science and Medicine, Palo Alto, California: In vitro characterization of a mutant β-actin expressed in a malignant human fibroblast cell line.

Cappuccinelli, P., Institute of Medical Microbiology, University of Sassari, Sardinia, Italy: Immunological heterogeneity of cytoskeletal proteins in eukaryotic microorganisms.

Owens, R.J., Totty, N., Waterfield, M.D., Crumpton, M.J., Imperial Cancer Research Fund, Lincoln's Inn Fields, London, United Kingdom: Structural characterization of p68, a new Ca^{2+}-binding protein of the cytoskeleton.

Maruta, H., Knoerzer, W., Isenberg, G., Max-Planck-Institute, Munich, Federal Republic of Germany: Nonpolymerizable variants regulate actin polymerization in *Physarum*.

SESSION 7 MOSTLY MAMMALIAN GENE EXPRESSION AND REGULATION

Chairperson: D. Cleveland, Johns Hopkins Medical School, Baltimore, Maryland

Cleveland, D.W.,[1] Pittenger, M.F.,[1] Feramisco, J.R.,[2] Havercroft, J.C.,[1] [1]Dept. of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Cold Spring Harbor Laboratory, New York: Autoregulated expression of vertebrate tubulins.

Lee, M.G.-S., Cowan, N.J., Dept. of Biochemistry, New York University Medical Center, New York: Modulation of mRNA levels transcribed from a human β-tubulin gene cloned in a eukaryotic shuttle vector.

J. Spudich

Baker, E., Bandziulis, R., Keller, L., Rosenbaum, J., Schloss, J., Dept. of Biology, Yale University, New Haven, Connecticut: Synthesis and accumulation of mRNAs for flagellar proteins during flagellar regeneration in *Chlamydomonas*.

Schedl, T.,[1] Burland, T.G.,[1] Gull, K.,[2] Dove, W.F.,[1] [1]McArdle Laboratory, University of Wisconsin, Madison; [2]Biological Laboratory, University of Kent, Canterbury, England: Regulation of tubulin expression in the *Physarum* cell cycle.

Landfear, S.M., Wirth, D.F., Harvard School of Public Health, Boston, Massachusetts: Structure and developmental regulation of tubulin genes in the parasitic protozoon *Leishmania enriettii*.

Farmer, S.R., Bond, J.F., Robinson, G.S., Mbangkollo, D., Fenton, M.J., Knight , G.B., Berkowitz, E.M., Dept. of Biochemistry, Boston University Medical School, Massachusetts: Differential expression of the β-tubulin multigene family during rat brain development—Identification of neural specific and constitutive mRNAs.

Sullivan, K.F., Lau, J.T.Y., Cleveland, D.W., Dept. of Physiological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, Maryland: Primary structure of a vertebrate β-tubulin gene family—At least three divergent β-tubulin isotypes are expressed in the chicken.

Havercroft, J.C., Cleveland, D.W., Dept. of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Programmed expression of β-tubulin genes during development and differentiation of the chicken.

Ginzburg, I., Littauer, U.Z., Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel: Expression and cellular regulation of microtubule proteins.

Drubin, D.,[1] Feinstein, S.,[2] Shooter, E.,[2] Kirschner, M.,[1] [1]Dept. of Biochemistry, University of California, San Francisco; [2]Dept. of Neurobiology, Stanford University, California: Expression of tau protein and tau mRNA during PC12 differentiation.

Holtzer, H., Friedman, T., Forry-Schaudies, S., Pacifici, M., Dept. of Anatomy, University of Pennsylvania Medical School, Philadelphia: Effects of Colcemid and taxol on the synthesis and assembly of tubulin, vimentin, and desmin during chondrogenesis and myogenesis.

Welch, W.J., Blose, S., Feramisco, J.R., Cold Spring Harbor Laboratory, New York: Heat-shock response and the cytoskeleton.

SESSION 8 MOSTLY MUSCLE GENE STRUCTURE AND EXPRESSION

Chairperson: S. Penman, Massachusetts Institute of Technology, Cambridge, Massachusetts

Penman, S., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Morphology and protein composition of the structural elements of cytoplasm and nucleus in cells and tissues.

Zimmer, W.E., Jr., Chang, K.S., Bergsma, D., Schwartz, R., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Organization and expression of the chicken actin multigene family.

Leavitt, J.,[1] Ng, S.-Y.,[1] Lin, C.-S.,[1] Gunning, P.,[2] Kedes, L.,[2] [1]Linus Pauling Institute of Science and Medicine, Palo Alto, California; [2]Dept. of Medicine, Stanford Medical School and Veterans Administration Medical Center, California: Isolation and characterization of wild-type and mutant human β-actin genes.

Gunning, P.,[1] Hickey, R.,[2] Ng, S.-Y.,[3] Ponte, P.,[1] Minty, A.,[1] Erba, H.,[1] Leavitt, J.,[3] Skoultchi, A.,[2] Kedes, L.,[1] [1]Stanford Medical School and Veterans Administration Medical Center, Palo Alto, California; [2]Albert Einstein School of Medicine, Bronx, New York; [3]Linus Pauling Institute of Science and Medicine, Palo Alto, California: Human actin-gene structure, evolution, and expression in heterologous cells.

Helfman, D.M., Feramisco, J.R., Kost, T.A., Yamawaki-Kataoka, Y., Ricci, W.M., Hughes, S.H., Cold Spring Harbor Laboratory, New York: Cloning by immunological screening of cDNA expression libraries—Analysis of smooth-muscle α- and β-tropomyosins.

MacLeod, A.R., Talbot, K., Ludwig Institute for Cancer Research, MRC, Cambridge, England: Human cytoskeletal tropomyosin genes.

G. Borisy, D. Cleveland, D. Murphy

Cooper, T., Ordahl, C., Dept. of Anatomy, University of California, San Francisco: Genomic structure of a troponin-T isogene expressed according to two regulatory programs in heart and skeletal muscle development.

Hammer, J.A. III, Korn, E.D., Paterson, B.M., National Institutes of Health, Bethesda, Maryland: Cloning of the *Acanthamoeba* myosin heavy-chain genes.

Leinwand, L.,[1] de Lozanne, A.,[2] Lewis, M.,[1] Saez, L.,[1] Spudich, J.,[2] [1]Albert Einstein College of Medicine, Bronx, New York; [2]Stanford University School of Medicine, California: Isolation and characterization of nonmuscle myosin heavy-chain cDNA clones from *Dictyostelium*.

Izant, J.G., Dept. of Genetics, Fred Hutchinson Cancer Research Center, Seattle, Washington: Antisense gene transcription as a tool for molecular analysis.

SESSION 9 ENTIRELY INTERMEDIATE FILAMENTS

Chairperson: **E. Fuchs,** University of Chicago, Chicago, Illinois

Fuchs, E., Marchuk, D., Tyner, A., McCrohon, S., Dept. of Biochemistry, University of Chicago, Illinois: Expression of keratin genes in human epithelia.

Steinert, P.,[1] Roop, D.,[2] [1]Dermatology Branch; [2]NCI, National Institutes of Health, Bethesda, Maryland: Amino acid sequences of epidermal keratins correlate with their developmental expression.

Jorcano, J.L.,[1,2] Rieger, M.,[2] Franz, J.K.,[2] Magin, T.M.,[2] Schiller, D.L.,[2] Franke, W.W.,[2] [1]Center of Molecular Biology, University of Heidelberg; [2]Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Principles of cytokeratin expression and cytokeratin relationships.

Zehner, Z.,[1] Paterson, B.,[2] [1]Medical College of Virginia, Virginia Commonwealth University, Richmond; [2]NCI, National Institutes of Health, Bethesda, Maryland: Sequence of the chicken vimentin gene.

Lazarides, E., Capetanaki, Y.G., Ngai, J., Division of Biology, California Institute of Technology, Pasadena: Vimentin—From gene to morphogenesis.

Quax, W., Quax-Jeuken, Y., Egberts, W.V., van den Heuvel, R., Hendriks, W., Bloemendal, H., Dept. of Biochemistry, University of Nijmegen, Netherlands: Genes for vimentin and desmin.

Ben-Ze'ev, A., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Differential control of cytokeratins and vimentin synthesis by cell-cell contact and cell spreading in cultured epithelial cells.

Roop, D.R.,[1] Toftgard, R.,[1] Kronenberg, M.S.,[2] Clark, J.H.,[2] Yuspa, S.H.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Changes in keratin gene expression during differentiation.

Closing Remarks: **D. Cleveland,** Johns Hopkins Medical School, Baltimore, Maryland

Ciliate Molecular Genetics

May 2–May 6

ARRANGED BY

Peter Bruns, Cornell University
Martin Gorovsky, University of Rochester

98 participants

Investigators from laboratories in the United States, Europe, and Japan attended the Ciliate Molecular Genetics meeting. Among the many high points of the meeting were: reports of telomere location for *Paramecium* surface antigens; indications from three different laboratories that ciliates may have a somewhat unique genetic code (unspliced termination codons in coding regions); the description of two remarkable cases (one partly characterized at the molecular level) of cytoplasmic mutants that alter macronuclear development; report of a *Tetrahymena* gene that is expressed only during conjugation and that appears to be homologous to the *zeste* locus in *Drosophila;* demonstration that *E. coli* CAP binds in a sequence-specific manner to the promoter of *Tetrahymena* rDNA; purification of gamones responsible for mating in a hypotrichous ciliate; and demonstration of a topoisomerase-like molecule bound to specific sequences in the promoter region of the *Tetrahymena* rDNA.

The meeting was reviewed both in *Nature* and *Genetics*.

Funding for this meeting was supplied in part by Amicon Corporation; J.T. Baker Chemical Co.; Boehringer Mannheim Biochemicals; Fotodyne, Inc.; H.P. Genenchem; Hana Biologics, Inc.; Kontes Scientific Glassware; Nikon, Inc.; Promega Biotech; Schleicher & Schuell; Society for Developmental Biology, Inc.; and the following divisions of the National Institutes of Health: Fogarty International Center and National Institute of General Medical Sciences.

SESSION 1 OVERVIEW

Chairperson: J. Engberg, University of Copenhagen, Copenhagen, Denmark

Gall, J.G., Dept. of Embryology, Carnegie Institution, Baltimore, Maryland: Ciliate molecular genetics.

Prescott, D.M., Swanton, M.T., Roth, M., Klobutcher, L.A., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Modification of DNA in ciliate development.

Preer, J., Jr., Dept. of Biology, Indiana University, Bloomington: Overview—Immobilization antigens.

Nanney, D.L., Dept. of Genetics and Development, University of Illinois, Urbana; Humboldt Fellow, Zoologisches Institut, Universität Münster, Federal Republic of Germany: Evolutionary genetics of the ciliates.

SESSION 2 GENOME ORGANIZATION AND REORGANIZATION

Chairperson: S.L. Allen, University of Michigan, Ann Arbor, Michigan

Klobutcher, L.,[1] Jahn, C.,[2] Prescott, D.,[3] [1]Dept. of Biochemistry, University of Connecticut Health Center, Farmington; [2]Dept. of Biological Sciences, University of Illinois, Chicago; [3]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: DNA sequence elimination and addition are both involved in gene maturation during macronuclear development in *Oxytricha nova*.

Carlinhour, S., Herrick, G., Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: Alternate juxtaposition of macronuclear sequences in *Oxytricha fallax*.

Lipps, H.J., Kraut, H., Institut für Biologie III, Tübingen, Federal Republic of Germany: DNA reorganization during macronuclear development of *Stylonychia*.

Yao, M.-C., Altschuler, M., Auster-

berry, C., Dept. of Biology, Washington University, St. Louis, Missouri: DNA rearrangement in *Tetrahymena*.

Blackburn, E., Bliska, J., Challoner, P., Cherry, J.M., Ryan, T., Spangler, E., Dept. of Molecular Biology, University of California, Berkeley: Sequence analysis and synthesis of macronuclear telomeres.

Diamond, C.H., Ohashi, P.S., Rose, A., Tsao, N.N.G.,Pearlman, R.E., Dept. of Biology, York University, Toronto, Canada: Analysis of a micronucleus limited sequence from *Tetrahymena thermophila*.

White, T., Allen, S., University of Michigan, Ann Arbor: Eliminated DNA sequences and a specific site of methylation in the macronucleus of *Tetrahymena thermophila*.

Yaeger, P.,[1] Orias, E.,[1] Larson, D.,[2] Howard, E.A.,[2] Blackburn, E.H.,[2] [1]Dept. of Biological Sciences, University of California, Santa Barbara; [2]Dept. of Molecular Biology, University of California, Berkeley: Mutations affecting rDNA amplification in *Tetrahymena thermophila*.

Olins, D.E., Allen, R.L., Cadilla, C.L., Olins, A.L., University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge: Macronuclear and chromatin structure in *Euplotes eurystomus*.

Gottschling, D.E., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: A complex at the molecular ends of *Oxytricha* macronuclear DNA imparts nucleosome phasing.

SESSION 3 POSTER SESSION

Ammermann, D., Institute of Biologie III, University of Tübingen, Federal Republic of Germany: Is the chromosome elimination in *Stylonychia lemnae* dependent on the micronuclear DNA content?

Bruns, P.J., Section of Genetics and Development, Cornell University, Ithaca, New York: Genetic maps of *Tetrahymena thermophila*.

Allen, S.L., Ervin, P.R., McLaren, N.C., Division of Biological Sciences, University of Michigan, East Lansing: Organization of the 5S rRNA gene clusters in the germ-line and somatic genomes of *Tetrahymena thermophila*.

Pederson, D.S., Bannon, G.A., Bowen, J.K., Shupe, K., Gorovsky, M.A., Dept. of Biology, University of Rochester, New York: Relationship of DNAse I hypersensitive sites to transcription of 5S and histone genes in *Tetrahymena thermophila*.

Hufschmid, J.-D., de Haller, G., Laboratory of Protozoology, University of Geneva, Switzerland: Studies on the DNA of the heterotrich ciliate *Climacostomum virens*.

Dawson, D., Herrick, G., Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: Rare internal C_4A_4 repeats in the micronuclear genome of *Oxytricha fallax*.

Tschunko, A., Allen, S., University of Michigan, Ann Arbor: Structure and genomic organization of an inverted repeated DNA sequence of *Tetrahymena thermophila*.

Poster Session

Haller, B.L., Orias, E., Dept. of Biological Sciences, University of California, Santa Barbara: Aphidicolin affects differential amplification of rDNA molecules in B/C3 heterozygotes of *Tetrahymena thermophila*.

Conover, R.K., Brunk, C.F., Dept. of Biology, University of California, Los Angeles: Isolation of telomeres from *Tetrahymena thermophila*.

Rogers, M., Karrer, K., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Adolescence in *Tetrahymena thermophila*.

Suhr-Jessen, P., Dept. of Anatomy and Cytology, University of Odense, Denmark: Stage-specific changes in protein synthesis pattern during conjugation in *Tetrahymena thermophila*.

Gutierrez, J.C., Orias, E., Dept. of Biological Sciences, University of California, Santa Barbara: Doublet cells of *Tetrahymena thermophila* originated after capsule induction on conjugating pairs.

Hamilton, E.P., Orias, E., Dept. of Biological Sciences, University of California, Santa Barbara: Macronuclear retention and other developmental effects induced by vinblastine in conjugating *Tetrahymena*.

Eustice, D.C., Wilhelm, J.M., Dept. of

Microbiology, University of Rochester, New York: Mechanism ofaction of aminoglycoside antibiotics with wild-type *Tetrahymena thermophila* and drug-resistant mutants.

Skelley, S., Wilhelm, J.M., Dept. of Microbiology, University of Rochester, New York: Multiple mutations in *Tetrahymena thermophila* conferring resistance to aminoglycoside antibiotics.

Sripati, C.E., Institut de Biologie physico-chimique, Paris, France: Invivo stability of ribosomes in *Tetrahymena thermophila*.

Cleffmann, G., Palissa, H., Institute of Animal Physiology, University of Giessen, Federal Republic of Germany: Division mutants as tools to study cell cycle control in *Tetrahymena*.

Smith-Sonneborn, J., Dept. of Zoology and Physiology, University of Wyoming, Laramie: Clonal age- and cell-specific response to low-frequency electromagnetic fields.

Dyer, B.D., Dept. of Biology, Boston University, Massachusetts: Anaerobic, sulfide-tolerant ciliates.

SESSION 4 CONJUGATION

Chairperson: A. Miyake, Università di Camerino, Camerino, Italy

Miyake, A., Dipartimento di Biologia Cellulare, Università di Camerino, Italy: Ciliate conjugation – Introductory remarks.

Heckmann, K., Zoological Institute, University of Münster, Federal Republic of Germany: Towards the molecular basis of cell recognition in *Euplotes octocarinatus*.

Luporini, P., Miceli, C., Dipartimento di Biologia Cellulare, Università di Camerino (MC), Italy: The molecular mechanism of sexual interaction in *Euplotes*.

Miceli, C., Luporini, P., Dipartimento di Biologia Cellulare, Università di Camerino, Italy: Isogamones in *Euplotes raikovi*.

Fujishima, M., Iwao, Y., Biological Institute, Faculty of Science, Yamaguchi University, Japan: Induction of reinitiation of meiosis in toad oocytes by injection of *Paramecium* extracts.

Eckert, W.A., Weiske-Benner, A., Institute of Zoology, University of Heidelberg, Federal Republic of Germany: Differentiation and transcriptional activity of nuclei during conjugation in *Tetrahymena thermophila*.

Allis, C.D., Wiggins, J.C., Richman, R., Chicoine, L., Wenkert, D., Baylor College of Medicine, Texas Medical Center, Houston: Histone rearrangements accompany nuclear differentiation and dedifferentiationin *Tetrahymena thermophila*.

Martindale, D.W.,[1,2] Goldberg, M.,[1] Martindale, H.,[1,2] Bruns, P.J.,[1,1] Dept. of Genetics and Development, Cornell University, Ithaca, New York; [2]Dept. of Microbiology, McGill University, Quebec, Canada: Sequence conservation of a conjugation-specific gene from *Tetrahymena*.

SESSION 5 RIBOSOMES, RIBOSOMAL GENES, AND MITOCHONDRIAL GENES

Chairperson: R. Hallberg, Iowa State University, Ames, Iowa

Amin, A.A., Pearlman, R.E., Dept. of Biology, York University, Toronto, Canada: Autonomously replicating segments from *Tetrahymena thermophila* rDNA – Sequence, structure, and function.

P.J. Bruns

Engberg, J., Nielsen, H., Jeppesen, C., Biochemical Institute B, University of Copenhagen, Denmark: The ribosomal RNA genes of *Tetrahymena* – Structure and function.

Higashinakagawa, T., Matsuura, T., Matsumoto, K., Matsuda, R., Dept. of Biology, Tokyo Metropolitan University, Japan: Structure and expression of *Tetrahymena* ribosomal DNA.

Niles, E.G., Matusick, L., Dept. of Biochemistry, State University of New York, Buffalo: CAP binding to the *Tetrahymena pyriformis* rDNA promoter.

Gocke, E., Bonven, B.J., Westergaard, O., Dept. of Molecular Biology and Plant Physiology, University of Aarhus, Denmark: Is rRNA synthesis in *Tetrahymena* regulated by a sequence-specific topoisomerase I framing the gene?

Kister, K.-P., Eckert, W.A., Dept. of

Physiology, Institute of Zoology, Heidelberg, Federal Republic of Germany: In vitro processing of precursor rRNA in isolated macronuclei of *Tetrahymena thermophila*.

Zaug, A.J., Kent, J.R., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: Auto-reopening of the circular intervening sequence of *Tetrahymena*—Implications for the mechanism of RNA self-splicing.

Wilhelm, J.M., Kelly, E., Skelly, S., Dept. of Microbiology, University of Rochester, New York: Ribosomal proteins from antibiotic-resistant strains of *Tetrahymena thermophila*—Absence of starvation-induced protein phosphorylation.

Suyama, Y., Dept. of Biology, University of Pennsylvania, Philadelphia: Genes and genome organization of *Tetrahymena* mitochondrial DNA.

Heinonen, T.Y.K., Young, P.G., Dept. of Biology, Queen's University, Kingston, Canada: Transcription map of *Tetrahymena* mitochondrial DNA.

SESSION 6 BEHAVIOR, CELL SURFACE, AND CELL ARCHITECTURE

Chairperson: C. Kung, University of Wisconsin, Madison, Wisconsin

Kung, C., Dept. of Genetics, Laboratory of Molecular Biology, University of Wisconsin, Madison: Behavioral genetics of *Paramecium*—Ancient and recent history.

Haga, N.,[1] Forte, M.,[2] Ramanathan, R.,[1] Saimi, Y.,[1] Kung, C.,[1] [1]Laboratory of Molecular Biology, University of Wisconsin, Madison; [2]Dept. of Biology, Case Western Reserve University, Cleveland, Ohio: Purification of a soluble protein controlling Ca^{++} channel activity in *Paramecium*.

Saimi, Y., Hinrichsen, R.D., Laboratory of Molecular Biology, University of Wisconsin, Madison: Genetic dissection of electrogenesis and characterization of mutants in *Paramecium*.

Schultz, J.E., Grünemund, R., Gierlich, D., Klumpp, S., Pharmaceutical Institute, University of Tübingen, Federal Republic of Germany: Ionic regulation of the cyclic nucleotide system in *Paramecium tetraurelia*.

Bonini, N.M., Gustin, M.C., Mason, P., Nelson, D.L., Piper, L., Dept. of Biochemistry, University of Wisconsin, Madison: A possible role for cyclic AMP in the regulation of the ciliary beat in *Paramecium tetraurelia*.

Van Houten, J., Schulz, S., Preston, R., Dept. of Zoology, University of Vermont, Burlington: Role of membrane in *Paramecium* chemoreception.

Kaneshiro, E.S., Matesic, D.F., Dept. of Biological Sciences, University of Cincinnati, Ohio: The sphingolipids of the *Paramecium baA* mutant.

Frankel, J., Jenkins, L.M., Nelsen, E.M., Lansing, T.J., Bakowska, J., Dept. of Biology, University of Iowa, Iowa City: Mutations affecting patterning of cell-surface structures in *Tetrahymena thermophila*.

SESSION 7 GENE ORGANIZATION AND EXPRESSION

Chairperson: E. Orias, University of California, Santa Barbara, California

Caron, F., Meyer, E., Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette, France: Cloning of the entire structural gene of the G surface antigen and its expression in *E. coli*.

Findly, R.C., Dept. of Biology, Yale University, New Haven, Connecticut: Genomic organization of the *Tetrahymena* hsp70 heat-shock genes.

Hallberg, R., Kraus, K., Dept. of Zoology, Iowa State University, Ames: Regulation of heat-shock protein mRNA utilization in *Tetrahymena thermophila*.

Galego, L., Rodriques-Pousada, C., Dept. of Microbiology, Instituto Gulbenkian de Ciencia, Oeiras, Portugal: Stress response in *Tetrahymena pyriformis*—Differences between starvation and heat shock.

Ness, J.C., Morse, D.E., Dept. of Biological Sciences, University of California, Santa Barbara: Biochemical analysis of the regulation of galactokinase gene expression in *Tetrahymena thermophila*.

Cupples, C., Pearlman, R.E., Dept. of Biology, York University, Toronto, Canada: Actinlike sequences in *Tetrahymena*.

Helftenbein, E., Institut für Biologie III, Universität Tübingen, Federal Republic of Germany: Nucleotide sequence of a macronuclear gene-sized DNA molecule coding for α-tubulin in *Stylonychia lemnae*.

Seyfert, H.-M., Institute of Animal Physiology, University of Giessen, Federal Republic of Germany:

M.A. Gorovsky

Regulation of tubulin synthesis throughout ciliary regeneration in *Tetrahymena*.

Calzone, F.J., Callahan, R.C., Gorovsky, M.A., Dept. of Biology, University of Rochester, New York: Regulation of tubulin synthesis during cilia regeneration in starved *Tetrahymena thermophila*.

Orias, E., Baum, M.P., Dept. of Biological Sciences, University of California, Santa Barbara: The "first replicated, most amplified" hypothesis for caryonidal mating type determination.

SESSION 8 SECRETION AND CYTOPLASMIC PHENOMENA

Chairperson: B.H. Satir, Albert Einstein College of Medicine, Bronx, New York

Adoutte, A.,[1] Garreau de Loubresse, N.,[2] Beisson, J.,[1] [1]Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette; [2]Centre de Cytologie Expérimentale du CNRS, Ivry-sur-Seine, France: Proteolytic cleavage and maturation of the crystalline secretion products of *Paramecium*.

Garofalo, R.S., Satir, B.H., Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Quantitative studies on trichocyst matrix expansion in vitro and its regulation by Ca^{++} and pH.

Aufderheide, K.J., Dept. of Biology, Institute of Developmental Biology, Texas A&M University, College Station: Genetic and cortical phenotypes associated with early clonal death in *Paramecium tetraurelia*.

Kanabrocki, J.A., Quackenbush, R.L., Dept. of Microbiology, University of South Dakota, School of Medicine, Vermillion, South Dakota: R-body synthesis in *E. coli*.

Hufnagel, L.A., Dept. of Microbiology, University of Rhode Island, Kingston, and Marine Biological Laboratory, Woods Hole, Massachussetts: Ciliary rosettes – A new type of intramembranous particle array in starved and mating *Tetrahymena thermophila*.

Forney, J.D., Epstein, L.M., Dept. of Biology, Indiana University, Bloomington: Cytoplasmic inheritance of a macronuclear deletion in *Paramecium*.

Williams, N.E.,[1] Ron, A.,[2] [1]Dept. of Zoology, University of Iowa, Iowa City; [2]Dept. of Anatomy and Embryology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Synthesis and deployment of immobilization antigen H3 in transforming *Tetrahymena thermophila* cells.

Meyer, E., Caron, F., Guiard, B., Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette, France: In vitro translation of *Paramecium* messenger RNAs is blocked at precise sites.

Kaney, A.R., Dept. of Biology, Bryn Mawr College, Pennsylvania: Unusual mating behavior in an amicronucleate strain of *Tetrahymena thermophila*.

Coffee break

SESSION 9 TRANSFORMATION WORKSHOP

Chairperson: R.E. Pearlman, York University, Toronto, Canada

Killeen, M.T.,[1] Duda, E.,[2] Pearlman, R.E.,[1] [1]Dept. of Biology, York University, Toronto, Canada; [2]Institute of Biochemistry, Hungarian Academy of Sciences, Szeged, Hungary: Attempts to genetically transform *Tetrahymena*.

Duda, E.G.,[1] Pearlman, R.,[2] [1]Institute of Biochemistry, Biological Research Center, Szeged, Hungary; [2]Dept. of Biology, York University, Downsview, Canada: Transformation of *Tetrahymena thermophila*.

Luehrsen, K.R., Orias, E., Dept. of Biological Sciences, University of California, Santa Barbara – Attempts to transform *Tetrahymena:* A progress report.

Brunk, C.F., Conover, R.K., Dept. of Biology, University of California, Los Angeles: Transformation of *Tetrahymena* to paromomycin resistance with pSV2-neo DNA.

Wünning, I.U., Lipps, H.J., Institut für Biologie III, Universität Tübingen, Federal Republic of Germany: A transformation system for the hypotrichous ciliate *Stylonychia mytilus*.

Molecular Biology of the Photosynthetic Apparatus

May 9–May 13

ARRANGED BY

Charles Arntzen, DOE/Plant Research Laboratory, Michigan State University
Lawrence Bogorad, The Biological Laboratories, Harvard University
Susan Bonitz, Cold Spring Harbor Laboratory

146 participants

The process of photosynthesis involves many reactions—light absorption, electron transfers, energy-coupling processes leading to ATP synthesis, and enzymatic reactions leading to CO_2 fixation and substrate and product cycling. The main purpose of this meeting was to emphasize the advancements that have occurred in the study of photosynthesis using new tools of molecular biology. Special emphasis was placed on organizing sessions in which specialists in the analysis of the functional components of the photosynthetic processes presented recent data in parallel with reports by molecular biologists dealing with the genetic basis and developmental events that lead to the synthesis and stable integration of these components.

This meeting was supported in part by the Advanced Genetic Sciences, Inc.; Amoco; Arco Solar, Inc.; CIBA-GEIGY; Dow Chemical Co.; Exxon Research and Engineering; National Science Foundation; N.A.T.O.; Rhone-Poulenc, Inc.; Shell Development Co.; Stauffer Chemical Co.; and U.S. Dept. of Agriculture.

SESSION 1 DNA REPLICATION, TRANSCRIPTION, AND PROTEIN SYNTHESIS IN PLASMIDS

Chairperson: J. Weil, University of Strasbourg, Strasbourg, France

Gillham, N.,[1] Palmer, J.,[1] Boynton, J.,[2] Harris, E.,[2] Depts. of [1]Zoology, [2]Botany, Duke University, Durham, North Carolina: Organization of plastid chromosomes.

Bogorad, L., Crossland, L.D., Russell, D., Stirdivant, S., Harvard University, Cambridge, Massachusetts: Transcription of chloroplast genes.

Hallick, R.B., Johanningmeier, U., Karabin, G.D., Nickoloff, J.A., Passavant, C., Dept. of Chemistry, University of Colorado, Boulder: Transcription of chloroplast genes.

Orozco, E.M., Jr., Hanley-Bowdoin, L., Poulsen, C., Mullet, J.E., Chua, N.-H., Laboratory of Plant Molecular Biology, Rockefeller University, New York, New York: In vitro transcriptional analysis of the *rL* and *atpB* genes from spinach and maize.

Rodermel, S.R., Bogorad, L., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Photoregulation of transcript levels during light-induced plastid development in maize.

Greenberg, B.,[1] Narita, J.,[1] DeLuca-Flaherty, C.,[1] Gruissem, W.,[2] Hallick, R.,[1] [1]Dept. of Chemistry, University of Colorado, Boulder; [2]Dept. of Botany, University of California, Berkeley: Properties of two chloroplast RNA polymerase activities.

Coffee break

SESSION 2 THE FUNCTIONAL ORGANIZATION OF THE PHOTOSYNTHETIC ENERGY TRANSDUCING APPARATUS

Chairperson: B. Cramer, Purdue University, West Lafayette, Indiana

Arntzen, C.J., DOE/Plant Research Laboratory, Michigan State University, East Lansing: Chloroplast membrane structure and function.

Andersson, B., Åkerlund, H.-E., Jansson, C., Dept. of Biochemistry, University of Lund, Sweden: Polypeptides on the oxidizing side of PSII.

Herrmann, R., Botanishes Institut, University of Dusseldorf, Federal Republic of Germany: Plastid genes for PSII proteins.

Erickson, J.M.,[1] Rochaix, J.-D.,[1] Mets, L.,[2] [1]Depts. of Molecular Biology and Plant Biology, University of Geneva, Switzerland; [2]Dept. of Biology, University of Chicago, Illinois: Using mutants to probe the functional structure of the 32 kilodalton protein of PSII.

B. Andersson, C. Arntzen, J. Bennett

Cohen, B.N., Bloom, M.V., Coleman, T., Weissbach, H., Roche Institute of Molecular Biology, Nutley, New Jersey: Analysis of in vitro chloroplast gene expression using a simplified dipeptide synthesis system.

Golden, S.S.,[1] Sherman, L.A.,[2] Haselkorn, R.,[1] [1]Dept. of Biophysics, University of Chicago, Illinois; [2]Division of Biological Sciences, University of Missouri, Columbia: Analysis of *A. nidulans* R2 *psbA* genes from wild-type and DCMU-resistant strains.

Mattoo, A.K.,[1] Marder, J.B.,[2] Edelman, M.,[1] [1]University of Maryland, College Park, and Plant Hormone Laboratory, USDA Agricultural Research Service, Beltsville, Maryland; [2]Dept. of Plant Genetics, Weizmann Institute of Science, Rehovot, Israel: Light and the 32K herbicide binding protein—Metabolism and function.

Kyle, D.J., DOE/Plant Research Laboratory, Michigan State University, East Lansing: Light-induced membrane damage and repair; photoinhibition.

SESSION 3 POSTER SESSION

Crossland, L., Rodermel, S., Bogorad, L., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Single gene for the large subunit of RuBP carboxylase in maize yields two mRNAs.

Gingrich, J.C., Hallick, R.B., Dept. of Chemistry, University of Colorado, Boulder: The *E. gracilis* ribulose-1,5-bisphosphate carboxylase gene—Nine introns in a chloroplast protein gene.

Passavant, C.W., Hallick, R.B., Dept. of Chemistry, University of Colorado, Boulder: Analysis of four structural genes encoded in the *E. gracilis* chloroplast chromosome.

Mullet, J.E., Boyer, S., Dept.of Biochemistry and Biophysics, Texas A&M University, College Station: In vitro transcription of the chloroplast *psbA* gene.

Hanley-Bowdoin, L., Chua, N.-H., Laboratory of Plant Molecular Biology, Rockefeller University, New York, New York: A 109-bp chloroplast sequence contains the promoter for the maize *rL* gene.

Woessner, J.,[1] Masson, A.,[3] Harris, E.,[2] Bennoun, P.,[3] Gillham, N.,[1] Boynton, J.,[2] Depts. of [1]Zoology, [2]Botany, Duke University, Durham, North Carolina; [3]Institut de Biologie Physico-Chimique, Paris, France: Molecular and genetic analysis of the chloroplast ATPase of *Chlamydomonas*.

Margulies, M.M., Smithsonian Environmental Research Center, Rockville, Maryland: The function of chloroplast membrane-bound ribosomes.

Edwards, C.A.,[1] Bellemare, G.,[2] Dharm, B.,[1] Chua, N.-H.,[1] [1]Laboratory of Plant Molecular Biology, Rockefeller University, New York, New York; [2]Dept. of Biochemistry, Laval University, Quebec, Canada: Chloroplast DNA-dependent RNA polymerase contains subunits that cross-react with the subunits of *E. coli* RNA polymerase.

Muskavitch, K.M.T., Krebbers, E.T., Bogorad, L., The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Light-stimulated transcription of a region of the *Z. mays* chloroplast genome.

McKown, R.L., Tewari, K.K., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Purification and properties of a pea chloroplast single-stranded DNA-binding protein.

Larrinua, I.M., Bogorad, L., The Biological Laboratories, Harvard University, Cambridge, Massachusetts: The characterization of translational signals for higher plant plastid genes.

Graan, T., Ort, D.R., Dept. of Plant Biology, USDA/ARS, University of Illinois, Urbana: Quantitation of the rapid electron donors to P700, the functional plastoquinone pool, and the ratio of the photosystems in spinach chloroplasts.

Wolber, P.K., Steinback, K.E., Advanced Genetic Sciences, Oakland, California: Analysis of a tryptic peptide photoaffinity-labeled by azidoatrazine.

Williams, J.C.,[1] Steiner, L.A.,[2] Simon, M.I.,[3] Feher, G.,[1] [1]University of California, San Diego; [2]Massachusetts Institute of Technology, Cambridge; [3]Agouron Institute, La Jolla, California: Sequencing of the genes encoding the subunits of the reaction center from *R. sphaeroides*.

Worland, S.,[1] Wilson, K.,[2] Hearst, J.,[1] Sauer, K.,[1] [1]Division of Chemical Biodynamics, Lawrence Berkeley Laboratory, and Dept. of Chemistry, University of California, Berkeley; [2]Cetus Corporation, Emeryville, California: Amino-terminal sequences of reaction-center polypeptides from *R. capsulata*.

Zsebo, K.M.,[1] Wu, F.,[2] Hearst, J.,[2] [1]Group in Comparative Biochemistry; [2]Dept. of Chemistry, University of California, Berkeley: Genetic-physical mapping of a photosynthetic gene cluster from *R. capsulata*.

Kiley, P.J., Kaplan, S., University of Illinois, Urbana: Identification of light-harvesting polypeptides within the chromatophores of *R. sphaeroides* by analysis of light-harvesting mutants.

Salvucci, M.E., Ogren, W.L., USDA/ARS and Agronomy Dept., University of Illinois, Urbana: Biochemical characterization of two *Chlamydomonas* Calvin cycle mutants.

Chattoo, B.B., Hohn, B., Friedrich Miescher-Institut, Basel, Switzerland: Molecular basis for an unstable chloroplast phenotype in petunia hybrida.

SESSION 4 BACTERIAL PHOTOSYNTHESIS

Chairperson: S. Kaplan, University of Illinois, Urbana, Illinois

Drews, G., Peters, J., Dierstein, R., Institute of Biology, Albert-Ludwigs University, Freiburg, Federal Republic of Germany: Organization and synthesis of the photosynthetic apparatus of *R. capsulata*.

Youvan, D.C., Cold Spring Harbor Laboratory, New York, and Exxon Research and Engineering Company, Clinton, New Jersey: Reaction-center, B870 and B800 and B850 genes and deduced polypeptide sequences from *R. capsulata*.

Feher, G., Allen, J.P., Dept. of Physics, University of California, San Diego: Crystallization of reaction centers from *R. sphaeroides;* Preliminary characterization.

Donohue, T.J.,[1] Chory, J.,[1] Varga, A.R.,[2] Staehelin, A.L.,[2] Kaplan, S.,[1] [1]University of Illinois, Urbana; [2]University of Colorado, Boulder: Biochemical and morphological studies of the induction of the photosynthetic membrane of *R. sphaeroides*.

Fuller, R.C., Redlinger, T.E., Dept. of Biochemistry, University of Massachusetts, Amherst: The development and topology of the photosynthetic apparatus of *C. aurantiacus*.

Hearst, J.E., University of California, Berkeley: The relationship between amino acid sequences and quinone binding in photosynthetic membranes.

Gilbert, C.W.,[1] Brown, A.E.,[2] Wydrzynski, T.J.,[1] Williams, K.,[1] Arntzen, C.J.,[1] [1]Michigan State University, East Lansing; [2]Auburn University, Alabama: Molecular analysis of triazine herbicide resistance in the photosynthetic bacterium *R. sphaeroides*.

Poster Discussion

Vannevar Bush Lecture Hall

SESSION 5 NUCLEAR GENE REGULATION

Chairperson: E. Tobin, University of California, Los Angeles, California

Boynton, J.,[1] Schmidt, R.,[1] Myers, A.,[2] Hosler, J.,[1] Gillham, N.,[2] Depts. of [1]Botany, [2]Zoology, Duke University, Durham, North Carolina: Biogenesis of chloroplast ribosomes—Cooperation of chloroplast and nuclear genes.

Jenkins, G.I.,[1] Bennett, J.,[2] [1]Dept. of Biological Sciences, University of Warwick, Coventry, England; [2]Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Photoregulation of chloroplast proteins—mRNA levels, protein stability, and phosphorylation.

Morelli, G., Coruzzi, G., Broglie, R., Moses, P., Chua, N.-H., Laboratory of Plant Molecular Biology, Rockefeller University, New York, New York: The expression of *rS* genes in vivo and in transformed plant cells.

Cashmore, A., Timko, M., Hand, M., Kausch, A., Fassler, J., Laboratory of Cell Biology, Rockefeller University, New York, New York: Nuclear genes encoding the light-harvesting chlorophyll *a/b*-binding polypeptides and the small subunit of ribulose-1,5-bisphosphate carboxylase.

Herrera-Estrella, L.,[1] Van den Broeck, G.,[1] Maenhaut, R.,[1] Timko, M.,[2] Cashmore, A.,[2] Van Montagu, M.,[1] Schell, J.,[1] [1]Laboratorium voor Genetica, Gent, Belgium; [2]Rockefeller University, New York, New York: Photoregulation of nuclear gene expression.

SESSION 6 POSTER SESSION

Chory, J., Muller, E.D., Kaplan, S., Dept. of Microbiology, University of Illinois, Urbana: Cloning, expression, and assembly of the form II ribulose-1,5-bisphosphate carboxylase from *R. sphaeroides*.

Andrews, T.J., Ballment, B., Australian Institute of Marine Science, Townsville: Reassembly of Rubisco from isolated subunits— Carbamate formation and 2-CABP binding by isolated large-subunit octamers.

Gutteridge, S., Sigal, I., Thomas, B., Arentzen, R., Cordova, A., Lorimer, G., Central Research and Development Dept., E.I. du Pont de Nemours and Company, Wilmington, Delaware: Directed mutagenesis of the carbamate-metal-ion-binding site of RuBP carboxylase.

Pierce, J., Reddy, G.S., Central Research and Development Division, E.I. du Pont de Nemours and Company, Wilmington, Delaware: A structure for the catalytic complex of RuBP carboxylase.

Coughlan, S.J., Clark, R.D., Hawkesford, M.J., Bennett, J., Hind, G., Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Identification of ferredoxin : NADP oxidoreductase as a component of a 5-polypeptide cytochrome *b/f* complex.

Sen, A., Dept. of Biophysics, Roswell Park Memorial Institute, Buffalo, New York: Polymorphic phase behavior of chloroplast thylakoid membrane lipids.

Chia, C.P., Arntzen, C.J., DOE/Plant Research Laboratory, Michigan State University, East Lansing: Accelerated membrane protein turnover in a chloroplast-encoded mutant.

Leto, K.J.,[1] Bell, E.,[2] McIntosh, L.,[2] [1]DuPont Company, Central Research and Development, Wilmington, Delaware; [2]Michigan State University, East Lansing: Failure to assemble PSII in a nuclear maize mutant is accompanied by accelerated turnover of chloroplast-encoded PSII polypeptides.

Clark, R.D., Bennett, J., Hind, G., Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Extraction and purification of a spinach thylakoid kinase that can phosphorylate light-harvesting chlorophyll *a/b* protein (LHCP) in vitro.

Abbott, M.S.,[1] Czarnecki, J.J.,[2] Selman, B.R.,[1] [1]Dept. of Biochemistry, [2]Institute for Enzyme Research, University of Wisconsin, Madison: Localization of the high-affinity binding site for ADP and ATP on the β subunit of the chloroplast CF1.

Merchant, S., Selman-Reimer, S., Abbott, M.S., Selman, B.R., Dept. of Biochemistry, University of Wisconsin, Madison: Analysis of the chloroplast CF1 in *C. reinhardtii*.

Tobin, E.M., Neumann, G.A., Kohorn, B.D., Thornber, J.P., Dept. of Biology, University of California, Los Angeles: Sequence and structure of an intron containing chlorophyll *a/b*-protein gene expressed in *L. gibba*.

Anderson, L.,[1] Eiserling, F.,[1,2] [1]Molecular Biology Institute,[2]Dept. of Microbiology, University of California, Los Angeles: Assembly interactions in *Synechocystis* 6701 phycobilisomes.

Kaufman, L.S., Watson, J.C., Briggs, W.R., Thompson, W.F., Carnegie Institution of Washington, Stanford, California: Photoregulation of nuclear genes in developing pea buds.

Wimpee, C.F., Tobin, E.M., Dept. of Biology, University of California, Los Angeles: Structure and expression of genes encoding the small subunit of RuBP carboxylase from *L. gibba*.

Yen, H.-C.B., Huggins, B.J., Georgen, K.N., Corporate Research, Standard Oil Company, Naperville, Illinois: Genetic transformation of foreign DNA in the photosynthetic bacterium *R. sphaeroides*.

Ausich, R.L., Murphy, R.M., Corporate Research, Standard Oil Company, Naperville, Illinois: Experiments on the transformation of *C. reinhardtii*.

Gold, B., Bogorad, L., The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Maize chloroplast DNA fragments are capable of acting as autonomously replicating sequences in yeast.

Cushman, J.C., Reardon, E.M., Miller, M.E., Price, C.A., Waksman Institute, Rutgers University, Piscataway, New Jersey: Proline mimics the effect of light in the regulation of plastid protein synthesis in *Euglena*.

SESSION 7 RUBISCO STRUCTURE AND FUNCTION

Chairperson: W. Ogren, University of Illinois, Urbana, Illinois

Ellis, R.J., Gallagher, T.F., Robinson, C., Jenkins, G.I., Hemmingsen, S., Dept. of Biological Sciences, University of Warwick, Coventry, England: Synthesis, processing, and assembly of Rubisco subunits.

Nierzwicki-Bauer, S.A.,[1] Curtis, S.E.,[2] Haselkorn, R.,[1] [1]Dept. of Biophysics, University of Chicago, Illinois; [2]Dept. of Genetics, North Carolina State University, Raleigh: Cotranscription of genes coding for the large and small subunits of RuBP carboxylase in the cyanobacterium *Anabaena* 7120.

Poster Discussion

Lorimer, G., Pierce, J., Gutteridge, S., Central Research and Development Division, E.I. du Pont de Nemours and Company, Wilmington, Delaware: RuBP carboxylase—Stereochemical aspects and the involvement of the carbamate-*metal-ion complex in catalysis.*

Hanks, J.F., Somerville, C.R., McIntosh, L., DOE/Plant Research Laboratory, Michigan State University, East Lansing: Site-specific mutagenesis of RuBP carboxylase/oxygenase.

SESSION 8 CYTOCHROME b_6f AND PHOTOSYSTEM I

Chairperson: W. Krogman, Purdue University, West Lafayette, Indiana

Hauska, G., Dept. of Biology, University of Regensburg, Federal Republic of Germany: Organization and function of the cytochrome b_6f complex.

Clark, R.D., Hind, G., Hawkesford, M.J., Bennett, J., Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Ferredoxin : NADP oxidoreductase of chloroplasts is also a protein kinase.

Ortiz, W., Lam, E., Malkin, R., Division of Molecular Plant Biology, University of California, Berkeley: Organization of the thylakoid PSI complex.

Lundell, D.J.,[1] Glazer, A.N.,[1] Melis, A.,[2] Malkin, R.,[2] [1]Dept. of Microbiology and Immunology; [2]Division of Molecular Plant Biology, University of California, Berkeley: Characterization of a cyanobacterial PS1 complex.

Fish, L.E.,[1] Kuck, U.,[2] Bogorad, L.,[1] [1]Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts; [2]Lehrstuhl für Allegemeine Botanik, Bochum, Federal Republic of Germany: Structural analysis of two adjacent light-inducible chloroplast genes in maize with partial amino acid homology encoding polypeptides of the CPI complex of PSI.

Jagendorf, A.T., Anthon, G., Section of Plant Biology, Cornell University, Ithaca, New York: Unresolved problems in photophosphorylation.

Selman-Reimer, S., Selman, B.R., Dept. of Biochemistry, University of Wisconsin, Madison: Reversible conformational changes of chloroplast CF1 induced by selective organic solvents.

Bottomley, W.,[1] Zurawski, G.,[2] Mason, J.G.,[1] Whitfeld, P.R.,[1] [1]Division of Plant Industry, CSIRO, Canberra, Australia; [2]DNAX Research Institute, Palo Alto, California: Organization and structure of the chloroplast-encoded subunits of ATP synthase.

Walker, J.E., Gay, N.J., Hampe, A., Falk, G., Runswick, M.J., Tybulewicz, V.L.J., MRC, Laboratory of Molecular Biology, Cambridge, England: Polypeptides of coupling factors and their genes.

H. Zuber

SESSION 9 LIGHT HARVESTING SYSTEMS IN CYANOBACTERIA

Chairperson: B. Zilinskas, Cook College, New Brunswick, New Jersey

Glazer, A.N., Dept. of Microbiology and Immunology, University of California, Berkeley: Phycobiliproteins as PBS components and as purified proteins.

Gantt, E., Redlinger, T., Lipschultz, C.A., Smithsonian Environmental Research Center, Rockville, Maryland: Phycobilisomes—A comparison of the terminal acceptor pigment in red algae and cyanobacteria.

Lemaux, P.G.,[1,2] Grossman, A.R.,[1] [1]Carnegie Institution of Washington. Stanford, California; [2]Dept. of Biological Sciences, Stanford University, California: Isolation of genes encoding phycobiliprotein subunits.

Chang, S.S.,[1] Kycia, J.H.,[1] Ledbetter, M.C.,[1] Feldmann, R.J.,[2] Siegelman, H.W.,[1] [1]Dept. of Biology, Brookhaven National Laboratory, Upton, New York; [2]Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland: Cyanobacterial PBS.

de Lorimier, R.,[1] Bryant, D.A.,[1] Porter, R.D.,[1] Fields, P.I.,[1] Liu, W.-Y.,[2] Jay,

L. Bogorad, C. Arntzen, S. Bonitz

SESSION 10 TRANSFORMATION SYSTEMS AND DIFFERENTIATION OF THE PHOTOSYNTHETIC APPARATUS

Chairperson: **I. Ohad,** Hebrew University, Jerusalem, Israel

Kolowsky, K.S., Williams, J.G.K., Szalay, A.A., Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York: Stable integration of foreign DNA into the chromosome of cyanobacteria.

Flores, E., Wolk, C.P., DOE/Plant Research Laboratory, Michigan State University, East Lansing: Approach to the genetic analysis of oxygenic photosynthesis—Transfer of plasmid vectors from *E. coli* to facultatively heterotrophic cyanobacteria.

Taylor, W., Mayfield, S., Martineau, B., Harpster, M., Nelson, T., Yamaguchi, J., Dept. of Genetics, University of California, Berkeley: Ontogenetically regulated genes.

Watson, J.C., Kaufman, L.S., Thompson, W.F., Dept. of Plant Biology, Carnegie Institution of Washington, Stanford, California: Organization and expression of light-regulated nuclear genes in pea and mung bean.

Horsch, R.B., Fraley, R.T., Rogers, S.G., Monsanto Company, St. Louis, Missouri: Inheritance of functional foreign genes in plants.

Concluding Discussion: The Use of Genetic Transformation in Understanding Photosynthetic Complexity

E.,[2] Stevens, S.E., Jr.,[1] [1]Microbiology Program, Pennsylvania State University, University Park; [2]Dept. of Chemistry, University of New Brunswick, Fredericton, Canada: Genes for the α and β subunits of PC.

Zuber, H., Sidler, W., Füglistaller, P., Brunisholz, R., Theiler, R., Institut für Molekularbiologie und Biophysik, Eidgen Technische Hochschule, Zürich, Switzerland: Structural studies on the light-harvesting polypeptides from cyanobacteria and bacteria.

Loach, P., Parkes, P., Miller, J., Hinchigeri, S., Callahan, P., Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois: Structure-function relationships of the light-harvesting complexes in photosynthetic bacteria.

RNA Processing Meeting

May 16–May 20

ARRANGED BY

Michael Mathews, Cold Spring Harbor Laboratory
Hugh Robertson, Rockefeller University
Aaron Shatkin, Roche Institute

258 participants

This year's meeting, the third of the series, attracted nearly 250 scientists and created a great deal of excitement. The field's prosaic-sounding name does less than justice to metamorphoses that transform RNA molecules both structurally

and functionally. Primary transcripts, simple copies of the nucleotide sequence in the gene, are modified by chemical substitution, by addition or deletion of stretches of RNA, and by splicing—the joining of previously distant RNA sequences.

One high point this year was the recognition of the catalytic role played by the RNA moiety of the RNase P enzyme in transfer RNA maturation. This molecule now joins what is likely to be a growing list of RNA molecules with catalytic function in cleavage and splicing reactions. The self-splicing reaction of the inaugural member of this group, *Tetrahymena* ribosomal RNA, was subjected to close scrutiny, and the candidacy of some further RNAs was advanced. A second peak was reached with the development of cell-free extracts capable of efficiently splicing mammalian messenger RNAs. Intense interest was occasioned by the discovery of unexpected chemical bonds and lariat-like intermediate structures formed during the splicing reaction, echoing illustrations of rope-makers' art on the cover of the abstract book. Progress was seen in areas such as ribonucleoprotein particle assembly and ribosomal RNA processing, and considerable debate was stirred by experiments implying the existence of a splicing mechanism in phage-infected bacteria. Particularly promising was the development of an in vitro system for the processing of mRNA 3′ ends, which will undoubtedly augment the elegant genetic studies of this event in the future.

This conference, the third to be held here in consecutive years, firmly established the need for an annual meeting on RNA processing. The series has created a vital forum for discussion of a range of topics related to RNA metabolism, and its success is founded on the dynamism and broad scope of the field. With some reluctance on this side of the ocean, it was decided that the 1985 meeting will be held in Rome. The meeting will return to its original venue in 1986, possibly launching an annual alternation between a European site and Cold Spring Harbor.

This meeting was supported in part by funds from the National Science Foundation and the following divisions of the National Institutes of Health: Fogarty International Center and National Institute of General Medical Sciences.

Introduction: **T. Cech,** University of Colorado, Boulder, Colorado

SESSION 1 RNA CATALYZED REACTIONS

Chairperson: J. Steitz, Yale University, New Haven, Connecticut

Price, J.,[1] Engberg, J.,[3] Kieft, G.,[2] Sievers, E.,[2] Kent, J.,[2] Morin, G.,[2] Been, M.,[2] Cech, T.,[1,2] Depts. of [1]Molecular, Cellular, and Developmental Biology; [2]Chemistry, University of Colorado Boulder; [3]Biochemical Institute, Panum Institute, University of Copenhagen, Denmark: The effects of deletions and insertions on the in vitro self-splicing of *T. thermophila* pre-rRNA.

Sullivan, F.X., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: The covalent addition of an oligoribonucleotide to the circular IVS of *Tetrahymena* is a fully reversible reaction mediated by the RNA.

Allen, L.C., Haydock, K., Dept. of Chemistry, Princeton University, New Jersey: Molecular mechanisms of catalysis by RNA.

Garriga, G., Collins, R., Lambowitz, A., Dept. of Biochemistry, St. Louis University School of Medicine, Missouri: RNA splicing in *Neurospora* mitochondria.

Perlman, P., Haldi, M., Jarrell, R., Morawiec, A., Dept. of Genetics, MCDB Program, Ohio State University, Columbus: Studies of mitochondrial intron structure.

Guerrier-Takada, C., Baer, M., Lawrence, N., Altman, S., Dept. of Biology, Yale University, New Haven, Connecticut: Structure and function of the RNA component of RNase P from *E. coli*.

Pace, N.R., Gardiner, K., Marsh, T.L., National Jewish Hospital and Research Center, and University of Colorado Health Sciences Center, Denver: The *B. subtilis* RNase P.

Prody, G.A., Bakos, J.T., Buzayan, J.M., Schneider, I.R., Bruening, G., Dept. of Biochemistry and Biophysics, University of California, Davis: Self-processing of multimeric forms of satellite tobacco ringspot virus RNA.

SESSION 2 STRUCTURE AND MECHANISM

Chairperson: O. Uhlenbeck, University of Illinois, Urbana, Illinois

Munroe, S.H., Dept. of Biology, Marquette University, Milwaukee, Wisconsin: Higher-order structure of RNA sequences at splice sites of adenoviral pre-mRNA.

Shelness, G.S., Williams, D.L., Dept. of Pharmacological Sciences, State University of New York, Stony Brook: Structure mapping of an estrogen-stabilized mRNA.

Lawrence, C., Browner, M., Ramsey, W.J., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Conserved secondary structure features in mRNA and hnRNA.

Moore, P., Kime, M.J., Abdel-Meguid, S., Dept. of Chemistry, Yale University, New Haven, Connecticut: Structure and properties of 5S RNA and its complexes with ribosomal proteins.

Filipowicz, W.,[1] Konarska, M.,[1] Strugala, K.,[1] Shatkin, A.J.,[2] [1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Mechanism of action of RNA 3′-terminal phosphate cyclase and identification of 2′, 3′-cyclic 3′-phosphodiesterase in extracts of HeLa cells.

Furneaux, H., Arenas, J., Perkins, K.K., Reinberg, D., Pick, L., Adhya, S., Carleton, S., Hurwitz, J., Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Purification and properties of an RNA cyclase and RNA ligase from HeLa cells.

Ohshima, Y.,[1] Tatei, K.,[1] Takemura, K.,[1] Maeda, A.,[1] Fujiwara, Y.,[1] Tanaka, H.,[2] [1]Institute of Biological Sciences, University of Tsukuba; [2]National Chemical Laboratory for Industry, Ibaraki, Japan: U1 RNA–protein complex preferentially binds to both 5′ and 3′ splice junction sequences.

Aebi, M., Reiser, J., Weissmann, C., Institut für Molekularbiologie I, Universität Zürich, Switzerland: Point mutations within the 5′ splice region of the rabbit β-globin large intron—Conversion of the "Chambon rule" GT to GC or GA does not preclude splicing.

Solnick, D., Dept. of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, Connecticut: Secondary structure and scanning mechanisms in mRNA splicing.

SESSION 3 POSTER SESSION

Andersen, J.,[1] Delihas, N.,[1] Hanas, J.S.,[2] Wu, C.-W.,[2] Depts. of [1]Microbiology; [2]Pharmacological Sciences, State University of New York, Stony Brook: 5S RNA structure and interaction with transcription factor A from *X. laevis* oocytes.

Ares, M., Mangin, M., Weiner, A.M., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Expression of human U2 genes in *Xenopus* oocytes.

Arrigo, A.-P., Simon, M., Darlix, J.-L., Khandjian, E.W., Spahr, P.-F., Dept. of Molecular Biology, University of Geneva, Switzerland: Heat-shock proteins of *D. melanogaster* are associated with both nuclear and cytoplasmic RNP particles.

Bark, C., Hammarström, K., Westin, G., Pettersson, U., Dept. of Medical Genetics, University of Uppsala, Sweden: Pseudogenes for human U4 RNA—Strong support in favor of an RNA-mediated mechanism of dispersion.

Barta, A.,[1] Steiner, G.,[1] Noller, H.F.,[2] Kuechler, E.,[1] [1]Institute of Biochemistry, University of Vienna, Austria; [2]Thimann Laboratory, University of California, Santa Cruz: Identification of a domain on 23S rRNA located at the peptidyl transferase site.

Bass, B., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: Specific interaction between the self-splicing RNA of *Tetrahymena* and its guanosine substrate.

Berrios, M., Fisher, P.A., Dept. of Pharmacological Sciences, State University of New York, Stony Brook: Is the major photolabelable ATPase associated with the *Drosophila* nuclear matrix-pore, complex-lamina fraction a form of myosin?

Blanchard, J.M., Piechaczyk, M., Panabières, F., Dani, C., Riad, S., Marty, L., Jeanteur, P., Université des Sciences et Techniques du Languedoc, and Centre P. Lamarque, Montpellier, France: Tissue-specific differences in the steady-state polyadenylation of mRNA coding for glyceraldehyde-3-*P*-dehydrogenase in various chicken tissues.

Bonitz, S., Cold Spring Harbor Laboratory, New York: Nuclear regulation of RNA processing in yeast mitochondria.

Breitenberger, C.A., Alzner-DeWeerd, B., Browning, K.S., RajBhandary, U.L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: tRNA sequences serve as signals in processing of mitochondrial rRNAs and mRNAs in *Neurospora crassa*.

Calvet, J.P., Smardo, F.L., Jr., Maser, R.L., Dept. of Biochemistry, University of Kansas Medical Center, Kansas City: Small RNAs that do and do not interact with rRNA.

Capasso, J.M., Docherty, M.A., Ray, A., Kaplan, E.D., Eliceiri, G.L., St. Louis University Medical School, Missouri: U1 small, nuclear RNA-like sequences in high-molecular-weight hnRNA.

Carrasco, A.E., Müller, M., De Robertis, E.M., Dept. of Cell Biology, University of Basel, Switzerland: Homeoticlike genes in vertebrates—Analysis of a gene that is expressed during early *Xenopus* development.

Chae, C.-B., Patton, J.R., Dept. of Biochemistry, University of North Carolina, Chapel Hill: Specific regions of U1 snRNP are sensitive and resistant to nuclease.

Chu, F.K., Belfort, M., Maley, F., Maley, G., Center for Laboratories and Research, New York State De-

partment of Health, Albany: Structure of T4 bacteriophage thymidylate synthase gene and its product.

Crouch, R.J., Itaya, M., Kanaya, S., NCI, National Institutes of Health, Bethesda, Maryland: *rnh* mutants are defective in cell growth.

de Massy, B.,[1] Fayet, O.,[2] Kogoma, T.,[3] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]CRBGC, Toulouse, France; [3]Dept. of Biology, University of New Mexico, Albuquerque: Activation of origins of DNA replication in *sdrA (rnh)* mutants of *E. coli* K-12.

Dihanich, M.,[1] Ellis, S.,[2] Najarian, D.,[2] Morales, M.,[2] Clark, R.,[2] Martin, N.C.,[2] Hopper, A.K.,[1] [1]Dept. of Biological Chemistry, 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.

Dinter-Gottlieb, G., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: Viroids contain homologies with the *Tetrahymena* IVS and may resemble group I introns in structure.

Drabkin, H., RajBhandary, U.L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Site-specific mutagenesis on a human initiator tRNA gene – Effect of A54 to T54 change on RNA processing and on modification of U55 to ψ55.

Earley, J., Roebuck, K.A., Stumph, W.E., Dept. of Chemistry and Molecular Biology Institute, San Diego State University, California: Some chicken U1 RNA genes are clustered and have limited regions of flanking DNA sequence homology.

Engberg, J., Nielsen, H., Jeppesen, C., Biochemical Institute B, University of Copenhagen, Denmark: The ribosomal RNA genes of *Tetrahymena* – Structure and function.

Falkenthal, S.,[1] Parker, V.P.,[2] Davidson, N.,[3] [1]Dept. of Genetics, Ohio State University, Columbus; [2]Amgen, Newberry Park, California; [3]Dept. of Chemistry, California Institute of Technology, Pasadena: An unexpected 3′ splice site is involved in differential splicing of RNA transcribed from the *Drosophila* MLC-ALK gene.

Forbes, D.,[1,3] Dahlberg, J.,[2] Lund, E.,[2] [1]Dept. of Biochemistry and Biophysics, University of California, San Francisco; [2]Dept. of Physiological Chemistry, University of Wisconsin, Madison; [3]Dept. of Biology, University of California, San Diego, La Jolla: Differential expression of multiple U1 RNA species in *Xenopus* oocytes and embryos.

Gupta, K.C., Kingsbury, D.W., Dept. of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Polytranscripts – An enigma in paramyxoviruses and rhabdoviruses.

Gurevitz, M., Apirion, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: The ribonuclease III processing site near the 5′ end of an RNA precursor of bacteriophage T4 and its effect on termination.

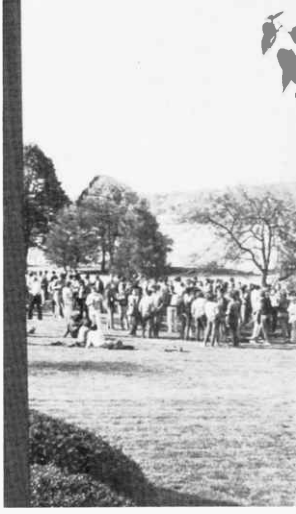
Hashimoto, C., Steitz, J.A., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: U4 and U6 RNAs coexist in a single small nuclear ribonucleoprotein particle.

Hay, N., Aloni, Y., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Efficient and accurate in vitro splicing of SV40-associated, small RNA (SAS-RNA).

Homison, G., Dieckmann, C.L., Koerner, T.J., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: Identification, isolation, and characterization of a yeast nuclear gene (*CBP1*) that is required for the proper processing of the 5′ end of the cytochrome *b* mRNA.

Hurt, D.J., Hopper, A.K., Hershey Medical Center, Pennsylvania: Cloning and characterization of *LOS1*, a gene of *S. cerevisiae* involved in tRNA processing.

Inoue, T., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: Secondary structure of the circular form of the *Tetrahymena* rRNA intervening sequence – A new technique for RNA structure analysis using chemical probes and reverse transcriptase.



Wine and cheese party

Itaya, M., Kanaya, S., Crouch, R.J., NCI, National Institutes of Health, Bethesda, Maryland: *E. coli* RNase H *(rnh)* mutations can be suppressed.

Kaltwasser, G., DiMaria, P., Goldenberg, C.J., Dept. of Pathology, Washington University, St. Louis, Missouri: Characterization and partial purification of an mRNA splicing activity.

Klein, B., Staden, A., Schlessinger, D., Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri: Alternate conformations in 16S rRNA.

Klootwijk, J., Kempers-Veenstra, A.E., Dekker, A.F., van Heerikhuizen, H., Musters, W., Oliemans, J., Planta, R.J., Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands: Expression of artificial rRNA genes in yeast.

Konarska, M., Padgett, R.A., Grabowski, P.J., Sharp, P.A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: mRNA splicing in vitro – Requirement for a cap structure.

Krainer, A.R., Ruskin, B., Green,

M.R., Maniatis, T., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: In vitro splicing of normal and mutant human β-globin pre-mRNAs.

Krowczynska, A., Albrecht, G., Brawerman, G., Dept. of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts: Endogenous cleavages in the polyribosomal β-globin mRNA of rabbit reticulocytes.

Krowczynska, A., Brawerman, G., Dept. of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts: Control of mRNA stability in mouse erythroleukemia cells.

Lahiri, D.K., Thomas, J.O., Dept. of Biochemistry, New York University School of Medicine, New York: What happens to nuclear RNA and its associated proteins during mitosis?

SESSION 4 tRNA PROCESSING

Chairperson: S. Altman, Yale University, New Haven, Connecticut

Tobian, J.A., Castano, J.G., Zasloff, M.A., NCI, National Institutes of Health, Bethesda, Maryland: Point mutations in a human $tRNA^{mer}_{f}$ *gene* – Effect on transport and processing.

Doersen, C., Gaines, G., Shuey, D., Attardi, G., California Institute of Technology, Pasadena: Synthesis and processing of human mRNA in in vitro systems.

Gandini-Attardi, D., Margarit, I., Tocchini-Valentini, G.P., Institute of Cell Biology, Rome, Italy: Role of the structure of the exon in tRNA splicing.

Hollingsworth, M.J., Martin, N.C., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: A mitochondrial gene is required for the expression of yeast mitochondrial RNase P.

Willis, I., Chisholm, V., Pearson, D., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Mutations affecting 5′-end maturation and splicing of an *S. pombe* dimeric tRNA precursor.

Atkinson, N.S., Dunst, R.W., Nolan, S.L., Hopper, A.K., Dept. of Biological Chemistry, Hershey Medical Center, Pennsylvania: Characterization of a yeast gene affecting RNA processing.

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*.

Reilly, R.M., RajBhandary, U.L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Biosynthesis of *E. coli* SuIII tRNA is defective in mutants encoding GAUC in place of the universal GTψC sequence.

Deutscher, M.P., Marlor, C.W., Zaniewski, R., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: The exoribonuclease family of *E. coli* – Identification and characterization of a new enzyme, RNase T.

SESSION 5 rRNA PROCESSING

Chairperson: J. Dunn, Brookhaven National Laboratory, Upton, New York

Dahlberg, A., Zwieb, C., Jemiolo, D., Jacob, W., Skinner, R., Stark, M., Section of Biochemistry, Brown University, Rhode Island: Effects of site-directed mutations on processing of *E. coli* rRNA.

Sirdeshmukh, R., Schlessinger, D., Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri: Maturation of 23S rRNA in vitro.

Szeberényi, J., Apirion, D., Dept. of Microbiology, Washington University, St. Louis, Missouri: Primary and secondary processing of 5S rRNA in *E. coli*.

Cole, J.R., Singer, P., Nomura, M., Depts. of Genetics and Biochemistry, University of Wisconsin, Madison: Translational regulation of ribosomal proteins in *E. coli* affects the half-lives of ribosomal protein mRNAs.

Bolla, R.,[1] Shiomi, Y.,[2] Braaten, D.,[2] Herbert, M.B.,[2] Schlessinger, D.,[2] [1]Dept. of Biology, University of Missouri, St. Louis; [2]Dept. of Microbiology and Immunology, Washington University School of

M. Mathews, A. Shatkin

Medicine, St. Louis, Missouri: Characterization of mouse L-cell core nucleoli.

Reddy, R., Lischwe, M., Epstein, P., Henning, D., Tan, E., Reichlin, M., Busch, H., Dept. of Pharmacology, Baylor College of Medicine, Houston, Texas: Nucleolar RNP antigens and U3 genes.

Tabak, H.F., Osinga, K.A., Groot Koerkamps, M.J.A., van der Horst, G.T.J., Section for Molecular Biology, University of Amsterdam, The Netherlands: Transcription of yeast mitochondrial DNA and processing of RNA.

Bachellerie, J.P., Michot, B., Hassouna, N., Raynal, F., Centre de Recherche de Biochimie et de Genetique Cellulaires du CNRS, Toulouse, France: Structure and processing of mouse rRNA precursors—Genuine spacers and spacerlike domains within mature rRNA.

Vance, V., Bowman, L.H., Dept. of Biology, University of South Carolina, Columbia: Transfection of mouse rDNA into rat cells—Faithful transcription and processing.

SESSION 6 POSTER SESSION

Lee, M.G.,[1] Young, R.A.,[2] Begs, J.D.,[1] [1]Dept. of Biochemistry, Imperial College of Science and Technology, London, England; [2]Dept. of Biochemistry, Stanford University School of Medicine, California: Cloning and analysis of the *RNA2* gene from *S. cerevisiae*.

Leser, G.P., Martin, T.E., Dept. of Biology, University of Chicago, Illinois: hnRNP core protein complements of cells examined with monoclonal antibodies.

Leys, E.J.,[1] Crouse, G.F.,[2] Frayne, E.G.,[1] Kellems, R.E.,[1] [1]Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: Identification of an RNA polymerase II transcription unit with an initiation site less than 200 bp upstream from the mouse *dhfr* gene.

Lockard, R.E., Garrett-Wheeler, E., Kumar, A., Dept. of Biochemistry, George Washington University Medical School, Washington, D.C.: Mapping of psoralen cross-linked nucleotides in RNA.

Mangin, M., Ares, M., Jr., Weiner, A.M., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: DNA conformation of snRNA genes—Mapping of hypersensitive sites on the human U1 and U2 transcription units.

Maran, A., Kalyanaraman, S., Shanmugam, G., School of Biological Sciences, Madurai Kamaraj University, India: Double-stranded RNA specific nuclease from germinating embryos of *Pennisetum typhoides*.

Mattaj, I.W., Zeller, R., DeRobertis, E.M., Dept. of Cell Biology, Biocenter, University of Basal, Switzerland: *Xenopus* U-snRNA gene transcription units.

Mecklenburg, K.,[1] Sass, P.,[2] Mahler, H.R.,[2] Perlman, P.S.,[1] [1]MCDB Program, Ohio State University, Columbus; [2]Dept. of Chemistry, Indiana University, Bloomington: Splicing defective mutants in the *Oxi3* gene of yeast mtDNA.

Miller, K., Zbrzezna, V., Pogo, A.O., Lindsey F. Kimball Research Institute, New York Blood Center, New York: RNA interstrand duplexes in murine erythroleukemia cells and *Drosophila* KC cells.

Morris, G.F., Marzluff, W.F., Dept. of Chemistry, Florida State University, Tallahassee: Biosynthesis of sea urchin N1 snRNA.

Noble, J., Prives, C., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: In vitro splicing and polyadenylation of an SV40 early pre-mRNA.

Orellana, O., Cooley, L., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Transcription and processing of a cloned $tRNA^{His}$ gene from *E. coli*.

Parker, R., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: A genetic analysis of mRNA splicing in yeast.

Patton, J.R., Chae, C.-B., Dept. of Biochemistry, University of North Carolina, Chapel Hill: Specific protein binding on β-globin RNA in whole nuclei, the nuclear matrix, and 40S hnRNP of chicken reticulocyte.

Pedersen, N., Hellung-Larsen, P., Engberg, J., Dept. of Biochemistry, Panum Institute, University of Copenhagen, Denmark: snRNAs in *Tetrahymena*.

Reinberg, D., Roeder, R.G., Rockefeller University, New York, New York: RNA polymerase II transcription system—A biochemical approach.

Reveillaud, I., Lelay-Taha, M.N., Sri-Widada, J., Brunel, C., Jeanteur, P., Laboratoire de Biochimie, Centre P. Lamarque, and Laboratoire de Biologie Moleculaire, USTL, Montpellier, France: Dramatic effect of Mg^{++} on snRNP conformation application to isolation of individual U1 and U5 snRNPs.

Roe, B.A., Ma, D.-P., Wong, J.F.H., Wilson, R.K., Ahmed, M.A., Dept. of Chemistry, University of Oklahoma, Norman: Structure and expression of several eukaryote, prokaryote, and organelle tRNA genes.

Rooney, R.J., Harding, J.D., Dept. of Biological Sciences, Columbia University, New York, New York: Kinetics of in vitro transcription and processing of mouse tRNA genes.

Ryner, L., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: A novel poly(A) polymerase activity in a cytoplasmic S100 extract of HeLa cells.

Samuels, D.S. Peebles, C.L., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Overproduction of pre-tRNAs.

Sarkar, S., Jayabaskaran, C., Dasgupta, S., Dept. of Muscle Research, Boston Biomedical Research Institute, Massachusetts: A novel cytoplasmic, translation-in-

hibitory 10S RNP particle of chick embryonic muscle.

Schmidt, F.J., Motamedi, H., Lee, Y., Dept. of Biochemistry, University of Missouri, Columbia: Tandem promoters preceding the gene for *E. coli* M1 RNA.

Schrier, W.H., Okarma, T.B., Stanford University, California: Induction of growth hormone transcription by triiodothyronine and dexamethasone is accompanied by increased 4S–8S RNA synthesis.

Shelness, G.S., Williams, D.L., Dept. of Pharmacological Sciences, State University of New York, Stony Brook: Apolipoprotein II mRNA – Alternate splicing and transcriptional heterogeneity yields six untranslated 5′ leader sequences.

Simon, M., Arrigo, A.-P., Spahr, P.-F., Dept. of Molecular Biology, University of Geneva, Switzerland: A cellular protein phosphorylated by RSV transforming product is associated with small cytoplasmic RNP particles.

Smith, D.B., Inglis, S.C., Dept. of Pathology, University of Cambridge, England: Influenza virus mRNA splicing is modulated during infection.

Strobel, M.C.,[1,2] Abelson, J.,[2] [1]Dept. of Biology, University of California, San Diego, La Jolla; [2]Division of Biology, California Institute of Technology, Pasadena: The intervening sequence directs modification of the SUP53 ($tRNA^{Leu}$) anticodon.

Subbarao, M.N., Watson, N., Apirion, D., Dept. of Microbiology, Washington University, St. Louis, Missouri: Processing reactions catalyzed by RNA in *E. coli*.

Talib, S., O'Brien-Vedder, C., Okarma, T.B., Stanford University, California: In vivo effects of psoralen cross-linking on U-snRNA gene expression.

Tanner, N.K., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: Probing the structure and function of RNA with ethidium bromide and methidiumpropyl-EDTA·Fe(II).

Thompson, W.S., Abernathy, E.C., Gross, R.H., Dept. of Biology, Dartmouth College, Hanover, New Hampshire: Stability and localization of liposome-introduced snRNAs and anti-snRNP antibodies in *Drosophila* cells.

Tomcsanyi, T., Apirion, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: A small RNA processed by RNase E is involved in plasmid DNA replication.

Tu, C.-P.D., Lai, H.-C.J., Li, N., Weiss, M.J., Reddy, C.C., Pennsylvania State University, University Park: Tissue-specific expression of a Y_a and a Y_c subunit of rat glutathione S-transferases.

Ullu, E., Weiner, A.M., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Efficient and accurate expression of a 7SL hnRNA gene is strongly dependent upon an element located in the 5′-flanking sequences.

Vold, B., Green, C., SRI International, Menlo Park, California: Large clusters of tRNA genes from *B. subtilis*.

Watson, N., Dallmann, G., Dallmann, K., Sonin, A., Apirion, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Cloning of genes for *E. coli* RNA processing enzymes.

Westin, G.,[1] Hammarström, K.,[1] Zabielski, J.,[1] Bark, C.,[1] Murphy, J.,[2] Lund, E.,[2] Dahlberg, J.,[2] Pettersson, U.,[1] [1]Dept. of Medical Genetics, University of Uppsala, Sweden; [2]Dept. of Physiological Chemistry, University of Wisconsin, Madison: Isolation and expression of genes for U2 hnRNA.

Willis, I., Frendewey, D., Nichols, M., Schaack, J., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: A single base change in the anticodon of an *S. pombe* serine tRNA affects RNase P cleavage and splicing in vitro.

Wollenzien, P.L.,[1] Expert-Bezancon, A.,[2] Cantor, C.R.,[1] [1]Dept. of Human Genetics and Development, Columbia University, New York, New York; [2]Institut de Recherche en Biologie Moléculaire, Paris, France: Three dimensional arrangement of the *E. coli* 16S rRNA in the 30S subunit.

SESSION 7 mRNA SPLICING

Chairperson: J. Manley, Columbia University, New York, New York

Belfort, M., Chu, F., Maley, G., Ehrenman, K., Pedersen-Lane, J., Maley, F., Center for Laboratories and Research, New York State Department of Health, Albany: Expression of an intron-containing prokaryotic thymidylate synthase gene.

Pikielny, C.W.,[1] Rodriguez, J.,[2] Rosbash, M.,[2] [1]Dept. of Biochemistry; [2]Dept. of Biology, Brandeis University, Waltham, Massachusetts: Possible intermediate in yeast mRNA splicing.

Rossi, J.J., McMahon, J., Cellini, A., Beckman Research Institute of the City of Hope, Los Angeles, California: Deletion of the ICS from the actin intron reduces but does not abolish splicing.

van Santen, V., Spritz, R.A., Dept. of Medical Genetics, University of Wisconsin, Madison: Sequence requirements for splicing of eukaryotic pre-mRNA – Deletion analysis of an intervening sequence.

Hwang, L., Park, J., Gilboa, E., Dept. of Molecular Biology, Princeton University, New Jersey: Mechanism of splicing of Mo-MLV *env* mRNA.

DiMaria, P., Kaltwasser, G., Goldenberg, C.J., Dept. of Pathology, Washington University, St. Louis, Missouri: In vitro Ad2 mRNA splicing of purified, synthetic RNA precursors.

Krämer, A.,[1] Hernandez, N.,[1] Reiss, B.,[1] Lahr, G.,[1] Frick, M.,[1] Keller, W.,[1] Rinke, J.,[2] Appel, B.,[2] Lührmann, R.,[2] [1]Institute of Cell and Tumor Biology, German Cancer

Research Center, Heidelberg; [2]Max-Planck-Institute, Berlin, Federal Republic of Germany: In vitro splicing of mRNA precursors.

Ruskin, B., Krainer, A., Maniatis, T., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: The fate of intron sequences during in vitro splicing.

Grabowski, P.J., Padgett, R.A., Sharp, P.A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: mRNA splicing in vitro—An excised intervening sequence and a potential intermediate.

SESSION 8 mRNA PROCESSING AND METABOLISM

Chairperson: W. Le Stourgeon, Vanderbilt University, Nashville, Tennessee and National Science Foundation, Washington, D.C.

Halbreich, A., Grandchamp, C., Foucher, M., Centre de Genetique Moléculaire du CNRS, Gif-sur-Yvette, France: Yeast mitochondria contain a linear RNA strand complementary to the circular, intronic bI1 RNA of cytochrome *b*.

Simon, M., Faye, G., Institut Curie, Orsay, France: Organization and processing of the OXI3/OLI2 multigenic transcript in yeast mitochondria.

Last, R., Woolford, J.L., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Evidence for related functions of the *RNA2, RNA3,* and *RNA4* gene products of *S. cerevisiae*.

Pape, L.K., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: A nuclear gene required for processing of the *S. cerevisiae* mitochondrial apocytochrome *b* pre-mRNA.

Anderson, K.P., Klessig, D.F., Dept. of Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City: Altered mRNA splicing in monkey cells abortively infected with human adenovirus may be responsible for inefficient synthesis of the virion fiber polypeptide.

Dreyfuss, G., Choi, Y.-D., Adam, S.A., Dept. of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, Illinois: Characterization of the protein complexes of hnRNA in vivo with monoclonal antibodies.

Raziuddin, A.K., Thomas, J.O., Szer, W., Dept. of Biochemistry, New York University School of Medicine, New York: Functional domains of HD40, the major core hnRNP protein of *Artemia*.

Greenberg, J.R., Carroll, E. III, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Reconstitution of functional mRNP-protein complexes.

Görlach, M., Schulze, G., Sarre, T., Hilse, K., Institute für Biology Universität, Freiburg, Federal Republic of Germany: Characterization of cytoplasmic mRNPs from two different erythropoietic systems (rabbit reticulocytes/murine erythroleukemia cells).

Katze, M.G., Chen, Y.-T., Krug, R.M., Memorial Sloan-Kettering Cancer Center, New York, New York: The processing and expression of RNA polymerase II transcripts in influenza virus infected cells.

H. Robertson

SESSION 9 SMALL RNAs AND RNPs

Chairperson: R. Gesteland, University of Utah, Salt Lake City, Utah

Patterson, B., Tollervey, D., Swerdlow, H., Wise, J.A., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: A genetic analysis of snRNA function in yeast.

Skuzeski, J.M.,[1] Lund, E.,[1] de Vegvar, H.E.N.,[1] Murphy, J.T.,[2] Steinberg, T.,[2] Dahlberg, J.E.,[1] [1]Dept. of Physiological Chemistry; [2]McArdle Laboratory, University of Wisconsin, Madison: Signals for transcription and maturation of U1 RNA.

Perez-Stable, C., Hess, J., Ayres, T.M., Shen, C.-K.J., Dept. of Genetics, University of California, Davis: Novel features of in vitro synthesis of human *alu* family small RNAs—Distinctive promoter organization and a new eukaryotic class III terminator.

Lührmann, R., Appel, B., Rinke, J., Reuter, R., Theissen, H., Bringmann, P., Max-Planck-Institut, Berlin, Federal Republic of Germany: Structure of U-snRNPs—Evidence for the existence of snRNAs U4 and U6 in a single RNP complex.

Fisher, D.E., Conner, G.E., Reeves, W.H., Blobel, G., Kunkel, H.G., Rockefeller University, New York, New York: SnRNP assembly is a two-step process, accompanied by posttranslational modification.

Kloetzel, P.M., Schult, C., Bautz, E.K.F., Dept. of Molecular Genetics, University of Heidelberg, Federal Republic of Germany: Analysis of specific RNP-com-

plexes in heat-shocked *Drosophila* cells.

Martin, T.E., Monsma, S.A., Leser, G.P., Dept. of Biology, University of Chicago, Illinois: Localization of Sm- and U1-specific snRNP antigens at transcription sites, including some lacking introns.

Masukata, H., Tomizawa, J., NIADDK, National Institutes of Health, Bethesda, Maryland: Alteration in RNA structure affects primer formation for ColE1 DNA replication.

SESSION 10 POLY A AND 3′ ENDS

Chairperson: R. Perry, Institute for Cancer Research, Philadelphia, Pennsylvania

Mott, J., Galloway, J.L. Farnham, P.J., Platt, T., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: RNA termination and processing *at* the 3′ end of tryptophan operon mRNA.

Higgins, C.F., Dept. of Biochemistry, University of Dundee, Scotland: A novel structure (the "REP" sequence) present in a large number of bacterial mRNA species may regulate mRNA processing and degradation.

Aloni, Y., Hay, N., Bengal, E., Laub, O., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Attenuation and transcription-termination in the control of eukaryotic gene expression—SV40 as a model system.

Frayne, E.G.,[1] Leys, E.J.,[1] Crouse, G.F.,[2] Kellems, R.E.,[1] [1]Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas; [2]NCI, Frederick, Maryland: Transcription of the *dhfr* gene proceeds uninterrupted through all seven poly(A) sites and terminates further downstream.

Wickens, M., Stephenson, P., Dept. of Biochemistry, University of Wisconsin, Madison: Point mutations in any one of three positions of the AAUAAA sequence prevent cleavage of SV40 late pre-mRNAs in *Xenopus* oocytes.

McDevitt, M.A., Imperiale, M.J., Ali, H., Nevins, J.R., Rockefeller University, New York, New York: Requirement of a downstream sequence for generation of a poly(A) addition site.

Sadofsky, M., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Deletions of SV40 DNA downstream from the late AAUAAA, including a region of U4 RNA homology, decrease the efficiency of 3′-end processing.

Moore, C., Sharp, P.A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Site-specific poladenylation in a cell-free reaction.

Manley, J.L., Yu, H., Ryner, L., Dept. of Biological Sciences, Columbia University, New York, New York: The hexanucleotide AAUAAA forms part of a "promoter" for mRNA polyadenylation in vitro.

Nordstrom, J.L., Kessler, M.M., Westhafer, M.A., Dept. of Biochemistry and Biophysics, Texas A&M University, College Station: The 3′ end of the sea urchin H2A gene serves as a polyadenylation signal in *COS* cells.

RNA Tumor Viruses

May 22–May 27, 1984

ARRANGED BY

Tony Hunter, Salk Institute
Steve Martin, University of California, Berkeley

434 participants

RNA tumor virus research continues to be an exciting and rapidly moving field. This was the ninth RNA tumor virus meeting, and its continuing popularity was demonstrated by the large number of attendees and presentations.

More than half of the presentations were concerned with work on viral and cellular oncogenes. Recurring themes included: the different mechanisms that result in the activation of proto-oncogenes; the use of in vitro mutagenesis techniques to define functional domains within viral transforming proteins; and the discovery of new, common integration sites for viruses without *onc* genes. The demonstration that mutations in the yeast RAS genes have marked phenotypic effects underscored the advantages of simple eukaryote models for studies on the cellular functions of oncogenes. Another exciting observation was the finding

that the Philadelphia translocation in chronic myelogenous leukemia results in a unique c-*abl* transcript and an altered and apparently activated c-*abl* protein.

Although work on oncogenes received the lion's share of attention, there were novel findings in other areas: the requirement for *pol* endonuclease activity for DNA integration; the role of the MLV *pol* protease in the processing of *gag* precursors; and the tissue specificity of the enhancer elements within viral LTRs as a basis for the tissue tropism of these viruses. Considerable progress was also reported in the characterization of the human T-cell lymphotropic viruses (HTLV and LAV) and their roles in T-cell leukemia and AIDS.

Funding was provided in part by the Cold Spring Harbor Laboratory Cancer Center Grant from the National Cancer Institute, National Institutes of Health.

SESSION 1 ONCOGENES (src, rel, fps, abl)

Chairperson: H. Hanafusa, Rockefeller University, New York, New York

Iba, H.,[1] Cross, F.R.,[1] Takeya, T.,[2] Hanafusa, T.,[1] Hanafusa, H.,[1] [1]Rockefeller University, New York, New York; [2]Institute for Chemical Research, Kyoto University, Japan: Low protein kinase activity in vivo may be correlated to the inability of $p60^{c\text{-}src}$ to transform CEF.

Yaciuk, P., Coussens, P.M., Shalloway, D., Molecular and Cell Biology Program, Pennsylvania State University, University Park: Effects of modifying the $pp60^{src}$ carboxyl terminus.

Coussens, P.M., Johnson, P.J., Shalloway, D., Molecular and Cell Biology Program, Pennsylvania State University, University Park: Highly efficient c-*src* expression plasmids induce low-level transformation.

Jakobovits, E.B., Majors, J.E., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: Hormonal regulation of the RSV *src* gene via the MMTV promoter defines a threshold dose for transformation.

Wilhelmsen, K.C., Tarpley, W.G., Miller, C.K., Temin, H.M., McArdle Laboratory, University of Wisconsin, Madison: Identification of some of the parameters governing transformation by oncogenes in retroviruses.

Yonemoto, W.,[1] Jarvis-Morar, M.,[1] Bolen, J.,[2] Israel, M.,[2] Brugge, J.,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]NCI, National Institutes of Health, Bethesda, Maryland: Association of the cellular *src* gene product with the middle T antigen of polyoma virus results in enhancement of associated tyrosine-specific kinase activity and modification of the cellular *src* protein.

Iijima, S., Wang, L.-H., Rockefeller University, New York, New York: Tissue-specific expression of c-*src* mRNA by alternative splicing.

Rohrschneider, L., Reynolds, S., Rothwell, V., Fred Hutchinson Cancer Research Center, Seattle, Washington: Regulation of cellular morphology by the RSV *src* gene product—Analysis of fusiform mutants.

Cross, F.R., Garber, E.A., Pellman, D., Hanafusa, H., Rockefeller University, New York, New York: The extreme amino terminus of the RSV-transforming protein is required for myristylation, membrane association, and transformation.

Garber, E.A., Cross, F.R., Pellman, D., Hanafusa, H., Rockefeller University, New York, New York: Myristylation of $p60^{src}$ and its role in membrane association—Comparison of wild-type RSV and in vitro constructed aminoterminal deletion mutants.

Snyder, M.A., Bishop, J.M., George William Hooper Research Foundation, Dept. of Microbiology and Immunology, University of California, San Francisco: A mutation at the major phosphotyrosine site in $pp60^{v\text{-}src}$ alters oncogenic potential.

Stone, J.C.,[1] Atkinson, T.,[2] Smith, M.,[2] Pawson, T.,[1] Depts. of [1]Microbiology, [2]Biochemistry, University of British Columbia, Vancouver, Canada: Identification of functional regions in the transforming protein of FuSV by in-phase insertion mutagenesis.

Weinmaster, G.,[1] Zoller, M.,[3] Smith, M.,[2] Pawson, T.,[1] Depts. of [1]Microbiology, [2]Biochemistry, University of British Columbia, Vancouver, Canada; [3]Cold Spring Harbor Laboratory, New York: Site-directed mutagenesis of FuSV reveals that tyrosine phosphorylation of $p130^{gag\text{-}fps}$ modulates its enzymatic and biological activities.

Foster, D.A., Shibuya, M., Hanafusa, H., Rockefeller University, New York, New York: The structure and transformation potential of cellular DNA sequences homologous to the *fps* gene of FuSV.

Feldman, R.A.,[1,2] Gabrilove, J.L.,[3] Tam, J.,[2] Moore, M.A.S.,[3] Hanafusa, H.,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Rockefeller University; [3]Memorial Sloan-Kettering Cancer Center, New York, New York: Differential expression of the cellular *fps/fes*-encoded protein in human hematopoietic tumors.

Mathey-Prevot, B., Baltimore, D., Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge: Effect of replacing the *abl* sequence by the avian sequences *src* or *fps* in Ab-MLV.

Prywes, R.,[1] Hoag, J.,[1] Rosenberg, N.,[2] Baltimore, D.,[1] [1]Whitehead Institute for Biomedical Research, Massachusetts Institute of Technol-

ogy, Cambridge; [2]Tufts University School of Medicine, Boston: The p15 region of *gag* is required for lymphoid transformation by Ab-MLV.

Foulkes, J.G.,[1] Baltimore, D.,[1] Maller, J.L.,[2] [1]Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Pharmacology, University of Colorado, School of Medicine, Denver: Microinjection of Ab-MLV tyrosyl-protein kinase into *Xenopus* oocytes induces phosphorylation of ribosomal protein S6 on serine residues.

SESSION 2 REPLICATION AND INTEGRATION

Chairperson: H. Varmus, University of California, San Francisco, California

Lobel, L.I., Tanese, N., Goff, S.P., Dept. of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York: Transfer of mutations from the 5′ LTR to the 3′ LTR during replication of Mo-MLV – Strong-stop DNAs may "jump" prematurely.

Smith, J.K., Cywinski, A., Taylor, J.M., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: The specificity of initiation of strong-stop plus DNA by RSV.

Resnick, R.M., Faras, A.J., Dept. of Microbiology, University of Minnesota, Minneapolis: Recognition binding sites for reverse transcriptase during RNase H-mediated (+) DNA synthesis in vitro.

Panganiban, A., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Sequences required for integration of SNV DNA.

Colicelli, J., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians and Surgeons, New York, New York: Mutants of Mo-MLV bearing a single base pair change at the LTR tip can still integrate into host DNA.

Longiaru, M.,[1] Duyk, G.,[2] Leis, J.,[2] Skalka, A.M..[1] [1]Dept. of Molecular Genetics, Hoffman-La Roche Inc., Roche Research Center, Nutley, New Jersey; [2]Case Western Reserve University School of Medicine, Cleveland, Ohio: Selective cleavage of wild-type and mutated double-strand retroviral LTR DNA by the endonuclease associated with AMV reverse transcriptase.

Duyk, G.,[1] Longiaru, M.,[2] DeHaseth, P.,[1] Terry, R.,[2] Shalka, A.,[2] Leis, J.,[1] [1]Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Sequence requirements for site selective cleavage of the RAV-2 LTR by the reverse transcriptase endonuclease.

Donehower, L.A., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: *pol* mutants of MLV are defective for integration.

Hippenmeyer, P.J., Grandgenett, D.P., Institute for Molecular Virology, St. Louis, Missouri: Amino terminus and mutational analysis of the ASV pp32 protein.

Levin, J.G.,[1] Hu, S.C.,[1] Rein, A.,[2] Messer, L.I.,[1] Gerwin, B.I.,[3] [1]NICHD, National Institutes of Health, Bethesda; [2]Frederick Cancer Research Facility, Frederick; [3]NIC, National Institutes of Health, Bethesda, Maryland: Functional mapping of MLV *pol* gene by characterization of a frameshift mutant.

Crawford, S., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians and Surgeons, New York, New York: A deletion mutation in the 5′ part of the *pol* gene of Mo-MLV prevents processing of the $Pr65^{gag}$ precursor polyprotein.

Yoshinaka, Y., Katoh, I., Copeland, T.D., Oroszlan, S., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Purification, biochemical and structural characterization of Mo-MLV protease encoded by the *gag-pol* gene.

Leis, J.,[1] Fu, X.,[1] Traugh, J.,[2] [1]Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]University of California, Riverside: The RNA-binding properties of the avian retrovirus pp12 are regulated by phosphorylation of a specific serine residue.

SESSION 3 POSTER SESSION I

Dolberg, D.S., Bissell, M.J., Dept. of Biology and Medicine, Lawerence Berkeley Laboratory, University of California, Berkeley: The role of wounding in RSV-induced tumor formation.

Kirschmeier, P.T., Perkins, A.S., Shane, E., Bilezikian, J.P., Weinstein, I.B., Columbia University College of Physicians and Surgeons, New York, New York: Suppression of the transformed phenotype by the *Salmonella typhimurium* adenyl cyclase gene.

Laugier, D.,[1] Poirier, F.,[1] Marx, M.,[2] Genvrin, P.,[1] Dambrine, G.,[3] Calothy, G.,[1] [1]Institut Curie, Orsay; [2]CNRS, ICIG, Villejuif; [3]Station de Pathologie Aviaire de l'INRA Monnaie, France: A RSV mutant with host cell-dependent transforming capacity.

Stoker, A.W., Enrietto, P.J., Wyke, J.A., Dept. of Tumour Virology, Imperial Cancer Research Fund, London, England: Temperature-sensitive mutations in functional domains of $pp60^{v\text{-}src}$.

Hughes, S.,[1] Mellstrom, K.,[2] Kosik, E.,[1] Tamanoi, F.,[1] Brugge, J.,[2] [1]Cold Spring Harbor Laboratory; [2] Dept. of Microbiology, State University of New York, Stony Brook: Mutation of a termination codon affects *src* initiation.

Perez, L., Wills, J.W., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Effects of upstream initiation codons on the expression of the RSV env gene.

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Parvin, J.,[1] Iijima, S.,[2] Wang, L.-H.,[2] [1]Mount Sinai School of Medicine; [2]Rockefeller University, New York, New York: Mechanisms for the generation of *src*-deletion mutants and recombination between viral and *c-src* sequences.

Kotler, M., Roguel, N., Relevy, H., Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Amplification of proviruses and their methylated state in avian sarcoma-transformed rat cells.

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Maness, P.,[1] Fults, D.,[2] [1]Dept. of Biochemistry, University of North Carolina School of Medicine, Chapel Hill; [2]Dept. of Neurosurgery, Bowman Gray School of Medicine, Winston-Salem: pp60$^{c\text{-}src}$ is localized in processes of developing cerebellar neurons.

Cotton, P.C., Brugge, J.S., Dept. of Microbiology, State University of New York, Stony Brook: Neural tissues express a modified form of the cellular *src* gene product pp60$^{c\text{-}src}$.

Gould, K.,[1] Cooper, J.A.,[1] Hunter, T.,[1] Bretscher, A.,[2] [1]Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California; [2]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Tyrosine protein kinase substrate p81 is homologous to a chicken microvillar core protein.

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Tidmarsh, G., Pillemer, E., Whitlock, C., Dailey, M., Weissman, I., Laboratory of Experimental Oncology, Stanford University School of Medicine, California: A novel surface antigen on Ab-MLV and spontaneous B-cell lymphomas.

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Neckameyer, W.,[1] Shibuya, M.,[2] Hsu, M.-T.,[1] Wang, L.-H.,[1] [1]Rockefeller University, New York, New York; [2]University of Tokyo, Japan: Nucleotide sequence of the transforming gene of ASV UR2 and structural analysis of its cellular homolog.

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Dean, M., Vande Woude, G.F., LBI-Basic Research Program, NCI, Frederick Cancer Research Facility, Frederick, Maryland: Multiple sites in front of the *v-mos* gene can serve as transcription initiation points.

Seth, A., Zweig, M., Showalter, S., Vande Woude, G., NCI, Frederick

Cancer Research Facility, Frederick, Maryland: Production of antisera with *v-mos* protein synthesized in *E.coli*.

Brizzard, B.L., Nash, M.A., Cizdziel, P.E., Murphy, E.C., Jr., Dept. of Tumor Biology/Virology Section, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Organization of viral DNA in MSV *ts*110-infected cell lines.

Singh, B.,[1] Escobedo, J.,[2] Dina, D.,[2] Arlinghaus, R.B.,[1] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla; [2]Chiron Corp., Emeryville, California: Reduced level of a 55,000-dalton cellular protein upon infection by Mo-MSV.

Papkoff, J., Ringold, G., Dept. of Pharmacology, Stanford University School of Medicine, California: Use of the MMTV LTR to promote steroid-inducible expression of *v-mos*.

Righi, M.,[1] Coll, J.,[1] Dozier, C.,[1] Galibert, F.,[2] Stehelin, D.,[1] [1]Oncologie Moléculaire, INSERM, Institut Pasteur, Lille; [2]Centre Hayem, Hopital St. Louis, Paris, France: Analysis of the *mil* oncogene of MH2 and its cellular counterpart.

S. Hughes

Kan, N.,[1] Rovigatti, U.,[1] Duesberg, P.,[2] Papas, T.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Molecular Biology, University of California, Berkeley: Avian carcinoma virus MH2 contains two *onc*-specific genes, *myc* and *mht*, which are closely related to the transforming genes of the avian MC29 and murine sarcoma 3611 viruses.

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Seeley, T.W., Mark, G.E., NCI, National Institutes of Health, Bethesda, Maryland: cDNA clones from human fetal liver homologous to the *raf* oncogene.

Mark, G.E.,[1] Flordellis, C.S.,[2] Rapp, U.R.,[2] Harris, C.C.,[1] [1]NCI, National Institutes of Health, Bethesda; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: The *raf* oncogene is transcribed in small cell lung carcinomas.

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Lewis, W., Raines, M., Wagner, R., Crittenden, L., Kung, H.J., Dept. of Biochemistry, Michigan State University, USDA Regional Poultry Research Laboratory, East Lansing: *c-erb-B* polymorphism – Cloning and characterization of a second *c-erb-B* allele.

Akiyama, T.,[1] Yamada, Y.,[1] Ogawara, H.,[1] Yamamoto, T.,[2] Kasuga, M.,[3] [1]Second Dept. of Biochemistry, Meiji College of Pharmacy; [2]Institute of Medical Science, [3]Third Dept. of Internal Medicine, University of Tokyo, Japan: Site-specific antibodies to *erb-B* oncogene product immunoprecipitate epidermal growth factor receptor.

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Najita, L., Rohrschneider, L., Fred Hutchinson Cancer Research Center, Seattle, Washington: Expression of the *v-fms* protein product at the cell surface.

Donoghue, D.J., Dept. of Chemistry, University of California, San Diego, La Jolla: Analysis of *v-sis* by deletion mutagenesis – Identification of regions required for biological activity.

Niman, H.L.,[1] Houghten, R.A.,[1] Bowen-Pope, D.F.,[2] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California; [2]Dept. of Pathology, University of Washington, Seattle: Widespread expression of high-molecular-weight forms of PDGF.

Thiel, H.-J., Hafenrichter, R., Federal Research Centre for Virus Diseases of Animals, Tübingen, Federal Republic of Germany: SSV transformation-specific glycopeptide – Immunological relationship to human PDGF.

Clark, J.,[1] Wagner, E.,[2] Gilboa, E.,[1] [1]Dept. of Molecular Biology, Princeton University, New Jersey; [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: The nature of the block in Mo-MLV replication in embryonal carcinoma cells.

DesGroseillers, L., Jolicoeur, P., Institut de Recherches Cliniques de Montréal, Université de Montréal, Quebec, Canada: Delayed replication of a MLV LTR mutant – Construction and biological characterization.

Chen, P.-J., Cywinski, A., Taylor, J.M., Institute for Cancer Research, Philadelphia, Pennsylvania: Endogenous reverse transcription of cellular RNA species – An approach to understanding the origin of certain pseudogenes and processed genes.

Van Beveren, C., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Novel eukaryotic initiation

codon (GUG) for murine glycosylated *gag* protein.

Pepinsky, R.B., Potts, W., Steeg, C.M., Southard, L., Vogt, V.M., Section of Biochemistry, Cornell University, Ithaca, New York: Structure and function of p19^{gag} and p19-related proteins of ASV and ALV.

Darlix, J.-L., Meric, C., Spahr, P.-F., Dept. of Molecular Biology, University of Geneva, Switzerland: The functions of RSV protein p12 in reverse transcription, translation, and packaging of the viral genome.

Katoh, I.,[1] Yoshinaka, Y.,[1] Oroszlan, S.,[1] Coward, J.,[2] Luftig, R.B.,[2] [1]Frederick Cancer Research Center, Frederick, Maryland; [2]Dept. of Microbiology and Immunology, Louisiana State University Medical Center, New Orleans: The effect of cerulenin on MLV assembly.

Ro, J.H.-S., Ghosh, H.P., Dept. of Biochemistry, McMaster University, Ontario, Canada: RSV reverse transcriptase gene contains promoter and ribosomal binding sequences needed for its expression in *E. coli*.

Deen, K.C., Sweet, R.W., Dept. of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania: Retroviral-related polymerase sequences in human DNA.

SESSION 4 MORE ONCOGENES (erbB, fms, raf/mil, mos, sis)

Chairperson: C. Sherr, St. Jude Childrens Hospital, Memphis, Tennessee

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: Structure and expression of activated c-*erb-B* genes in ALV-induced erythroblastosis.

Raines, M.,[1] Fadly, A.,[2] Crittenden, L.B.[2] Kung, H.J.,[1] [1]Dept. of Biochemistry, Michigan State University; [2]USDA Regional Poultry Research Laboratory, East Lansing, Michigan: Biological characterization of ALV-induced erythro-blastosis—A leukemia associated with c-*erb-B* activation.

Xu, Y.-H.,[1] Ishii, S.,[1] Clark, A.,[1] Richert, N.,[1] Ito, S.,[1] Roe, B.,[2] Yamamoto, T.,[3] Merlino, G.,[1] Pastan, I.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Chemistry, University of Oklahoma, Norman; [3] Institute of Medical Science, University of Tokyo, Japan: Cloning of cDNA encoding the human EGF receptor.

Merlino, G.,[1] Ishii, S.,[1] Xu, Y.-H.,[1] Clark, A.,[1] Richert, N.,[1] Ito, S.,[1] Yamamoto, T.,[2] Pastan, I.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Institute of Medical Science, University of Tokyo, Japan: Correlation of enhanced expression and amplification of the EGF receptor gene in A431 cells.

Gilmore, T., DeClue, J., Dehazya, P., Martin, G.S., Dept. of Zoology, University of California, Berkeley: Tyrosine phosphorylation induced by the *v-erb-B* protein in vivo and in vitro.

Rettenmier, C.W., Roussel, M.F., Anderson, S.J., Look, A.T., Sherr, C.J., St. Jude Children's Research Hospital, Memphis, Tennessee: Cell-surface localization of some *v-fms*-coded glycoproteins is required for transformation.

Rettenmier, C.W., Roussel, M.F., Sherr, C.J., St, Jude Children's Research Hospital, Memphis, Tennessee: In vitro kinase activity associated with the *v-fms* glycoprotein.

Rapp, U.R.,[1] Jansen, H.W.,[2] Bister, K.,[2] [1]NCI Frederick Cancer Research Facility, Frederick, Maryland; [2]Max-Planck-Institut, Berlin, Federal Republic of Germany: Transforming ability of retroviruses containing the *v-raf/mil* and *v-myc* oncogenes.

Biegalke, B.,[1] Morrow, M.,[1] Rapp, U.,[2] Linial, M.,[1] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington: [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: The *v-mil* gene product is not required for transformation by MH2.

Béchade, C.,[1] Calothy, G.,[1] Coll, J.,[2] Saule, S.,[2] Ghysdaël, J.,[2] Stehelin, D.,[2] [1]Institut Curie, Orsay; [2]INSERM University, Institut Pasteur, Lille, France: Expression of the *v-mil*-encoded p100$^{gag\text{-}mil}$ is required for transforming and mitogenic properties of MH2 in chick embryo neuroretinal cells.

Hannink, M., Dept. of Chemistry, University of California, San Diego, La Jolla: Oligonucleotide mutagenesis of viral oncogenes.

Blair, D.G.,[1] Seth, A.,[2] Dean, M.,[2] Zweig, M.,[3] Vande Woude, G.,[2] [1]NCI; [2]LBI; [3]PRI, NCI, Frederick Cancer Research Facility, Frederick Maryland: Expression of MSV *v-mos* human c-*mos* hybrid recombinants in transfected NIH-3T3 cells.

McGeady, M.L.,[1] Wood, T.G.[2] Vande Woude, G.F.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Biochemistry, Georgetown University, Washington, DC: Inhibition of *mos* expression by normal mouse cell sequences.

Hamelin, R.,[1] Murphy, E.C., Jr., [2] Arlinghaus, R.B.,[1] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California; [2]Dept. of Tumor Biology, M.D. Anderson Hospital, Houston, Texas: Transcription products of the *ts*110 Mo-MSV and its wild-type revertants.

Maxwell, S.A., Kloetzer, W.S., Arlinghaus, R.B., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: P85$^{gag\text{-}mos}$ encoded by *ts*110 Mo-MSV contains a thermolabile kinase activity associated with the *mos* sequences.

Gazit, A., Igarashi, H., Chiu, I.-M., Srinivasan, A., Yaniv, A., Tronick, S., Robbins, K., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: Activation of the normal human c-*sis*/PDGF-2 proto-oncogene as a transforming gene.

Chiu, I.-M., Tronick, S,R., Igarashi, H., Reddy, E.P., Givol, D., Robbins, K.C., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning and nucleotide sequence analysis of the human c-*sis* PDGF gene.

Westin, E., Clarke, M., Gorse, K., Rat-

ner, L., Josephs, S., Reitz, M., Gallo, R.C., Wong-Staal, F., NCI, National Institutes of Health, Bethesda Maryland: Characterization of cells transformed by a cDNA clone of the human c-*sis* gene.

Josephs, S.,[1] Ratner, L.,[1] Westin, E.,[1] Clarke, M.,[2] Reitz, M.S.,[1] Wong-Staal, F.,[1] [1]NCI, National Institutes of Health; [2]NIADDKD, Bethesda, Maryland: Nucleotide sequence analysis of transforming human c-*sis* cDNA.

Josephs, S.,[1] Lautenberger, J.,[1] Westin, E.,[1] Vergilio, L.,[1] Ratner, L.,[1] Clarke, M.,[2] Reitz, M.S.,[1] Wong-Staal, F.,[1] Papas, T.S.,[1] [1]NCI, National Institutes of Health; [2]NIADDKD, Bethesda, Maryland: Expression in bacteria of human c-*sis* protein – Evidence against a polyprotein precursor including chain A and chain B of human PDGF.

Deuel, T.F., Huang, S.S., Huang, J.S., Jewish Hospital, Washington University Medical Center, St. Louis, Missouri: Human PDGF – A product of the human proto-oncogene *c-sis* which interacts with cells through a PDGF receptor/protein tyrosine kinase.

SESSION 5 YET MORE ONCOGENES (ras, SIMPLE EUKARYOTES AND NEW oncs)

Chairperson: **D. Lowy,** National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Willumsen, B.M.,[1] Christensen, A.,[1] Hubbert, N.L.,[2] Papageorge, A.G.,[2] Lowy, D.R.,[2] [1]Fibiger Institute, Copenhagen, Denmark; [2]NCI, National Institutes of Health, Bethesda, Maryland: The carboxyl terminus of the Ha-MSV p21 protein is required for three functions – Cellular transformation, membrane association, and lipid binding.

Tambourin, P.E., Chattopadhyay, S.K., Lowy, D.R., NCI, National Institutes of Health, Bethesda, Maryland: HaSV – Noncoding and p21-coding sequences affect its oncogenic activity.

Vousden, K.H., Phillips, D.H., Marshall, C.J., Chester Beatty Laboratories, Institute of Cancer Research, London, England: Activation of a cellular proto-oncogene by in vitro modification with a chemical carcinogen.

Santos, E., Barbacid, M., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Frequent genetic rearrangements of the *Ki-ras* locus in human tumors.

Gibbs, J.B.,[1] Ellis, R.W.,[1] Sigal, I.S.,[1] Stein, R.B.,[1] Poe, M.,[2] Scolnick, E.M.,[1] [1]Virus and Cell Biology Research, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania; [2]Dept. of Biophysics, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey: Autophosphorylation of *v*-Ha-*ras* p21 is modulated in whole cells and in vitro by amino acid residue 12.

Der, C.J.,[1] Finkel, T.,[2] Cooper, G.M.,[1] [1]Dept. of Pathology, Harvard Medical School; [2]Committee on Biophysics, Harvard University, Boston, Massachusetts: Structural and functional analyses of normal and activated cellular *ras* proteins.

Wigler, M.,[1] Powers, S.,[1] Kataoka, T.,[1] Fasano, O.,[1] Taparowsky, E.,[1] Birnbaum, D.,[1] Goldfarb, M.,[1] Strathern, J.,[1] Broach, J.,[2] [1]Cold Spring Harbor Laboratory; [2]Dept. of Microbiology, State University of New York, Stony Brook: Structure, genetics, and activation of yeast and mammalian *ras* genes.

Tamanoi, F., Walsh, M., Rao, M., Powers, S., Kataoka, T., Wigler, M., Cold Spring Harbor Laboratory, New York: Yeast *ras* proteins.

MacAuley, A., Auersperg, N., Weeks, G., Pawson, T., Depts. of Microbiology and Anatomy, University of British Columbia, Vancouver, Canada: Expression and functional activity of *ras* proteins are regulated in developmental systems from simple and higher eukaryotes.

Simon, M.A.,[1] Drees, B.,[1] Kornberg, T.,[1] Bishop, J.M.,[2] [1]Dept. of Biochemistry and Biophysics, [2]George William Hooper Foundation, University of California, San Francisco: The expression of three *src* homologs during *Drosophila* development.

Mark, G.E.,[1] Jackman, M.,[1] MacIntyre, R.,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Cornell University, Ithaca, New York: *Drosophila melanogaster* contains unique loci related to the *raf* oncogene.

Besmer, P.,[1] Murphy, J.E.,[1] Lader, E.,[1] George, P.C.,[1] Snyder, H.W., Jr.,[3] Zuckerman, E.E.,[2] Hardy, W.D.,

E. Ruley, J. Lautenberger, N. Teich

Jr.,[2] Laboratories of [1]Molecular Oncology, [2]Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York; [3]Pacific Northwest Research Foundation, Seattle, Washington: New acute transforming feline retroviruses—The HZ4-FeSV contains unique retroviral oncogene *v-kit*--The HZ5-FeSV is a new *fms* virus.

Müller, R.,[1] Müller, D.,[1] Mölders, H.,[1] Defesche, J.,[1] Bonner, T.I.,[2] Rapp, U.R.,[2] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]NCI, National Institutes of Health, Frederick Cancer Research Facility, Frederick, Maryland: An alternative strategy for the identification of potential c-*onc* genes.

Cooper, C.S.,[1] Park, M.,[2] Blair, D.G.,[2] Tainsky, M.A.,[2] Vande Woude, G.F.,[2] [1]Laboratory of Molecular Carcinogenesis, Dana-Farber Cancer Institute, Boston, Massachusetts; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: Molecular cloning of a new transforming gene from a chemically transformed human cell line.

SESSION 6 POSTER SESSION II

Guerrero, I.,[1] Villasante, A.,[1] D'Eustachio, P.,[2] Corces, V.,[3] Pellicer, A.,[1] Depts. of [1]Pathology, [2]Biochemistry, New York University Medical Center, New York, New York; [3]Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: In vivo activation of mouse *ras* oncogenes.

Tainsky, M.A.,[1] Cooper, C.S.,[2] Blair, D.G.,[1] Giovanella, B.C.,[3] Vande Woude, G.F.,[4] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Dana-Farber Cancer Institute, Boston, Massachusetts: [3]Stehlin Foundation for Cancer Research, Houston, Texas; [4]Director, LBI Basic Research Program, Frederick Cancer Research Facility, Frederick, Maryland: An activated N-*ras* gene is detected in late- but not early-passage human PA1 teratocarcinoma cells.

Westin, E., Wong-Staal, F., Gorse, K., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: Activation of *N-ras* in human acute leukemias.

Souyri, M., Furth, M., Fleissner, E., Memorial Sloan-Kettering Cancer Center, New York, New York: Active N-*ras* oncogenes from human T-cell leukemias—Structural characteristics and pathogenic properties.

Gambke, C., Moroni, C., Friedrich Miescher-Institut, Basel, Switzerland: Identification and characterization of transforming genes from human leukemias.

Padua, R.A., Barrass, N., Currie, G.A., Marie Curie Memorial Foundation Research Institute, Oxted, England: Transforming genes in malignant melanoma.

Kraus, M., Yuasa, Y., Aaronson, S., NCI, National Institutes of Health, Bethesda, Maryland: A position 12-activated Ha-*ras* oncogene in all HS578T mammary carcinosarcoma cells but not normal cells of the same patient.

Robins, T., Showalter, S., Vande Woude, G., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Biological properties of a murine retrovirus expressing the human EJ bladder oncogene.

Chang, E.H.,[1] Morgan, P.L.,[1] White, E.A.,[1] Tsichlis, P.N.,[2] Patrick, D.H.,[1] [1]Uniformed Services University of the Health Sciences, Bethesda, Maryland; [2]Fox Chase Cancer Center, Philadelphia, Pennsylvania: Pathogenicity of retroviruses containing either human *c-Ha-ras-1* or EJ/T24 *ras* gene.

Lee, E.J., Chang, E.H., Dept. of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland: Characterization and cDNA cloning of human c-Ha-*ras* RNA transcripts.

Anderson, G.R., Stoler, D.L., Saavedra, R.A., Dept. of Cell and Tumor Biology, Roswell Park, Buffalo, New York: The KiSV, its anoxic shock genes, and LDH_x.

Ulsh, L.S., Shih, T.Y., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Metabolic turnover of human c-*ras* p21 of EJ bladder carcinoma and its normal cellular homolog.

Srivastava, S.K., Yuasa, Y., Kraus, M.H., Fijita, J., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: Comparative analysis of p21 molecules encoded by human *ras* oncogenes.

Gallick, G.E.,[1] Kurzrock, R.,[2] Gutterman, J.U.,[2] Depts. of [1]Tumor Biology, [2]Clinical Immunology, UTSCC, M.D. Anderson Hospital, Houston, Texas: Expression of *ras* gene products in primary human colon tissues and metastatic lesions.

Kurzrock, R.,[2] Gallick, G.E.,[1] Liang, J.,[3] Gutterman, J.U.,[2] Depts. of [1]Tumor Biology, Section of Virology, [2]Clinical Immunology, [3]Dept. of Genetics, UTSCC, M.D. Anderson Hospital, Houston, Texas: Expression of *ras* gene products in primary human lung tumors.

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Novel infectious endogenous viruses in chickens.

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Kotler, M.,[1] Burstein, H.,[1] Gilead, M.,[2] [1]Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem; [2]Poultry Diseases Laboratory, Beit Shemesh, Israel: Viral etiology of hemangiosarcoma outbreaks among layer hens.

Ewert, D.L., Wistar Institute, Philadelphia, Pennsylvania: ALV infection and tumor induction in infection-resistant and susceptible chimeric chickens.

Dunwiddie, C., Faras, A., Dept. of Microbiology, University of Minnesota, Minneapolis: The presence of avian retroviral *gag*-, *pol*-, and *env*-related gene sequences in ALV lacking chick embryo fibroblasts and quail embryo fibroblasts.

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SESSION 7 CARCINOGENESIS AND LEUKEMOGENESIS

Chairperson: **R. Risser,** McArdle Laboratory, University of Wisconsin, Madison, Wisconsin

Nusse, R., van Ooyen, A., Dept. of Molecular Biology, The Netherlands Cancer Institute, Amsterdam: Structure, nucleotide sequence and mode of activation of *int-1* – Implications for mammary tumorigenesis.

Fung, Y.-K.T., Seeger, C., Shackelford, G., Varmus, H.E., Dept of Microbiology and Immunology, University of California, San Francisco: Molecular cloning of *int-1* cDNA and its expression in *E. coli*.

Moore, R., Brookes, S., Casey, G., Dickson, C., Peters, G., Imperial Cancer Research Fund Laboratories, London, England: The structure and expression of the MMTV *int-2* locus in mice.

Gallahan, D., Robbins, J., Callahan, R., NCI, National Institutes of Health, Bethesda, Maryland: Evidence for a new common region for MMTV integration in mammary tumors of feral *Mus musculus musculus*.

Hankins, W.D.,[1] Thorgeirsson, S.,[1] Tabin, C.,[2] Cones, R.,[2] Weinberg, R.,[2] Mulligan, R.,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Massachusetts Institute of Technology, Boston: Transformation of hemopoietic precursors by replication-defective, helper-free virions carrying the bladder carcinoma cellular oncogene, EJ-1.

Rein, A.,[1] Ihle, J.,[1] Medicus, R.,[1] Holmes, K.,[2] Schultz, A.,[1] Keller, J.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Immortalization of a class of hematopoietic cells by infection with HaSV.

Boettiger, D.,[1] Dexter, T.M.,[2] [1]Dept. of Microbiology, University of Pennsylvania, Philadelphia; [2]Paterson Laboratories, Manchester, England: Infection of murine hemopoietic stem cells with a recombinant retrovirus containing the *src* gene from RSV alters their self-renewal capacity.

Palmieri, S., NIAID, National Institutes of Health, Hamilton, Montana: Erythroid cell transformation by RSV.

Heisterkamp, N.,[1] Stephenson, J.R.,[1] Grosveld, G.,[2] de Klein, A.,[2] Groffen, J.,[1] [1]Oncogene Science Inc., Mineola, New York; [2]Erasmus University, Rotterdam, The Netherlands: Cellular (onco)genes involved in chronic myelocytic leukemia.

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Konopka, J.B., Watanabe, S.M., Witte, O.N., Dept. of Microbiology and Molecular Biology Institute,

S. Martin

University of California, Los Angeles: Activation of the human c-*abl* oncogene.

Wolf, D., Harris, N., Rotter, V., Dept. of Cell Biology, Weizmann Institute of Science, Rehovot, Israel: Reconstitution of p53 expression of Ab-MLV-transformed L12 cells containing a nonfunctional gene by transfection of a genomic p53 clone.

Shen-Ong, G.L.C., Reddy, E.P., Mushinski, J.F., Potter, M., NCI, National Institutes of Health, Bethesda, Maryland: Disruption and activation of the *c-myb* locus by Mo-MLV insertion in plasmacytoid lymphosarcomas induced by pristane and Abelson virus.

SESSION 8 ENHANCERS AND VECTORS

Chairperson: G. Vande Woude, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

Celander, D., Haseltine, W.A., Dana-Farber Cancer Institute, Boston, Massachusetts: The role of the transcriptional enhancer element in cell tropism and leukemogenicity of murine leukemia viruses.

Lenz, J.,[1] Cloyd, M.,[2] Rosen, C.,[3] Celander, D.,[3] Haseltine, W.,[3] [1]Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York; [2] Dept. of Surgery, Duke University, Durham, North Carolina; [3]Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Leukemogenicity and thymotropism of a murine retrovirus and determined by enhancer-related sequences in the viral LTR.

Rosen, C.,[1] Lenz, J.,[2] Cloyd, M.,[3] Haseltine, W.,[1] [1]Dana-Farber Cancer Institute, Boston, Massachusetts; [2]Albert Einstein College of Medicine, Bronx, New York; [3]NIAID, Hamilton, Montana: Elements that confer tissue tropism reside within the U_3 region of the LTR of Fr-MLV.

DesGroseillers, L., Villemur, R., Jolicoeur, P., Institut de Recherches Cliniques de Montréal, Université de Montréal, Quebec, Canada: The tandem direct repeats within the LTR of MLV are the primary determinant of their leukemogenic potential.

Clark, S.P., Mak, T.W., Ontario Cancer Institute, Toronto, Canada: Comparison of murine retroviral enhancers as transcriptional promoters.

Davis, B.,[1] Chao, E.,[1] Overhauser, J.,[1] Linney, E.,[2] Fan, H.,[1] [1]Dept. of Molecular Biology and Biochemistry, University of California, Irvine; [2]La Jolla Cancer Research Foundation, California: Deletion and substitution mapping within the Mo-MLV LTR.

Raymond, K.J.,[1] Berg, P.,[2] Ju, G.,[1] [1]Dept. of Molecular Genetics, Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey: [2]National Institutes of Health, Bethesda, Maryland: Localization of enhancer sequences in the RSV LTR.

Norton, P.A., Coffin, J.M., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Transcriptional control sequences in the RSV LTR.

Robins, T., Vande Woude, G., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Cloning and expression of intronless genes using a retrovirus "shuttle" vector.

Jhappan, C.,[1] Chirikjian, J.,[1] Vande Woude, G.,[2] Robins, T.,[2] [1]Dept. of Biochemistry, Georgetown University, Washington, DC; [2]Frederick Cancer Research Facility, Frederick, Maryland: Direct molecular cloning of G418^R "shuttle" vector proviral DNA mediated by cell fusion.

Gilboa, E., Park, J., Hwang, L., Seiberg, M., Yu, S., Dept. of Molecular Biology, Princeton University, New Jersey: Strategies of gene transfer with retrovirus-derived vectors.

Hwang, L., Park, J., Gilboa, E., Dept. of Molecular Biology, Princeton University, New Jersey: Mechanism of splicing of Mo-MLV *env* mRNA – Role of intron-contained sequences.

Reik, W., Wolf, B., Harbers, K., Jaenisch, R., Heinrich-Pette Institut für Experimentelle Virologie und Immunologie, Hamburg, Federal Republic of Germany: Vectors for the analysis of retroviral integration – Construction of replication competent Mo-MLV derivatives selectable in bacteria.

Miller, A.D., Eckner, R.J., Ong, E.S., Evans, R.M., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Gene transfer into cultured cells and animals using retroviral vectors.

Cone, R.D., Reilly, E., Weber, A., Eisen, H.N., Mulligan, R.C., Center for Cancer Research and Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Passage- and tissue-specific expression of small genomic sequences inserted into murine retroviral vectors.

Mann, R., Baltimore, D., Whitehead Institute of Biomedical Research, Massachusetts Institute of Technology, Cambridge: The position of the Mo-MLV packaging site can be varied.

Mann, R., Baltimore, D., Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge: Packaging of spliced retroviral RNAs.

SESSION 9 POSTER SESSION III

Yoshimura, F., Hedin, K., Fred Hutchinson Cancer Research Center, Seattle, Washington: Tissue-specific expression of enhancer activity of an MCF MLV LTR.

Overhauser, J., Fan, H., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Insertion of DNA fragments into the

Moloney LTR—Effects on promoter activity and viral expression.

Herman, S.A., Coffin, J.M., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Analysis of downstream transcription from randomly integrated proviruses.

Keshet, E., Rotman, G., Itin, A., Dept. of Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Diversity of LTR units associated with a murine "retrovirus-like" (VL30) gene family.

Khan, A.S.,[1] Kessel, M.,[2] [1]NIAID, [2]NCI, National Institutes of Health, Bethesda, Maryland: Structural and functional analysis of the LTRs associated with a cloned endogenous retrovirus genome isolated from African green monkey DNA.

Morgan, R.A., Huang, R.C.C., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Undermethylation of intracisternal A-particle genes is correlated with transcriptional activity in murine plasmacytomas but not in NIH-3T3 embryo fibroblasts.

Honigman, A., Bar-Shira, A., Panet, A., Hebrew University Hadassah Medical School, Jerusalem, Israel: Processing of the 3′ ends of Mo-MLV RNA.

Ridgway, A.A.,[1] Swift, R.A.,[2] Dodgson, J.B.,[3] Kung, H.-J.,[2] Fujita, D.J.,[1] [1]Cancer Research Laboratory, University of Western Ontario, Canada; Depts. of [2]Biochemistry, [3]Microbiology, Michigan State University, East Lansing: Activation of the *c-myc* gene by chicken syncytial virus—Transcription in vitro initiates from two sites within the viral LTR.

Stoltzfus, C.M., Lorenzen, S.K., Chang, L.-J., Dept. of Microbiology, University of Iowa, Iowa City: Comparison of *src* mRNA splicing in cells infected with different strains and intron deletion mutants of RSV.

Robinson, H., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Retroviral transduction of proto-oncogene sequences.

Niwa, O., Dept. of Experimental Radiology, Faculty of Medicine, Kyoto University, Japan: Expression of the transfecting Mo-MLV genome and the *neo* gene fused to the LTR of Mo-MLV in teratocarcinoma cells.

Taketo, M.,[1,2] Gilboa, E.,[3] Sherman, M.I.,[1] [1]Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Jackson Laboratory, Bar Harbor, Maine; [3]Dept. of Biochemical Sciences, Princeton University, New Jersey: Expression in embryonal carcinoma cells of neomycin resistance gene placed under the control of Mo-MLV LTR promoter.

Anderson, C., Dept. of Chemistry, University of California, San Diego, La Jolla: Retroviral vectors and gene expression.

Weber-Benarous, A., Cone, R., London, I.M., Mulligan, R., Research; Massachusetts Institute of Technology, Cambridge: Expression of K562 β-globin gene in mouse erythroleukemia cells.

Cone, R.D., Mulligan, R., Center for Cancer Research and Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Construction of a broad host-range packaging line for murine retroviral vectors.

Armentano, D., Shimamura, A., Gilboa, E., Dept. of Molecular Biology, Princeton University, New Jersey: The packaging signal of Mo-MLV.

Olsen, J., Swanstrom, R., Dept. of Biochemistry, Cancer Research Center, University of North Carolina, Chapel Hill: Analysis of plasmids from a RSV-derived shuttle vector rescued in bacteria.

Lautenberger, J.,[1] Court, D.,[1] Jorcyk, C.,[1] Kan, N.,[1] Seth, A.,[1] Vergilio, L.,[1] Josephs, S.,[2] Papas, T.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick; [2]NCI, National Institutes of Health, Bethesda, Maryland: High-level expression of oncogene proteins in *E. coli* using plasmid pJL6 and its variants.

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Yang, W.K.,[1] Boone, L.R.,[1,2] Ou, C.Y.,[1] Yang, D.M.,[1] Myer, F.E.,[1] Tennant, R.W.,[1] [1]Biology Division, Oak Ridge National Laboratory, Tennessee; [2]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: A distinct viral p30^{gag}-directed host restriction of endogeneous murine type-C retroviruses.

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Yuen, P.H., Malehorn, D., Nau, C., Knupp, C., Wong, P.K.Y., Dept. of Microbiology, University of Illinois, Urbana: Localization of the paralytogenic determinant(s) of *ts*1, a temperature-sensitive mutant of Moloney murine leukemia virus.

Ikeda, H., Laigret, F., Repaske, R., Theodore, T., Kozak, C.A., Martin, M.A., NCI, National Institutes of Health, Bethesda, Maryland: Characterization of molecularly cloned retroviral sequence associated with *Fv-4* resistance.

Kozak, C., NIAID, National Institutes of Health, Bethesda, Maryland: Susceptibility of cells from wild mice to exogenous xenotropic mouse leukemia virus—Regulation by a single chromosome 1 gene.

Silver, J., NIAID, National Institutes of Health, Bethesda, Maryland: AKR genes on chromosomes 7 and 15 affect susceptibility to T-cell lymphoma.

Dandekar, S., Rositto, P., Gardner, M., Dept. of Pathology, School of Medicine, University of California, Davis: Mechanism of an ecotropic MLV restriction gene, *Akvr-1*^{R}, in wild mice.

Levine, K., Yoshimura, F., Fred Hutchinson Cancer Research Center, Seattle, Washington: Characterization of molecular clones of AKR thymoma-specific proviruses.

Hays, E.F.,[1] Levy, J.A.,[2] [1]Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles; [2]Cancer Research Institute, University of California, San Francisco: Differences in lymphomagenic properties of AKR mouse retroviruses.

Wolff, L., Ruscetti, S., NCI, National Institutes of Health, Bethesda, Maryland: Loss of the U_3 region

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Sitbon, M., Evans, L., Nishio, J., Wehrly, K., Chesebro, B., NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Difference in virulence of two related Fr-MLV is not due to difference in their ability to replicate or to induce MCF viruses.

Evans, L.H.,[1] Cloyd, M.W.,[2] [1]NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana; [2]Dept. of Surgery, Duke University, Durham, North Carolina: Specificity of recombination of ecotropic MLVs with endogenous murine retrovirus sequences to generate MCF viruses.

Villemur, R., DesGroseillers, L., Jolicoeur, P., Institut de Recherches Cliniques de Montréal, Université de Montréal, Quebec, Canada: Attempts to detect specific integration site of ecotropic and MCF-type proviruses in gross passage of a MLV-induced mouse thymoma.

Thomas, C.Y., Boykin, B.J., Coppola, M.A., University of Virginia School of Medicine, Charlottesville: Recombinant ecotropic and class-II polytropic viruses from CW mice accelerate the development of splenic lymphomas.

Cloyd, M.W.,[1] Evans, L.H.,[2] [1]Dept. of Surgery, Duke University, Durham, North Carolina; [2]NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Primary murine leukemia cells lack xenotropic MLV *env* products but do express MCF-like proteins – Type of MCF *env* varies with mouse strain and ecotropic virus inoculated.

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Horowitz, J.M., Kaehler, D., Risser, R., McArdle Laboratory for Cancer Research, Madison, Wisconsin: Molecular and biological characterization of the endogenous ecotropic provirus of BALB/c mice.

Horowitz, J.M., Kaehler, D,, Risser, R,, McArdle Laboratory for Cancer Research, Madison, Wisconsin: Rescue of endogenous MLV genomes by transfection with subgenomic viral fragments.

Jolicoeur, P.,[1] Villeneuve, L.,[1] Kozak, C.,[2] [1]Institut de Recherches Cliniques de Montreal, Universite de Montreal, Quebec, Canada; [2]NIAID, National Institutes of Health, Bethesda, Maryland: The RMoInt-1, a locus rearranged in several Mo-MLV-induced rat thymomas, maps on mouse chromosome 15.

Economou-Pachnis, A., Lohse, M., Tsichlis, P.N., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Polymorphism in *Mlvi-2* and the immunoglobulin heavy-chain locus in the rat due to individual members of a family of highly repeated elements.

Merregaert, J.,[1] Janowski, M.,[1] Boniver, J.,[2] Maisin, J.R.,[1] [1]Dept. of Radiobiology, CEN/SCK; [2]Laboratory of Pathological Anatomy, University of Liège, Belgium: The proviral genome of the murine radiation leukemia virus – Integration in cellular DNA, characterization of an infectious molecular clone, and nucleotide sequence of the LTR.

Pal, B.K., Cooper, R.E., Dept. of Biological Sciences, California State Polytechnic University, Pomona: Methylation, virogene expression,

and stages of differentiation of B cells transformed by wild mouse retrovirus.

Sitbon, M., Nishio, J., Wehrly, K., Chesebro, B., NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: A new sensitive focal immunofluorescence assay with monoclonal antibodies allowing detection of individual strains of MLV in complex virus mixtures.

Wolfe, J.H., Janssen, W.E., Pozsgay, J., Blank, K.J., Dept. of Pathology, University of Pennsylvania School of Medicine, Philadelphia: Alterations in viral protein processing in MLV-induced cell lines from H-2-congenic mice.

Zijlstra, M.,[1] de Goede, R.E.Y.,[1] Schoenmakers, H.J.,[1] Radaszkiewics, T.,[2] Melief, C.J.M.,[1] [1]Central Laboratory of Blood Transfusion Service, Amsterdam, The Netherlands; [2]Institute of Pathology, Vienna, Austria: The H-2 complex of the mouse influences the phenotype of MLV-induced lymphomas.

SESSION 10 MORE CARCINOGENESIS AND LEUKEMOGENESIS

Chairperson: E. Fleissner, Memorial Sloan-Kettering Cancer Center, New York, New York

Famulari, N.G.,[1] Holland, C.A.,[2] Hopkins, N.H.,[2] O'Donnell, P.V.,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Dept. of Biology, Massachusetts Institute of Technology, Boston: The contribution of gp70, p15(E), and LTR sequences of MCF 247 virus to thymotropism and pathogenicity in AKR mice.

Holland, C.,[1] Chatis, P.,[1] Hartley, J.,[2] Hopkins, N.,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]NCI, National Institutes of Health, Bethesda, Maryland: The 3′ end of nondefective Friend virus confers erythroleukemogenesis on MoLV.

Oliff, A., Memorial Sloan-Kettering Cancer Center, New York, New York: Both LTR and *env* gene sequences contribute to leukemogenesis by the helper-independent Friend viruses.

O'Donnell, P.V., Koehne, C., Woller, R., Lonial, H., Fleissner, E., Memorial Sloan-Kettering Cancer Center, New York, New York: Identification of a clonal population of cells with limited transformation capacity during the latent period of MCF virus-accelerated leukemia in AKR/J mice.

Li, Y.,[1] Holland, C.,[1] Hopkins, N.,[1] Hartley, J.,[2] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]NCI, National Institutes of Health, Bethesda, Maryland: Viral integration near c-myc in about 15% of MCF247-induced AKR thymomas.

Selten, G.,[1] Cuypers, H.T.,[1] Quint, W.,[1] Zijlstra, M.,[2] Maandag, E.R.,[1] Boelens, W.,[1] van Wezenbeek, P.,[1] Melief, C.,[2] Berns, A.,[1] [1]Dept. of Biochemistry, University of Nijmegen; [2]CLB, Amsterdam, The Netherlands: MLV-induced T-cell lymphomagenesis in mice – Proviral activation of c-*myc* and *pim-1*.

Steffen, D., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Proviruses adjacent to c-*myc* in Mo-MLV-induced lymphomas.

Tsichlis, P.N., Strauss, P.G., Lohse. M., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Mo-MLV-induced rat thymomas contain at least three common regions of provirus integration.

Neil, J.C.,[1] Stewart, M.,[1] Wilkie, N.,[1] Lees, G.,[2] Jarrett, O.,[2] Onions, D.,[2] [1]Beatson Institute for Cancer Research; [2]Dept. of Veterinary Pathology, University of Glasgow, Scotland: Multiple mechanisms of *myc* activation in feline T-cell leukemias.

Mullins, J.I., Doggett, D.L., Broda, D.S., Binari, R.C., Jr., Cotter, S.M., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: c-*myc* transduction in naturally occurring feline T-cell leukemias.

Bestwick, R., Machida, C., Kabat, C., Oregon Health Sciences University, Portland: Seed oncogenes that cause progressive leukemogenesis – Membrane glycoprotein genes of SFFV.

Breindl, M., Harbers, K., Jaenisch, R., Heinrich-Pette-Institut for Experimental Virologie, Hamburg, Federal Republic of Germany: Retrovirus-induced lethal mutation in mice is associated with an altered chromatin structure.

Levy, D.,[1,2] Lerner, R.,[2] Wilson, M.,[2] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla: Tissue-specific transcription of defective endogenous retroviruses under coordinate genetic control.

SESSION 11 THE LAST ONCOGENE SHOW (*fos*, *myc*, *myb*, *ski*)

Chairperson: R. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, Washington

Meijlink, F.C.P.W., Curran, T., Miller, A.D., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Transformation by the c-*fos* gene – Intramolecular interaction in the 3′ region regulates expression.

Curran, T.,[1] Van Beveren, C.,[2] Verma, I.M.,[2] [1]Dept. of Molecular Genetics, Hoffmann-LaRoche Inc., Roche Research Center, Nutley, New Jersey; [2]Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Viral and cellular *fos* proteins are complexed with a 39,000-dalton cellular protein.

Wiman, K.G.,[1] Hayday, A.C.,[2] Saito, H.,[2] Tonegawa, S.,[2] Hayward, W.S.,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Massachusetts Institute of Technology, Boston: Mechanism of activation of a translocated but intact c-*myc* gene that is not close to an immunoglobulin enhancer element.

Vennström, B.,[1] Kahn, P.,[1] Adkins, B.,[1] Enrietto, P.,[2] Hayman, M.,[2] Graf, T.,[1] Luciw, P.[3] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]Imperial Cancer Research Fund, London, England; [3]Chiron Corp., Emeryville, California: Transformation capacities of a murine retrovirus encoding an avian *myc* oncogene.

Ramsay, G.,[1] Evan, G.,[1] Lerner, R.,[2] Bishop, J.M.,[1] [1]George William Hooper Foundation, University of California, San Francisco; [2]Scripps Clinic and Research Foundation, La Jolla, California: Identification of the human c-*myc* protein using sera raised against a series of c-*myc*$^{(human)}$ peptides.

Hann, S., Eisenman, R., Fred Hutchinson Cancer Research Center, Seattle, Washington: Protein products of the human c-*myc* oncogene.

Heaney, M.L., Parsons, J.T., Dept. of Microbiology, University of Virginia, Charlottesville: Site-directed mutagenesis of the v-*myc* gene—Analysis of regions that share homology with the v-*myb* and E1A genes.

Morgan, J.H., Parsons, J.T., Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Analysis of the *myc* protein from normal and transformed cells.

Lee, W., Schwab, M., Westaway, D., Bishop, J.M., Varmus, H., Depts. of Medicine, Microbiology and Immunology, University of California, San Francisco: Transformation-promoting activity of c-*myc* genes derived from normal and tumor tissues.

Schwab, M.,[1] Busch, M.,[2] Rosenau, W.,[2] Brodeur, G.,[3] Varmus, H.E.,[1] Bishop, J.M.,[1] Depts. of [1]Microbiology, [2]Pathology, University of California, San Francisco; [3]Dept. of Pediatrics, St. Louis, Missouri: Amplification and overexpression of N-*myc* may be related to malignant progression rather than to cellular transformation in human neuroblastoma.

Pelicci, P.G., Lanfrancone, L., Brathwaite, M.D., Wolman, S.R., Dalla-Favera, R., Dept. of Pathology, Kaplan Cancer Center, New York University School of Medicine, New York: Oncogene (c-*myc*, c-*myb*) amplification and acute myelogenous leukemia—Molecular and cytogenetic studies.

Klempnauer, K.-H.,[1] Symonds, G.,[1] Evan, G.I.,[2] Bishop, J.M.,[1] [1]George William Hooper Research Foundation, Dept. of Microbiology and Immunology, University of California Medical Center, San Francisco; [2]Ludwig Institute for Cancer Research, MRC Centre, Cambridge, England: Subcellular localization of *myb* gene products and the fate of p45$^{v\text{-}myb}$ during the differentiation of AMV-transformed myeloblasts.

Lipsick, J.S., Boyle, W.J., Lampert, M.A., Ong, J., Baluda, M.A., Jonsson Comprehensive Cancer Center, Dept. of Pathology, University of California School of Medicine, Los Angeles: Both the E26 and AMV viral oncogene products are nuclear proteins.

Brodeur, D., Barkas, A., Stavnezer, E., Molecular Biology and Virology Program, Memorial Sloan-Kettering Cancer Center, New York, New York: v-*ski* polyproteins are located in the nuclei of SKV-transformed cells.

SESSION 12 POSTER SESSION IV

Müller, R., Wagner, E.F., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Induction of differentiation after transfer of a cellular oncogene (c-*fos*) into F9 teratocarcinoma stem cells.

Shibuya, M.,[1] Yokota, J.,[1] Ueyama, Y.,[2] [1]Dept. of Genetics, Institute of Medical Science, University of Tokyo; [2]Dept. of Pathology, Tokai University, Central Institute for Experimental Animals, Japan: Amplification of a cellular oncogene (c-*myc*) in human gastric adenocarcinoma cell lines.

Rovigatti, U.,[1] Blair, D.,[1] Helson, L.,[2] Papas, T.,[1] Rader, J.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick Maryland; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Oncogene alterations in human neuroblastoma and primitive neuroectodermal tumors.

Sklar, M.,[1,2] Rosson, D.,[2] Tereba, A.,[2] Depts. of [1]Radiation Therapy, [2]Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Oncogenic activity of v-*myc* and a human c-*myc*-related sequence in a mouse bone marrow-derived cell line and primary mouse macrophages.

Brightman, B.K., Fan, H., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Construction of a recombinant murine leukemia virus carrying MC29 v-*myc* gene.

Matthews, E.A., Bishop, J.M., George William Hooper Research Foundation, Dept. of Microbiology and Immunology, University of California, San Francisco: A recombinant virus containing cellular and viral *myc* sequences.

Van Beneden, R.,[2] Watson, D.,[2] Sonstegard, R.,[1] Chen, T.,[2] [1]Dept. of Biology, McMaster University, Ontario, Canada; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: Isolation and characterization of fish c-*myc*.

Soe, L.H., Roy-Burman, P., Depts. of Pathology and Biochemistry, University of Southern California School of Medicine, Los Angeles: Possible association between DNA polymorphism at the c-*myc* locus and tumors in the domestic cat.

Shih, C.-K.,[1,2] Clurman, B.E.,[1] Goodenow, M.M.,[1] Linial, M.,[3] Hayward, W.S.,[1] [1]Memorial Sloan-Kettering Cancer Center; [2]Rockefeller University, New York, New York; [3]Fred Hutchinson Cancer Center, Seattle, Washington: Characterization

virus-productive adult T-cell leukemia cell line.

Eiden, M.,[1] Fisher, A.,[1] Newman, M.,[2] Mann, D.,[1] Mullins, J.,[3] Howley, P.,[1] Reitz, M.,[1] Gallo, R.C.,[1] [1]NCI, National Institutes of Health; [2]Uniformed Services University of the Health Sciences, Bethesda, Maryland; [3]Harvard School of Public Health, Boston, Massachusetts: Expression of cloned HTLV-I small *env* protein in transfected mouse cells.

Homma, T.,[1] Kanki, P.J.,[1,2] King, N.W., Jr.,[2] Hunt, R.D.,[2] Essex, M.,[1] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston; [2]New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts: Antibodies to cell membrane antigens associated with HTLV in macaques with lymphoma.

Newman, M.,[1] Baker, I.,[1] Reitz, M.,[2] Eiden, M.,[2] Blattner, W.,[2] Harris, C.,[2] Mann, D.,[2] [1]Uniformed Services University of the Health Sciences; [2]NCI, National Institutes of Health, Bethesda, Maryland: Detection of viral *env* protein on HTLV-infected cells using monoclonal antibodies.

Sarngadharan, M.G.,[1] Popovic, M.,[2] Salahuddin, Z.,[2] Bruch, L.,[1] Markham, P.D.,[1] Schuepbach, J.,[2] Gallo, R.C.,[2] [1]Litton Bionetics, Kensington; [2]NCI, National Institutes of Health, Bethesda, Maryland: Antibodies reactive with a human T-lymphotropic retrovirus (HTLV-III) in the sera of patients with AIDS.

Narayanan, R.,[1] Srinivasan, A.,[2] Kalyanaraman, V.,[1] Getchell, J.,[1] Francis, D.,[1] Holloway, B.,[1] Cabradilla, C.,[1] [1]Centers for Disease Control, Molecular Virology Branch, Atlanta, Georgia; [2]NCI, National Institutes of Health, Bethesda, Maryland: Survey of tissues from AIDS patients for the expression of cellular homologs of retroviral oncogenes.

Mullins, J.I., Riedel, N., Dept. of Cancer Biology, Harvard School of Public Health, Boston. Massachusetts: Identification of proviral DNA weakly related to HTLV in cells derived from a patient with AIDS.

Mullins, J.I.,[1] McLane, M.F.,[1] Essex, M.,[1] Groopman, J.,[2] Gold, J.,[4] Schooley, R.,[3] Ho, D.,[3] Hirsch, M.,[3] Feorino, P.,[5] Cabradilla, C.,[5] [1]Harvard School of Public Health; [2]New England Deaconess Hospital; [3]Massachusetts General Hospital, Boston; [4]Memorial Sloan-Kettering Cancer Center, New York, New York; [5]Centers for Disease Control, Atlanta, Georgia: A search for HTLV-related DNA sequences in AIDS.

Ehrlich, G.,[1,2] Merl, S.,[2,3,4] Han, T.,[5] Tomar, R.,[2] Barcos, M.,[5] Moore, J.,[2,3,4] Blair, D.,[2] Zamkoff, K.,[2,3] Vournakis, J.,[1] Comis, R.,[2,4] Poiesz, B.,[2,3,4] [1]State University of New York; [2]State University of New York Medical Center; [3]Veterans Administration Medical Center, Syracuse, New York; [4]Barbara Kopp Research Center. Auburn, New York; [5]Roswell Park Memorial Institute, Buffalo, New York: HTLV-I homologous sequences in fresh and cultured cells from AIDS and LAS patients.

Barker, C.,[1] Willis, J.W.,[1] Bradac, J.,[1] Tainsky, M.,[2] Hunter, E.,[1] [1]Dept. of Microbiology, University of Alabama, Birmingham; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: The molecular cloning and characterization of the genome of Mason-Pfizer monkey virus.

Kashmiri, S.V.S., Mehdi, R., Ferrer, J.F., University of Pennsylvania, Philadelphia: Hypermethylation of bovine leukemia proviral DNA in leukemic cells and infected lymphocytes.

O'Neill, R.R., Buckler, C.E., Theodore, T., Martin, M.A., Repaske, R., NIAID, National Institutes of Health, Bethesda, Maryland: The complete nucleotide sequence of *env* and LTR regions of an infectious xenotropic MLV—Relationship to MCF and ecotropic MLV sequences.

Friedrich, R.,[1] Hackl, B.,[1] Oliff, A.,[2] [1]Institute of Immunobiology, University of Freiburg, Federal Republic of Germany; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Structure of the *env* gene of the nonleukemogenic murine retrovirus Ampho 4070.

Steele, P., Rabson, A., Maloy, L., Colligan, J., Bryan, T., Martin, M., NIAID, National Institutes of Health, Bethesda, Maryland: Human endogenous *env* sequences are shared by a novel class of primate endogenous retroviral DNAs and are expressed in human tissue.

Elder, J.H.,[1] Mullins, J.I.,[2] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California; [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Comparative analysis of the *env* genes of FeLV-B.

Katz, R.A., Skalka, A.M., Dept. of Molecular Genetics, Hoffman-La Roche Inc., Roche Research Center, Nutley, New Jersey: Biochemical analysis of a defect in the mRNA leader region of the endogenous avian retrovirus ev-1.

Bennett, D.D.,[1] Wright, S.E.,[1,2] [1]Viral Oncology Laboratory, Veterans Administration Medical Center; [2]Depts. of Medicine and Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City: Immunization with envelope glycoprotein of an avian RNA tumor virus protects against sarcoma virus tumor induction—Role of subgroup.

Nunberg, J.H.,[1] Gilbert, J.H.,[1] Rodgers, G.,[1] Snead, R.M.,[1] Winston, S.,[2] [1]Cetus Corporation, Emeryville, California; [2]Syngene Products and Research, Fort Collins, Colorado: Localization of a determinant of virus neutralization on FeLV gp70.

Schultz, A.M., Copeland, T.D., Oroszlan. S., NCI, Frederick Cancer Research Facility, Frederick, Maryland: The *env* proteins of BLV—Purification and sequence analysis.

Pinter, A., Honnen, W.J., Memorial Sloan-Kettering Cancer Center, New York, New York: Functional roles of the carbohydrates of MLV *env* proteins—Studies with inhibitors of oligosaccharide processing.

Soong, M.M.,[1] Wong, P.,[2] Tompkins, W.,[1] Depts. of [1]Pathobiology, [2]Microbiology, University of Illinois, Urbana: Evidence for processing of Mo-MLV gPr80^{env} in association with the cell cytoskeleton.

Suni, J.I.,[1] Närvänen, A.,[1] Wahlström, T.,[2] Vaheri, A.,[1] Copeland, T.,[3] Cohen, M.,[3] Oroszlan, S.,[3] Depts.

of [1]Virology, [2]Pathology, University of Helsinki, Finland; [3]NCI, Frederick Cancer Research Facility, Frederick, Maryland: Retrovirus-related *M*, 75,000 polypeptide from cultured choriocarcinoma cells and renal adenocarcinoma tissue.

Simonneau, L.,[1] Crisanti, P.,[2] Lorinet, A.M.,[2] Calothy, G.,[3] Courtois, Y.,[1] Pessac, B.,[2] [1]INSERM; [2]INSERM, Hôpital Broussais, Paris, [3]Institut Curie-Biologie, Orsay, France: Regulation of crystallins expression and lentoid bodies formation by oncogenic retroviruses in quail embryo neuroretinal cultures.

Rossomando, A., Meruelo, D., Dept. of Pathology, New York University Medical Center, New York, New York: A common 300-bp viral/genomic DNA sequence is associated with minor and major histocompatibility (*H*) genes.

Ou, C.Y.,[1] Koh, C.K.,[1] Boone, L.R.,[2] Callahan, R.,[3] Yang, W.K.,[1] [1]Biology Division, Oak Ridge National Laboratory, Tennessee; [2]NIEHS, Research Triangle Park, North Carolina; [3]National Institutes of Health, Bethesda, Maryland: An apparent novel family of retroviral sequences in the mouse genome.

Rabson, A.B., Steele, P.E., Hamagishi, Y., Martin, M.A., NIAID, National Institutes of Health, Bethesda, Maryland: Structural analysis of human endogenous retroviral mRNAs.

SESSION 13 HTLV, BLV, AND D TYPES

Chairperson: W. Haseltine, Dana-Farber Cancer Institute, Boston, Massachusetts

Sodroski, J.,[1] Perkins, D.,[1] Briggs, D.,[1] Patarca, R.,[1] Wong-Staal, F.,[2] Gallo, R.,[2] Haseltine, W.,[1] [1]Dana-Farber Cancer Institute, Boston, Massachusetts; [2]National Institutes of Health, Bethesda, Maryland: Structural differences between HTLV-I and HTLV-II.

Sodroski, J., Rosen, C., Haseltine, W.A., Dana-Farber Cancer Institute, Boston, Massachusetts: Functional differences between the LTRs of HTLV types I and II.

Chen, I.S.Y.,[1] Wachsman, W.,[1] Shimotohno, K.,[2] Golde, D.W.,[1] [1]Dept. of Medicine, University of California, Los Angeles; [2]National Cancer Center, Tokyo, Japan: Molecular genetic studies of the HTLV-II LTR and the pX region.

Shimotohno, K.,[1] Takahashi, Y.,[1] Golde, D.W.,[2] Miwa, M.,[1] Sugimura, T.,[1] Chen, I.S.Y.,[2] [1]National Cancer Center Research Institute, Tokyo, Japan; [2]Dept. of Medicine, University of California, Los Angeles: Nucleotide sequence analysis of HTLV type II.

Ratner, L., Josephs, S.F., Shaw, G.M., Hahn, B.H., Wong-Staal, F., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: pX deletion in HTLV isolates from an African adult T-cell leukemia patient.

Yoshida, M.,[1] Kiyokawa, T.,[1] Yoshikura, H.,[2] Hattori, S.,[1] Seiki, M.,[1] [1]Dept. of Viral Oncology, Cancer Institute, Tokyo; [2]Dept. of Bacteriology, Faculty of Medicine, University of Tokyo, Japan: Identification and production of HTLV env protein and cytotoxic antisera to virus-positive cells.

Hattori, S.,[1] Imagawa, K.-I.,[2] Shimizu, F.,[2] Hashimura, E.,[2] Seiki, M.,[1] Yoshida, M.,[1] [1]Dept. of Viral Oncology, Cancer Institute, Tokyo; [2]Otsuka Assay Laboratory, Otsuka Pharmacy Co. Ltd., Tokushima, Japan: Identification of *gag* and *env* gene products of HTLV.

Copeland, T.D., Tsai, W.P., Schultz, A.M., Oroszlan, S., LBI-Basic Research Program, NCI, Frederick Cancer Research Facility, Maryland: Human HTLV *gag* and env gene products—Structural and antigenic relatedness to type-C and type-D retroviruses.

Franchini, G., Popovic, M., Mann, D., Wong-Staal, F., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: HTLV sequences in the T cells and B cells of a patient with adult T-cell leukemia.

Franchini, G., Wong-Staal, F. Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: HTLV-I transcripts in fresh and cultured cells of patients with adult T-cell leukemia.

Popovic, M.,[1] Sarngadharan, M.G.,[2] Read, E.,[1] Gallo, R.C.,[1] [1]NCI, National Institutes of Health, Bethesda; [2]Litton Bionetics, Kensington, Maryland: Rescue and continous production of human T-cell lymphotropic retrovirus (HTLV-III) from patients with AIDS.

Popovic, M.,[1] Flomenberg, N.,[3] Volkman, D.,[2] Mann, D.,[1] Fauci, A.S.,[2] Dupont, B.,[3] Gallo, R.C.,[1] [1]NCI, National Institutes of Health, [2]NIAID, Bethesda, Maryland; [3]Dept. of Human Immunogenetics, Memorial Sloan-Kettering Cancer Center, New York, New York: Alteration of specific T-cell function by infection with HTLV-I or HTLV-II.

Barré-Sinoussi, F., Chermann, J.C., Montagnier, L., Viral Oncology Unit, Institut Pasteur, Paris, France: Characteristics of a new human T-lymphotropic retrovirus associated with acquired immune deficiency syndrome.

Lee, T.H.,[1] McLane, M.F.,[1] Tachibana, N.,[1 2,3] Francis, D.P.,[2,3] Homma, T.,[1] Essex, M.,[1] [1]Dept. of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts; [2]Miyazaki Medical School, Japan; [3]Center for Disease Control, Atlanta, Georgia: Relative immunogenicity of HTLV-specific proteins in adult T-cell leukemia/lymphoma patients, healthy carriers, asymptomatic hemophiliacs, and AIDS patients.

Lee, T.H.,[1] Coligan, J.E.,[2,3] Homma, T.,[1] McLane, M.F.,[1] Tachibana, N.,[1,2,3] Essex, M.,[1] [1]Dept. of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts; [2]NIAID, National Institutes of Health, Bethesda, Maryland; [3]Miyazaki Medical School, Japan: env gene-encoded antigens detected in cells transformed by different strains of HTLV.

Gelmann, E.,[1] Fleurdelys, B.,[1] Kettmann, R.,[2] Burny, A.,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biologie Moléculaire, Université Libre de Bruxelles, Belgium: Molecular cloning of integrated BLV proviral DNA from lymphoma tissue.

Sagata, N.,[1] Yasunaga, T.,[2] Ogawa, Y.,[1] Tsuzuku-Kawamura, J.,[1] Ikawa, Y.,[1] [1]Laboratory of Molecular Oncology, [2]Computation Center, Institute of Physical and Chemical Research (RIKEN), Wako, Japan: BLV–Unique structural features of its LTRs and evolutional relationship to HTLV.

Stromberg, K.,[1] Arthur, L.O.,[1] Rabin, H.,[1] Giddens, W.E.,[2] Benveniste, R.E.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Pathology, University of Washington, Seattle: Characterization of an exogenous type-D retrovirus isolated from macaques with simian AIDS and retroperitoneal fibromatosis.

Desrosiers, R.,[1] Daniel, M.D.,[1] Butler, C.,[1] King, N.,[1] Wills, J.,[2] Barker, C.,[2] Hunter, E.,[2] Hunt, R.,[1] Letvin, N.,[1] [1]New England Regional Primate Research Center, Harvard Medical School, Southboro, Massachusetts; [2]Laboratory for Special Cancer Research, University of Alabama, Birmingham: Characterization of a new type-D retrovirus–Is it the cause of the immunodeficiency syndrome of macaques?

SV40, Polyoma, and Adenoviruses

August 15–August 19

ARRANGED BY

Terri Grodzicker, Cold Spring Harbor Laboratory
Michael Botchan, University of California, Berkeley

401 participants

The meeting on the molecular biology of SV40, polyoma, and adenoviruses, which is held in alternate years at the Cold Spring Harbor Laboratory, was attended by more than 400 scientists. The small DNA tumor viruses continue to be intensively studied model systems for investigating mechanisms of eukaryotic gene control, replication, and transformation. Many papers were presented on active areas of research, such as: control of transcription and RNA splicing both in vivo and in vitro; analysis and purification of viral and cellular transcription factors; properties of viral and cellular enhancers; regulation of gene expression by the adenoviral E1A protein; control of translation of VA RNAs; replication of viral DNAs in vitro; the role of viral transforming proteins in immortalization, transformation, and cooperation with other oncogenes; and association of tumor antigens with cellular proteins such as p53 and c-*src*. Several viral transforming proteins have been overproduced by using a variety of prokaryotic and eukaryotic expression vectors. The adenovirus E1A protein and SV40 T antigen have been purified and their functions in a variety of cells as well as in in vitro transcription and replication systems are being intensively investigated.

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

SESSION 1 ADENOVIRUSES: TRANSCRIPTION

Chairperson: J. Manley, Columbia University, New York, New York

Hearing, P., Shenk, T., Dept. of Microbiology, State University of New York, Stony Brook: Transcription of Ad5 E1A is regulated in *cis* by two separate sequences and in *trans* by the E1A gene products.

Kingston, R.E., Baldwin, A.S., Sharp, P.A., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Regulation of heat-shock-protein-70 gene expression by the adenoviral E1A region and by *c-myc*.

Kumar, R., Gong, S.-S., Subramanian, K.N., Dept. of Microbiology and Immunology, University of Illinois Health Sciences Center, Chicago: Transcriptional regulatory elements located at the left terminus of Ad5.

Stein, R., Ziff, E.B., Kaplan Cancer Center, New York University Medical Center, New York: HeLa cell β-tubulin gene transcription is stimu-

lated by Ad5 in parallel with viral early genes by an E1A-dependent mechanism.

Gaynor, R.B.,[1] Yoshinaga, S.,[2] Han, M.,[2] Feldman, L.,[3] Berk, A.,[2] Depts. of [1]Medicine, [2]Microbiology, [3]Microbiology and Immunology, University of California, Los Angeles: Adenoviral E1A protein enhances transcription of genes transcribed by RNA polymerase III.

Hoeffler, W.K., Roeder, R.G., Rockefeller University, New York, New York: Extraction and quantitation of pol III transcription factors from adenovirally infected HeLa cells.

Moore, C.L., Sharp, P.A., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Site-specific utilization of viral polyadenylation sites in a cell-free reaction.

McDevitt, M.A., Hart, R.P., Imperiale, M.J., Ali, H., Nevins, J.R., Rockefeller University, New York, New York: Requirement of a downstream sequence for generation of a poly(A) addition site.

Falck-Pedersen, E.,[1] Logan, J.,[2] Shenk, T.,[2] Citron, B.,[1] Darnell, J.E., Jr.,[1] [1]Rockefeller University, New York, New York; [2]Dept. of Microbiology, State University of New York, Stony Brook: The use of recombinant mutants of Ad5 *dl*309 for examination of transcriptional control DNA elements—Termination of the E1A gene by a mouse β-major globin termination sequence.

Natarajan, V., Madden, M.J., Salzman, N.P., NIAID, NCI, National Institutes of Health, Bethesda, Maryland: Proximal and distal domains that control in vitro transcription of the adenoviral IVa_2 gene.

SESSION 2 SV40, POLYOMA: TRANSCRIPTION

Chairperson: W. Folk, University of Michigan, Ann Arbor, Michigan

Gidoni, D.,[1] Dynan, W.,[1] Saffer, J.,[2] Singer, M.,[2] Tjian, R.,[1] [1]Dept. of Biochemistry, University of California, Berkeley; [2]NCI, National Institutes of Health, Bethesda, Maryland: Protein-DNA interactions between transcription factor Sp1 and regulatory sequences in viral and cellular promoters.

Mishoe, H.,[1] Brady, J.N.,[2] Radonovich, M.,[1] Salzman, N.P.,[1] [1]Laboratory of Biology of Viruses, [2]Laboratory of Molecular Virology, NCI, National Institutes of Health, Bethesda, Maryland: SV40 GC-rich sequences function as an independent transcriptional control element in vitro.

Keller, J.M., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Elements of the SV40 late promoter and transactivation by T antigen.

Brady, J., Khoury, G., NCI, National Institutes of Health, Bethesda, Maryland: SV40 late transcription—Induction by T antigen.

Chandrasekharappa, S., Hartzell, S., Byrne, B., Subramanian, K., Dept. of Microbiology and Immunology, University of Illinois Health Sciences Center, Chicago: Two noncontiguous domains of the SV40 late promoter are required for its activation with T antigen.

Michaeli, T., Prives, C., Dept. of Biological Sciences, Columbia University, New York, New York: Regulation of SV40 late gene expression in *Xenopus laevis* oocytes.

Mertz, J.E., McArdle Laboratory, University of Wisconsin, Madison: The SV40 late leader region encodes a regulated transcriptional terminator.

Tseng, R.W., Acheson, N.H., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Transcription is terminated at multiple sites on the L strand of polyoma virus DNA during lytic infection.

Sadofsky, M., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Deletions downstream from the SV40 late AAUAAA decrease the efficiency of 3′-end processing.

Kern, F.G., Dailey, L., Basilico, C., Dept. of Pathology, New York University School of Medicine, New York: Effects of deletions and polyadenylation signal substitution on transcription initiating from the late promoter in rat cells transfected with chimeric plasmids.

F. Sherman, M. Botchan, T. Grodzicker

Duprey, E., Hearing, P., Shenk, T., Dept. of Microbiology, State University of New York, Stony Brook: The bidirectional nature of the Ad5-E1A enhancer is environment specific.

Dickerson, I., Jones, N.C., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Sequence requirements for splicing of Ad5 E1A pre-mRNA.

Tremblay, M.L., Yee, S.-P., Persson, R., Smiley, J.R., Branton, P.E., Dept. of Pathology, McMaster University, Hamilton, Canada: Lack of complementation between Ad5 mutants and mouse cell lines constitutively expressing the ICP4 immediate early gene of HSV-1.

Leff, T., Sassone-Corsi, P., Zajchowski, D., Boeuf, H., Goding, C., Jalinot, P., Kédinger, C., Chambon, P., INSERM, Faculté de Médecine, Strasbourg, France: Sequence elements controlling the expression of adenoviral E2A and E3 transcription units.

Murthy, S., Bhat, G., Thimmappaya, B., Cancer Center, Northwestern University Medical School, Chicago, Illinois: Adenoviral E2A early promoter contains two transcriptional control regions but lacks nucleotide sequences specific for its transactivation by E1A.

Huang, D.-H., Roeder, R.G., Rockefeller University, New York, New York: Localization of upstream elements that modulate transcription of the adenoviral E2A early genes.

Imperiale, M.J., Hart, R.P., Nevins, J.R., Rockefeller University, New York, New York: Mapping and properties of Ad5 E2 transcriptional regulatory sequences.

Berger, S.L.,[1] Folk, W.,[1,2] [1]Cell and Molecular Biology Program, [2]Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Differential activation of an RNA polymerase II and an RNA polymerase III–transcribed gene by the polyoma virus enhancer and the adenoviral E1A gene products.

Yoder, S.S., Berget, S.M., Dept. of Biochemistry, Rice University, Houston, Texas: Control of *dhfr* gene expression by Ad2.

Khalili, K., Weinmann, R., Wistar Institute, Philadelphia, Pennsylvania: Shutoff of host protein synthesis after adenoviral infection.

Reich, N.C.,[1] Babich, A.,[1] Weinberger, C.,[2] Darnell, J.,[1] [1]Rockefeller University, New York, New York; [2]State University of New York, Stony Brook: Nuclear-cytoplasmic transport of viral and cellular mRNAs late in adenoviral infection.

Iwamoto, S.,[1] Eggerding, F.A.,[2] Falck-Pedersen, E.,[1] Darnell, J.E., Jr.,[1] [1]Rockefeller University, New York, New York; [2]Dept. of Pathology, University of California School of Medicine, Los Angeles: Transcription of the Ad2 major late transcript at early and late times after infection.

Ko, J.-L., Harter, M.L., Dept. of Microbiology, University of Medicine and Dentistry of New Jersey, Newark: In vitro characterization of a bacterially produced Ad2 E1A-like protein–DNA binding and transcriptional activation.

Abmayr, S.M., Roeder, R.G., Rockefeller University, New York, New York: The effect of pseudorabies virus infection on in vitro transcription.

Bondopadhyay, S., Spector, D.J., Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Effect of template conformation on transcription of adenoviral promoters in vitro.

Samuels, M., Carthew, R., Sharp, P.A., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Purification of specific RNA polymerase II transcription factors.

Freyer, G.A., O'Neill, K.E., Tohill, K.A., Roberts, R.J., Cold Spring Harbor Laboratory, New York: In vitro and in vivo studies of Ad2 major late mRNA splicing.

Lee, R., Concino, M., Weinmann, R., Wistar Institute, Philadelphia, Pennsylvania: The effect on in vitro transcription of single base changes in the adenoviral major late promoter.

Mirza, A., Institut für Molekularbiologie, Universitätsklinikum Essen, Federal Republic of Germany: Protein kinase(s) as positive and negative modulators of adenoviral transcription.

Mok, M., Maderious, A., Chen-Kiang, S., Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Cornell University Graduate School of Medical Sciences, New York, New York: Premature termination of transcription occurs temporally and is inducible in adenoviral infection.

Fraser, N.W., Owen, J.L., Dressler, G.R., Wistar Institute, Philadelphia, Pennsylvania: Transcriptional termination in Ad2.

Bertuch, A., Hansen, U., Dana-Farber Cancer Institute, Boston, Massachusetts: Nitrocellulose filter-binding assay for the cellular protein(s) specifically binding the 21-bp repeat region of the SV40 promoters.

Nobel, J., Prives, C., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: In vitro splicing and polyadenylation of an SV40 early pre-mRNA.

Hay, N., King, D., Aloni, Y., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Efficient and accurate in vitro excision of SV40-associated small RNA (SAS-RNA).

Somasekhar, M.B., Mertz, J.E., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Factors involved in splicing of the SV40 late transcripts.

Laub, O., Hay, N., Bengal, E., Aloni, Y., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Attenuation and transcription-termination in the control of SV40 gene expression.

Grass, D.S., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: Insertion of the Ad2 late promoter into the SV40 late leader region and its effect on late mRNA synthesis.

Lamarche, M.F., Bourgaux-Ramoisy, D., Bourgaux, P., Faculté de Médecine, Université de Sherbrooke, Canada: Activity of a late promoter in integrated polyoma DNA.

Bergeron, J.L., Acheson, N.H., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Viable insertion mutants of

polyoma virus containing a second copy of the signal for polyadenylation of RNA.

Skarnes, W.C., Tessier, D.C., Acheson, N.H., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Pausing or premature termination of RNA polymerase II nearby the early and late promoters of polyoma virus RNA.

Cunningham, T.P., Pipas, J.M., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: SA12 is a BK-like papovavirus which replicates in monkey cells.

Delmas, V.,[1] Bastien, C.,[1] Scherneck, S.,[2] Feunteun, J.,[1] [1]IRSC, Villejuif, France; [2]Academy of Sciences of the German Democratic Republic, Berlin: Sequence and genetic organization of HapV, a papovavirus causing lesions in Syrian hamsters.

Davies, B.,[1] Arrand, J.,[2] Griffiths, M.,[1] Fried, M.,[1] [1]Dept. of Tumor Virus Genetics, Imperial Cancer Research Fund, London; [2]Paterson Laboratories, Christie Hospital, Manchester, England: Sequence of enhancer and origin regions of polyoma virus defectives.

von Hoyningen-Huene, V.,[1] Ruley, E.,[2] Davies, B.,[1] Griffiths, M.,[1] Fried, M.,[1] [1]Dept. of Tumour Virus Genetics, Imperial Cancer Research Fund, London, England; [2]Cold Spring Harbor Laboratory, New York: Enhancer activity of duplicated sequences in the polyoma virus noncoding region.

Herbomel, P., Bourachot, B., Yaniv, M., Dept. of Molecular Biology, Pasteur Institute, Paris, France: Homologous and heterologous transactivation and transrepression of early and late transcription.

Passananti, C., Maione, R., Pozzi, L., De Simone, V., Amati, P., Dept. di Biopatologia Umana, Università La Sapienza, Roma, Italy: Polyoma mutants selected in differentiated cell lines.

Boehnlein, E., Chowdhury, K., Gruss, P., Institute for Microbiology, ZMBH, Heidelberg, Federal Republic of Germany: Structural and functional analysis of a polyoma enhancer/promoter mutation required for growth in embryonal carcinoma cells.

Templeton, D.,[1] Eckhart, W.,[1] Blangy, D.,[2] [1]Salk Institute, San Diego, California; [2]IRSC, Villejuif, France: Mouse sequences can functionally substitute polyoma enhancer sequences.

Dandolo, L., Aghion, J., Blangy, D., IRSC, Villejuif, France: Regulation of polyoma virus late expression in embryonal carcinoma cells.

Zenke, M., Grundström, T., Wintzerith, M., Matthes, H., Chambon, P., INSERM, Faculté de Médecine, Strasbourg, France: Point mutation scanning of the SV40 enhancer.

Hen, R., Borelli, E., Chambon, P., INSERM, Faculté de Médecine, Strasbourg, France: Blocking of SV40 and polyoma enhancer activity by the Ad2 E1A products.

Axelrod, N., Farabaugh, P., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Can SV40 enhancer sequences substitute for yeast enhancerlike sequences (upstream activation sites)?

Sergeant, A.,[1] Bohmann, D.,[1] Zentgraf, H.,[2] Keller, W.,[1] [1]Institute of Cell and Tumor Biology, [2]Institute of Virology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: The effects of chromatin assembly and of the SV40 enhancer on transcription of homologous and heterologous genes in vitro.

Kenny, S.,[1] Natarajan, V.,[1] Strike, D.,[1] Khoury, G.,[2] Salzman, N.P.,[1] [1]Laboratory of Biology of Viruses, [2]Laboratory of Molecular Virology, National Institutes of Health, Bethesda, Maryland: JC virus enhancer active in human brain cells.

Dabrowski, C.E., Blankenhorn, E.P., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania Medical School, Philadelphia: Analysis of the efficiency of translational initiation in SV40-containing transient expression vectors.

Litzkas, P., Jha, K.K., Ozer, H.L., Dept. of Biological Sciences, Hunter College, City University of New York, New York: Efficient transfer of clones SV40 and nonviral DNA into human diploid cells.

Dalie, B., Harter, M.L., Dept. of Microbiology, University of Medicine and Dentistry of New Jersey, Newark: Expression of Ad2 E1A and E1B gene products from an SV40 vector.

Lebkowski, J., DuBridge, R., Greisen, K., Antell, E., Calos, M., Dept. of Genetics, Stanford University School of Medicine, California: Studying mutation with viral vectors.

Reddy, V.B., Garramone, A.J., Beck, A.K., Vellussi, V., Bernstine, E.G., Integrated Genetics, Framingham, Massachusetts: Expression of dimeric, biologically active human choriogonadotropin (hCG) in CV-1 cells, using a single SV40 vector.

SESSION 4 POSTER SESSION II

Glenn, G.M., Ricciardi, R.P., Wistar Institute, Philadelphia, Pennsylvania: Location of the functional nucleotide defects in Ad5 E1A host-range mutants.

Smith, D., Velcich, A., Kegler, D., Ziff, E., Kaplan Cancer Center, Dept. of Biochemistry, New York University Medical Center, New York: Complementation of the Ad5 E1A mutant *d*l312 by vectors that express linker insertion mutants of the Ad5 E1A gene.

Krippl, B.,[1] Ferguson, B.,[2] Westphal, H.,[1] Rosenberg, M.,[2] Jones, N.,[3] [1]NICHD, NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania; [3]Purdue University, West Lafayette, Indiana: Functional characterization of the E1A proteins of human adenovirus.

Schmitt, R.C., Fahnestock, M.L., Lewis, J.B., Fred Hutchinson Cancer Research Center, Seattle, Washington: Ad2 E1A proteins localized using antipeptide antisera.

Tsukamoto, A., Berk, A.J., Molecular Biology Institute, University of California, Los Angeles: A colony blot method for the selection of mono-

clonal antibodies to adenoviral E1A proteins and their use in purification.

Harlow, E., Schley, C., Cold Spring Harbor Laboratory, New York: Monoclonal antibodies to the adenoviral E1A proteins.

Yee, S.-P., Tremblay, M.L., McGlade, J., Branton, P.E., Dept. of Pathology, McMaster University, Hamilton, Canada: Analysis of Ad5 E1 proteins, using antipeptide sera.

Subramanian, T.,[1] Kuppuswamy, M.,[1] Gysbers, J.,[2] Mak, S.,[2] Chinnadurai, G.,[1] [1]Institute for Molecular Virology, St. Louis University Medical Center, Missouri; [2]Dept. of Biology, McMaster University, Hamilton, Canada: 175R T antigen coded by adenoviral E1B plays a dual role in viral DNA replication.

D'Halluin, J.C., Milleville, M., Laboratoire de Virologie Moléculaire, INSERM, Lille, France: An Ad2 E1B gene product is involved in the control of cell endonuclease activity.

Barker, D., Berk, A.J., Molecular Biology Institute, University of California, Los Angeles: The role of Ad2 E1B gene products during infection and transformation.

Virtanen, A., Pettersson, U., Dept. of Medical Genetics, University of Uppsala, Sweden: Novel mRNAs from the transforming region E1B of human Ad2.

Deutscher, S.L.,[1] Kapoor, Q.S.,[2] Cladaras, C.,[2] Wold, W.S.M.,[2] [1]Edward A. Doisy Dept. of Biochemistry; [2]Institute for Molecular Virology, St. Louis University Medical School, Missouri: The 19K glycoprotein coded by region E3 of adenovirus—Purification, characterization, and structural analysis.

Chatterjee, D., Maizel, J.V., Jr., NICHD, NCI, National Institutes of Health, Bethesda, Maryland: The adenoviral E3 glycoprotein is homologous to HLA-DR heavy chain.

Cutt, J.R., Halbert, D.N., Shenk, T.E., Dept. of Microbiology, State University of New York, Stony Brook: Analysis of adenoviral E4 mutants.

Lewis, J.B.,[1] Fahnestock, M.L.,[1] Hardy, M.M.,[2] Anderson, C.W.,[2] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Nonvirion protein products resulting from expression of the Ad2 major late promoter at early times after the infection of HeLa cells.

Silverman, L.,[1] Johnston, J.M.,[1] Anderson, K.P.,[1] Deppert, W.,[2] Klessig, D.F.,[1] [1]Dept. of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City; [2]Dept. of Biochemistry, University of Ulm, Federal Republic of Germany: Characterization of the block to replication of human adenovirus in abortively infected monkey cells.

Bhat, R.A., Domer, P., Thimappaya, B., Cancer Center, Northwestern University Medical School, Chicago, Illinois: Structural requirements of adenoviral VA_1 RNA for its translation enhancement function.

Rohan, R.M., Ketner, G., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Point mutations in the adenoviral VA_1 promoter region.

Horvath, J., Weber, J., Dept. de Microbiologie, Faculté de Médecine, Université de Sherbrooke, Canada: Ad2 complementary DNA sequences in human B and T lymphocytes.

Signäs, C.,[1] Akusjärvi, G.,[1] Pettersson, U.,[2] Depts. of [1]Microbiology, [2]Medical Genetics, University of Uppsala, Sweden: The gene for Ad3 fiber.

van Loon, A.E., Rozijn, T.H., Sussenbach, J.S., Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands: General characterization of DNAs of the enteric Ad40 and Ad41.

Belak, S.,[1] Virtanen, A.,[3] Berencsi, G.,[2] Pettersson, U.,[3] [1]Dept. of Epizootiology, University of Veterinary Science, [2]Institute of Microbiology, Semmelweis Medical University, Budapest, Hungary; [3]Dept. of Medical Genetics, University of Uppsala, Sweden: Sequence homology between bovine adenoviruses with different species specificity—Importance of region E3.

Becerra, S.P.,[1] Anderson, C.W.,[2] Rose, J.A.,[1] [1]NIAID, NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Direct mapping and mechanism of origin of the adeno-associated viral structural proteins.

Ko, J.-L., Coles, R.S., Harter, M.L., Dept. of Microbiology, University of Medicine and Dentistry of New Jersey, Newark: Improved methods for maximizing expression of cloned Ad2 E1A and E1B genes in *E. coli*.

Vasavada, R.,[1] Barbanti-Brodano, G.,[2] Ricciardi, R.,[1] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Institute of Microbiology, University of Ferrara, Italy: Construction of human cell lines that express the Ad12 E1A proteins, using a novel BK-plasmid vector.

VanDoren, K., Solnick, D., Reichl, H., Gluzman, Y., Cold Spring Harbor Laboratory, New York: Helper independent Ad5 vectors.

Green, P.,[1] Hearing, P.,[2] Fowlkes, D.,[1] [1]Dept. of Pathology, University of North Carolina, Chapel Hill; [2]Dept. of Microbiology, State University of New York, Stony Brook: Expression of a heterologous gene in adenovirus.

Haj-Ahmad, Y., Graham, F.L., Depts. of Biology and Pathology, McMaster University, Hamilton, Canada: Packaging constraints in human Ad5.

Hearing, P., Samulski, R.J., Shenk, T., Dept. of Microbiology, State University of New York, Stony Brook: Identification of the sequences required for efficient packaging of Ad5 DNA into virions.

Franza, R.,[1] Garrels, J.,[1] Furth, M.,[2] Ruley, E.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Cooperating oncogenes are required for transformation of established REF52 cells.

Senear, A.W., Lewis, J.B., Fred Hutchinson Cancer Research Center, Seattle, Washington: Efficient transformation of rodent cell lines by the adenoviral E1A gene.

Kuppuswamy, M., Hurwitz, D.R., Chinnadurai, G., Institute for Molecular Virology, St. Louis University Medical Center, Missouri: A single E1A T antigen (243R) can immortalize and fully transform rat embryo fibroblasts in cooperation with E1B.

Moran, B., Whyte, P., Zerler, B., Maruyama, K., Grodzicker, T., Ruley,

E., Cold Spring Harbor Laboratory, New York: Analysis of the transforming functions of adenoviral E1A.

Haley, K.,[1] Jones, N.C.,[1] Babiss, L.,[2] Ginsberg, H.S.,[2] [1]Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana; [2]Dept. of Microbiology, College of Physicians & Surgeons, Columbia University, New York, New York: The role of Ad5 E1A gene products in transformation of rat cells.

Jochemsen, A.G., Bernards, R., Bos, J.L., van der Eb., A.J., Dept. of Medical Biochemistry, State University of Leiden, The Netherlands: Oncogenic transformation by adenoviral E1A regions in the presence of the c-H*ras* oncogene.

Eager, K.,[1] Williams, J.,[2] Appella, E.,[3] Ricciardi, R.,[1] [1]Wistar Institute, [2]Carnegie-Mellon University, Pittsburgh, Pennsylvania; [3]NCI, National Institutes of Health, Bethesda, Maryland: Expression of class-I cell-surface antigens of Ad12-transformed mouse cells is highly reduced.

Akagi, K.,[1] Cook, J.L.,[3] Patch, C.T.,[1] Kato, T.,[1] Lewis, A.M.,[2] Levine, A.S.,[1] [1]NICHD, [2]NIAID, NCI, National Institutes of Health, Bethesda, Maryland; [3]National Jewish Hospital and Research Center, Denver, Colorado: Dose-dependent correlation of Ad2 gene expression and sensitivity to lysis by nonspecific effector cells among hybrid cells formed from SV40- and Ad2-transformed cells.

Yoshida, K., Ren, C.S., Yamashita, T., Fujinaga, K., Cancer Research Institute, Sapporo Medical College, Japan: The transforming gene of canine Ad1.

Klimkait, T., Doerfler, W., Institute of Genetics, University of Cologne, Federal Republic of Germany: Ad2 or Ad5 E1 functions elicit replication and late expression of Ad12 DNA in hamster cells.

Wojcik, B., Dermody, J., LaBella, F., Radna, R., Ozer, H.L., Dept. of Biological Sciences, Hunter College, City University of New York, New York: Viral DNA synthesis in wild-type and mutant Chinese hamster cells.

Pierce, W.C., Eggerding, F.A., Dept. of Pathology, University of California, Los Angeles: Expression of Ad2 replicative functions in semi-permissively infected cells.

Korn, R.,[1] Friefeld, B.R.,[1] deJong, P.,[2] Sninsky, J.J.,[2,3] Horwitz, M.S.,[1,2] Depts. of [1]Cell Biology, [2]Microbiology and Immunology, [3]Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Expression of a domain of the adenoviral DNA polymerase in a prokaryotic vector.

Wang, K., Pearson, G.D., Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Adenoviral sequences required for replication in vivo.

Munz, P.L.,[1] Volkert, F.C.,[2] Young, C.S.H.,[1] [1]Dept. of Microbiology, Columbia University, New York, New York; [2]Dept. of Molecular Biology, Princeton University, New Jersey: The characterization of adenoviral overlap recombination.

Kinloch, R., Hay, R., Mautner, V., Medical Research Council Virology Unit, Glasgow, Scotland: Recombination between Ad2 and Ad5 hexon-coding regions.

Dery, C.V.,[1] de Murcia, G.,[3] Lamarre, D.,[2] Poirier, G.,[2] Weber, J.,[1] Depts. of [1]Microbiologie, [2]Biologie, Université de Sherbrooke, Canada; [3]Institut de Biologie Moléculaire et Cellulaire de CNRS, Strasbourg, France: PolyADP-ribosylation of adenoviral core proteins.

Wang, C.-N.J., Chen-Kiang, S., Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Cornell University Graduate School of Medical Sciences, New York, New York: Characterization of transcriptionally active Ad2 chromatin during lytic infection.

Silver, L.S., Hafemann, B., Anderson, C.W., Dept. of Biology, Brookhaven National Laboratory, Dept. of Microbiology, State University of New York, Stony Brook: Acquisition of Ad2 receptors by normal, peripheral human lymphocytes.

SESSION 5 ADENOVIRUSES: REGULATION OF GENE EXPRESSION

Chairperson: N. Jones, Purdue University, West Lafayette, Indiana

Jones, N.C.,[1] Andrisani, O.,[1] Richter, J.,[2] Ferguson, B.,[3] Rosenberg, M.,[3] [1]Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana; [2]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [3]Dept. of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania: Functional and biochemical characterization of purified Ad5 and Ad12 E1A proteins.

Spindler, K.R., Eng, C.Y., Berk, A.J., Dept. of Microbiology and Molecular Biology Institute, University of California, Los Angeles: Maximal adenoviral DNA replication in growth-arrested cells requires the product of the E1A 12S mRNA.

Moran, E., Zerler, B., Ruley, E., Mathews, M.B., Grodzicker. T., Cold Spring Harbor Laboratory, New York: Analysis of wild-type and mutant Ad2 E1A cDNAs.

Pilder, S., Logan, J., Shenk, T., Health Sciences Center, State University of New York, Stony Brook: Functional analysis of the Ad5 E1B gene products.

White, E., Grodzicker, T., Stillman, B.W., Cold Spring Harbor Laboratory, New York: Mutations in the nuclear envelope-associated adenoviral E1B 19K tumor antigen cause the degradation of chromosomal DNA.

Weinberg, D.H., Ketner, G., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Characterization of an adenoviral E4 mutant defective in viral late gene expression.

Svensson, C., Larsson, S., Akusjärvi, G., Dept. of Medical Genetics, University of Uppsala, Sweden: Adenoviral VA_1RNA – A general stimulator of mRNA translation.

Schneider, R.J., Weinberger, C., Shenk, T., Dept. of Microbiology, State University of New York,

Stony Brook: Translational regulation by adenoviral VA_I RNA – Analysis of a human α-globin gene in $VA_I^{+/-}$ backgrounds. Analysis of a VA_I^{-}/VA_{II}^{-} double mutant.

Mathews, M.B.,[1] O'Malley, R.,[1] Reichel, P.A.,[1] Merrick, W.C.,[2] Sierkierka, J.,[3] [1]Cold Spring Harbor Laboratory, New York; [2]Case Western Reserve University, Cleveland, Ohio; [3]Roche Institute of Molecular Biology, Nutley, New Jersey: Adenoviral VA_I RNA maintains the activity of protein synthesis initiation factor eIF-2.

Rice, S., Klessig, D., Dept. of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City: Construction and analysis of adenoviral E2A deletion mutants.

SESSION 6 SV40, POLYOMA: REGULATION OF GENE EXPRESSION

Chairperson: G. Khoury, National Institutes of Health, Bethesda, Maryland

Lewis, D., Manley, J., Dept. of Biological Sciences, Columbia University, New York, New York: Replication-defective mutants restore expression of the SV40 early region in human 293 cells.

Robbins, P., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: T-antigen can stimulate the effects of the SV40 enhancer on the HSV TK promoter.

Velcich, A., Ziff, E., Kaplan Cancer Center, New York University Medical Center, New York: An Ad2 E1A expression vector reduces transcription from the early SV40 promoter.

Lebkowski, J., Greisen, K., Calos, M., Dept. of Genetics, Stanford University School of Medicine, California: Can SV40 replication antagonize transcription?

Hertz, G.Z., Mertz, J.E., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: SV40 sequences required for transcription and viral DNA replication.

Herr, W., Gluzman, Y., Cold Spring Harbor Laboratory, New York: Duplications of a mutated SV40 enhancer restore its activity.

Piette, J., Yaniv, M., Dept. of Molecular Biology, Pasteur Institute, Paris, France: In vivo and in vitro studies of the interactions between proteins of 3T6 cells and the polyoma enhancer.

Wildeman, A.G., Sassone-Corsi, P., Chambon, P., Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Faculté de Médecine, Strasbourg, France: The SV40 enhancer stimulates transcription in vitro from both homologous and heterologous promoters by interacting with specific factor(s).

Schöler, H.R.,[1] Sergeant, A.,[2] Keller, W.,[2] Gruss, P.,[1] [1]Institute for Microbiology, ZMBH, Im Neuenheimer; [2]Division of Molecular Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Cell-specific transcriptional enhancement in vitro.

Melin, F., Pinon, H., Kress, C., Blangy, D., IRSC, Villejiuf, France: Polyoma mutants with extended host-range and high lytic potential on EC cells.

SESSION 7 POSTER SESSION III

Asselin, C., Bastin, M., Dept. of Microbiology, University of Sherbrooke, Canada: Immortalization of secondary rat embryo cells by truncated forms of the large T genes of SV40 and polyoma virus.

de Ronde, A., Sol, C., MacDonald, M., ter Schegget, J., van Strien, A., van der Noordaa, J., Dept. of Virology, Academical Medical Centre, Amsterdam, The Netherlands: Host range for transformation of BKV and SV40.

Kelly, F.,[1] Agha, M.E.,[2] Livingstone, D.,[2] [1]Institut Pasteur, Paris, France; [2]Dana-Farber Cancer Institute, Boston, Massachusetts: Immortalization of mouse fibroblasts with small T antigen.

Chiang, L.-C., Silnutzer, J., Spence, S.L., Greenwood, D., Pipas, J.M., Barnes, D.W., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Selection of SV40- and polyoma virus–transformed cells in a serum-free media.

Hutchinson, N., Chang, L.S., Pater, M.M., di Mayorca, G., Dept. of Microbiology, University of Medicine and Dentistry of New Jersey, Newark: Discovery of a *ts* mutant located at 0.455 map units in the SV40 large T antigen that transforms F111 rat cells to anchorage independence at the nonpermissive temperature for lytic growth.

Gurney, T.,[1,2] Gurney, E.,[1,2] Blanck, G.,[3] [1]Oncologie Moléculaire, IRSC du CNRS, Villejuif, France; [2]Dept. of Biology, University of Utah, Salt Lake City; [3]Dept. of Biology, Columbia University, New York, New York: Particular rearrangements within SV40 DNA accompany reversion and retransformation in clones of SVT2 cells.

O'Neill, F.J., Miller, T.H., Renzetti, L., Veterans Administration Medical Center, University of Utah, Salt Lake City: Inhibition of transformation of permissive cells following infection with SV40 stocks containing defectives with reiterated origins and termini.

Vesco, C.,[1] Fischer Fantuzzi, L.,[2] [1]Instituto di Biologia Cellulare, [2]Centro Studi Acidi Nucleici, CNR, Roma, Italy: A 43-amino-acid deletion in the amino-terminal half of the SV40 large T antigen results in a non-karyophilic protein capable of transforming established cells.

Kennett, S., Samad, A., Blum, A., Carroll, R.B., Dept. of Pathology, New York University Medical Center, New York: Characterization of a stable aminoterminal fragment of SV40 large T antigen.

Montenarh, M., Kohler, M., Stürzbecher, H.-W., Schürmann, C., Müller, D., Henning, R., Dept. of Biochemistry, University of Ulm,

Federal Republic of Germany: Dissection of structural modifications of SV40 T antigen with regard to viral replication and transformation.

Hand, R., Whittaker, L., Ismail, A., McGill Cancer Centre, Montreal, Canada: Membrane and nuclear forms of SV40 large T antigen differ in immunological and biochemical characteristics.

Jarvis, D.L., Butel, J.S., Dept. of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: Modification of SV40 T antigen by glycosylation.

Tack, L.C.,[1] Tack, B.F.,[2] [1]Molecular Biology and Virology Laboratory, Salk Institute, [2]Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Identification of an active ester bond in SV40 T antigen.

Montano, X., Lane, D.P., Dept. of Biochemistry, Imperial College, London, England: Cellular localization and immunochemistry of SV40 small T, using the small-T-specific monoclonal antibody pAβ280.

Levine, S., O'Driscoll, K., Wolowodiuk, V., Maltzman, W., Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey: Cellular DNA synthesis and gene expression are necessary for the interaction of SV40 T antigen with the p53 cellular tumor antigen.

Winberry, L., Friderici, K., Fluck, M., Dept. of Microbiology, Michigan State University, East Lansing: Transformation by polyoma *tsA* mutants—Characterization of the temperature-sensitive phenotype.

Clark, K., Folk, W., Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Transformation of rat cells by a polyoma-gastrin recombinant.

Markland, W.,[1] Cheng, S.H.,[1] Oostra, B.A.,[1] Markham, A.,[2] Smith, A.E.,[1] [1]National Institute for Medical Research, London; [2]ICI Pharmaceuticals Division, Macclesfield, England: In vitro mutagenesis of polyoma middle T transformation-associated domains.

Bolen, J.B., Yonemoto, W., Brugge, J.S., Israel, M.A., NCI, National Institutes of Health, Bethesda, Maryland: Increased specific activity of $pp60^{c\,src}$ phosphotransferase in rodent cells following polyoma virus infection and transformation.

Dalianis, T., Ramqvist, T., Klein, G., Dept. of Tumor Biology, Karolinska Institutet, Stockholm, Sweden: Studies on the polyoma virus–induced tumor-specific transplantation antigen (TSTA)– Does middle or large T antigen play a role?

Street, A.J., Griffin, B.E., Imperial Cancer Research Fund, London, England: Purification and characterization of polyoma virus middle T antigen.

Pater, M.M., di Mayorca, G., Pater, A., Dept. of Microbiology, University of Medicine and Dentistry of New Jersey Medical School, Newark: Transformation of primary human embryonic kidney cells with BKV, a human papovavirus.

Major, E., Miller, A., Mourrain, P., Traub, R., NINCDS, NCI, National Institutes of Health, Bethesda, Maryland: JCV multiplication in a line of human fetal glial cells, SVG, established with an origin-defective mutant of SV40.

Shaw, P., Keller, W., Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Identification of cell-viral hybrid mRNA and proteins expressed by the SV40-transformed rat cell line 14B.

Conrad, S.E.,[1] Ito, M.,[1] Stewart, C.,[1] Stuart, P.,[1] [1]Dept. of Microbiology, Michigan State University, East Lansing; [2]Dept. of Molecular Biology, University of California, Berkeley: SV40-induced expression of cellular thymidine kinase.

Sauer, G., Amtmann, E., Dann, M., Hoss, A., Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Reversion of the transformed phenotype in the presence of the SV40 T antigen by an antiviral xanthate compound.

Matlashewski, G.,[1] Lamb, P.,[1] Pim, D.,[1] Benchimol, S.,[2] Crawford, L.,[1] [1]Molecular Virology Laboratory, Imperial Cancer Research Fund, London, England; [2]Division of Biological Research, Ontario Cancer Institute, Toronto, Canada: Analysis of p53 genomic and cDNA clones from human cells.

Schmieg, F., Simmons, D., School of Life and Health Sciences, University of Delaware, Newark: Intracellular location and kinetics of complex formation between SV40 T antigen and p53.

Van Roy, F., Liebaut, G., Fiers, W., Laboratory of Molecular Biology, State University of Ghent, Belgium: Complexes between viral large T antigen and cellular p53 are dispensable for most SV40-induced transformation characteristics.

Rundell, K., Bossert, A., Dept. of Microbiology and Immunology, Northwestern University, Chicago, Illinois: Recognition of cellular proteins by small T antigen produced in bacteria.

de Villiers, J.,[1] Schaffner, W.,[1] Tyndall, C.,[2] Lupton, S.,[2] Kamen, B.,[2] [1]Institut für Molekularbiologie II, Zurich, Switzerland; [2]Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, England: Enhancer function is essential for polyoma virus DNA replication.

Österlund, M., Risuleo, G., Magnusson, G., Dept. of Virology, Uppsala University Biomedical Center, Sweden: Effect of polyoma virus early gene expression and DNA synthesis of mutations in the enhancer region and base sequences 3′ to the poly(A) site.

Garcea, R.L.,[1] Raptis, L.,[2] Liang, J.,[2] Benjamin, T.,[2] [1]Dana-Farber Cancer Institute, [2]Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Separating the functions of polyoma's transforming genes.

Cowie, A., Kamen, R., Genetics Institute, Boston, Massachusetts: Protein-DNA interactions between polyoma virus large T antigen and the viral DNA.

Triezenberg, S.J., Folk, W.R., Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Essential nucleotides in the polyoma virus origin region.

Manos, M.,[2] Gluzman, Y.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Molecular Genetics, Cetus Corporation, Emeryville, California: Genetic and biochemical analysis of SV40 large T antigen mutants.

Scheller, A., Covey, L., Prives, C., Dept. of Biological Sciences, Columbia University, New York, New

York: Binding of polyoma and SV40 large T antigens to viral and cellular DNA.

Hinzpeter, M., Deppert, W., Dept. of Biochemistry, Ulm, Federal Republic of Germany: Nuclear subclasses of SV40 large T exhibit different SV40 ORI DNA–binding properties.

Tack, L.C., Molecular Biology and Virology Laboratory, Salk Institute, La Jolla, California: Loss of T antigen from replicating SV40 chromosomes is associated with asynchronous progression of replication forks.

Fields-Berry, S.,[1] Weaver, D.,[2] DePamphilis, M.,[1] [1]Dept. of Biological Chemistry, Harvard Medical School, [2]Dept. of Biology, Massachusetts Institute of Technology, Boston: Physiological conditions and DNA sequence determine the pathway for segregation of SV40 DNA replicative intermediates.

Hsu, M.-T. Dept. of Molecular Cell Biology, Rockefeller University, New York, New York: Study of SV40 DNA repair and replication by two-dimensional gel electrophoresis and psoralane cross-link.

Paxton, W.B., Subramanian, K.N., Dept. of Microbiology and Immunology, University of Illinois Health Sciences Center, Chicago: Chromatin structure of the early SV40 origin region.

Innis, J.W., Scott, W.A., Dept. of Biochemistry, University of Miami School of Medicine, Florida: DNA replication and chromatin structure of SV40 insertion mutants.

Milavetz, B., Cyze, C., Payne, C., Hopkins-Davis, T., Depts. of Biochemistry, Microbiology and Immunology, University of Western Ontario, London, Canada: Changes in chromatin associated with the early stages in SV40 encapsidation.

Ambrose, C., Blasquez, V., Bina, M., Dept. of Chemistry, Purdue University, West Lafayette, Indiana: The virion assembly process plays an active role in the organization of SV40 chromatin.

Ng, S.-C.,[1] Mertz, J.E.,[2] Bina, M.,[1] [1]Dept. of Chemistry, Purdue University, West Lafayette, Indiana; [2]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: SV40 assembly in cells infected with mutants deleted in the agnogene.

Lutter, L.C., Quasney, M., Cellular and Molecular Biology Program, Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Immunoprecipitation of SV40 replication and transcription complex, using monoclonal antibodies against large T antigen.

Gerard, R.D., Harlow, E., Cold Spring Harbor Laboratory, New York: Monoclonal antibodies to SV40 nucleoprotein complexes.

Mole, S.E., Lane, D.P., Dept. of Biochemistry, Imperial College of Science and Technology, London, England: Fusion proteins between β-galactosidase and SV40 large T in an immunochemical investigation of T antigen.

Simanis, V., Gannon, J., Lane, D.P., CRC Eukaryotic Molecular Genetics Group, Dept. of Biochemistry, Imperial College of Science and Technology, London, England: An immunoaffinity purification procedure for SV40 large T antigen.

Milavetz, B., Hopkins-Davis, T., Payne, C., Cancer Research Laboratory, Depts. of Biochemistry, Microbiology and Immunology, University of Western Ontario, London, Canada: Purification of SV40 T antigen.

Gerard, R.D., Gluzman, Y., Cold Spring Harbor Laboratory, New York: Inducible expression of SV40 T antigen.

Kruczek, I., Kühnel, H., Fanning, E., Institute of Biochemistry, Munich, Federal Republic of Germany: Small rodent RNAs and higher molecular weight RNAs of SV40-infected and SV40-transformed monkey cells have sequences homologous to the integration site of Ad12 DNA in a transformed hamster cell.

Hand, R.,[1] Zeng, G.C.,[1] Ozer, H.,[2] [1]McGill Cancer Centre, Montreal, Canada; [2]Hunter College, City University of New York, New York: Inhibition of an early step in DNA chain elongation of polyoma DNA in ts20, a DNA[ts] mutant of BALB/3T3 cells, and correlation of that defect with a heat-labile topoisomerase I activity from mutant cells.

Lambert, M.,[1] Pelligrini, S.,[2] Gattoni-Celli, S.,[1] Weinstein, I.B.,[1] [1]Institute of Cancer Research, Columbia University; [2]Dept. of Pathology, New York University School of Medicine, New York, New York: A carcinogen induces asynchronous replication of polyoma DNA in rat cells via a *trans*-acting factor.

Bullock, P., Miller, J., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: Analysis of the sequences used during SV40 excision.

Roth, D.B., Wilson, J.H., Verna and Marrs McLean Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas: Measurement of the relative rates of homologous and nonhomologous recombination in transfected SV40 DNA.

Nepveu, A., Wallenburg, J., Gusew, N., Chartrand, P., Dept. de Microbiologie, Faculté de Médecine, CHUS, Sherbrooke, Canada: Evidence that a polyoma virus genome containing an insertion of highly repetitive cellular DNA integrates via homologous recombination.

Manor, H., Yarom, R., Neer, A., Baran, N., Dept. of Biology, Technion–Israel Institute of Technology, Haifa: Recombination between integrated viral DNA and flanking cellular sequences occurs within "onion skin" replication intermediates in polyoma-transformed cells.

Friderici, K., Priehs, C., Fluck, M.M., Dept. of Microbiology and Public Health, Michigan State University, East Lansing: Superinfection rescue of integrated polyoma sequences from Py-6 cells.

Oh, S.Y., Fluck, M.M., Dept. of Microbiology and Public Health, Michigan State University, East Lansing: Differences in integration patterns of polyoma virus genomes between Fischer rat cells and baby hamster kidney cells.

Priehs, C., Fluck, M.M., Dept. of Microbiology, Michigan State University, East Lansing: High frequency of recombination between viral genomes in the integration pathway of polyoma virus.

SESSION 8 WORKSHOP: VIRAL VECTORS

Chairperson: John Hassell, McGill University, Montreal, Canada

Berkner, K.L.,[1,2] Sharp, P.A.,[1] Roberts, T.M.,[3] Schaffhausen, B.,[4] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]ZymoGenetics, Inc., Seattle, Washington; [3]Dana-Farber Cancer Institute, [4]Tufts New England Medical Center, Boston, Massachusetts: Abundant expression of active polyoma middle T in 293 cells infected with an Ad5 (*pymT*) recombinant.

Massie, B.,[1] Gluzman, Y.,[2] Hassell, J.A.,[1] [1]Dept. of Microbiology and Immunology, McGill University, Montreal, Canada; [2]Cold Spring Harbor Laboratory, New York: Construction of a helper-free adenovirus-polyomavirus hybrid that expresses polyomavirus large T antigen from the Ad2 major late promoter.

Logan, J., Shenk, T., Dept. of Microbiology, State University of New York, Stony Brook: The adenoviral tripartite leader sequence enhances translation of viral mRNAs late after infection.

Mansour, S.,[1] Tjian, R.,[1] Grodzicker, T.,[2] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Cold Spring Harbor Laboratory, New York: Construction of defective adenoviral recombinants that express polyoma T antigens.

Yamada, M., Grodzicker, T., Cold Spring Harbor Laboratory, New York: An adenoviral vector that expresses the protein product of a nonselected foreign gene at a high level.

Jat, P., Cepko, C., Sharp, P.A., Center for Cancer Research, Dept. of Biology, Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge: Construction of recombinant retroviruses encoding transforming genes from DNA tumor viruses.

Cherington, V.,[1] Mahoney, M.,[1] Kaplan, D.,[1] Corbley, M.,[1] Morgan, B.,[1] Schaffhausen, B.,[2] Roberts, T.,[1] [1]Dana-Farber Cancer Institute, [2]Tufts New England Medical Center, Boston, Massachusetts: Retroviral shuttle vector for polyoma early region proteins and neomycin resistance.

Roberts, T.M.,[1] Kaplan, D.,[1] Pallas, D.,[1] Mahoney, M.,[1] Bockus, B.,[2] Bolen, J.,[3] Schaffhausen, B.,[2] [1]Dana-Farber Cancer Institute, [2]Dept. of Biochemistry and Pharmacology, Tufts University, Boston, Massachusetts; [3]NCI, National Institutes of Health, Bethesda, Maryland: Isolation of polyoma T antigens from prokaryotic and eukaryotic sources.

SESSION 9 SV40, POLYOMA: REPLICATION

Chairperson: C. Prives, Columbia University, New York, New York

Veldman, G.,[1] Lupton, S.,[2] Kamen, R.,[1] [1]Genetics Institute, Boston, Massachusetts; [2]Dept. of Microbiology, State University of New York, Stony Brook: The polyoma enhancer contains multiple, redundant sequence elements that activate both viral DNA replication and gene expression.

Muller, W.J., Hassell, J.A., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Structural requirements for the function of the polyoma virus origin for DNA replication.

Lee, G.J., Woodworth-Gutai, M., Dept. of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: Eukaryotic regulation elements that enhance DNA replication.

Magnusson, G., Nilsson, S., Sandberg, G., Dept. of Virology, Uppsala University Biomedical Center, Sweden: Activities of polyoma virus T antigens in lytic infection.

Vakalopoulou, E., Vogt, B., Schneider, J., Mertz, R., Sperka, S., Huber, B., Winnacker, E.-L., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: Binding of SV40 T antigen to viral and synthetic DNA sequences.

Chalifour, L.E., Wirak, D.O., Wassarman, P.M., DePamphilis, M.L., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Replication and expression of SV40 and polyoma virus DNA in mouse embryonic cells.

Bradley, M., Hudson, J., Livingston, D., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Specific in vitro adenylation of SV40 large T antigen.

Clertant, P.,[1] Guizani, I.,[1] May, E.,[2] Cuzin, F.,[1] [1]INSERM, Centre de Biochimie, Universite dé Nice; [2]Institut de Recherche Scientifique sur le Cancer du CNRS, Villejuif, France: The nucleotide binding activity of polyoma and SV40 large T proteins.

Levitt, A., Chen, S., Blanck, G., George, D., Pollack, R., Dept. of Biological Sciences, Columbia University, New York, New York: Two integrated, partial repeats of SV40 together code for a super-T antigen.

Cereghini, S., Yaniv, M., Dept. of Molecular Biology, Pasteur Institute, Paris, France: Chromatin assembly of transfected DNA—Structural changes in the origin/promoter/enhancer region of SV40 upon replication.

Bryan, P.,[1] Folk, W.,[2] [1]Dept. of Molecular Genetics, Genex Corporation, Gaithersburg, Maryland; [2]Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Sequences required for the DNase I hypersensitive structure in polyoma.

SESSION 10 TRANSFORMATION. I

Chairperson: J. Butel, Baylor College of Medicine, Houston, Texas

Butel, J.S.,[1] Lanford, R.E.,[2] [1]Dept. of Virology and Epidemiology, Baylor College of Medicine, Houston; [2]Dept. of Microbiology, Southwest Foundation for Biomedical Research, San Antonio, Texas: Transforming potential of an SV40 mutant unable to transport T antigen to the nucleus.

Richardson, W.D., Kalderon, D., Roberts, B., Paucha, E., Colledge, W.H., Smith, A.E., National Institute for Medical Research, London, England: Sequence requirements for nuclear location of SV40 large T.

Kriegler, M.,[1] Perez, C.F.,[1] Hardy, C.,[1] Feinberg, M.,[2] Botchan, M.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Radiology, Stanford University Medical Center, California: Transformation mediated by the SV40 T antigens—Separation of the overlapping SV40 early genes with a retroviral vector.

Ryan, K., Brockman, W., University of Michigan Medical School, Ann Arbor: Isolation of a cell line resistant to transformation by SV40 T antigens.

Sharma, S., Rodgers, L., Cold Spring Harbor Laboratory, New York: Cell lines that express exclusively a membrane-bound form of SV40 large T antigen.

Raptis, L., Benjamin, T.L., Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Regulated expression of middle T antigen in a line of rat fibroblasts.

Gélinas, C., Bouchard, L., Bastin, M., Dept. of Microbiology, University of Sherbrooke, Canada: Stabilization of polyoma middle T antigen by a cellular factor is required for transformation.

Dailey, L.,' Brugge, J.S.,[2] Basilico, C.,[1] [1]Dept. of Pathology, New York University Medical Center, New York; [2]Dept. of Microbiology, State University of New York, Stony Brook: The amino terminus of polyoma middle T antigen is required for formation of active *c-src*/middle T complexes and polyoma-mediated transformation.

Schaffhausen, B.,[1] Parsons, S.,[3] Roberts, T.,[2] Bockus, B.,[1] [1]Dept. of Biochemistry and Pharmacology, Tufts Medical School, [2]Dana-Farber Cancer Institute, Boston, Massachusetts; [3]Dept. of Microbiology, University of Virginia, Charlottesville: Association of polyoma middle T with *c-src*.

Grussenmeyer, T.,[1] Walter, G.,[2] Hutchinson, M.A.,[3] Templeton, D.,[3] Eckhart, W.,[3] [1]Institut für Immunbiologie, Universität Freiburg, Federal Republic of Germany; [2]Dept. of Pathology, University of California, San Diego, La Jolla; [3]Salk Institute, San Diego, California: Properties of polyoma virus medium T antigen protein kinase purified by affinity chromatography using two antipeptine monoclonal antibodies.

Wilson, J.B.,[1] Courtneidge, S.,[2] Griffiths, M.,[1] Fried, M.,[1] [1]Imperial Cancer Research Fund, [2]Medical Research Council, London, England: Analysis of unstable, flat revertants of a polyoma-transformed cell line.

SESSION 11 TRANSFORMATION. II

Chairperson: M. Fried, Imperial Cancer Research Fund, London, England

Kimelman, D.,[1] Miller, J.,[1] Cepko, C.,[2] Mulligan, R.,[2] Roberts, B.,[1] [1]Dept. of Biological Chemistry, Harvard Medical School, [2]Center for Cancer Research, Massachusetts Institute of Technology, Boston: Individual Ad5 E1A gene products elicit distinct alterations of cellular morphology and gene expression.

Maruyama, K., Ruley, E., Cold Spring Harbor Laboratory, New York: Adenoviral E1A blocks in vitro differentiation of rat PC12.

Kao, H.-T., Capasso, O., Heintz, N., Nevins, J.R., Rockefeller University, New York, New York: Control of cellular gene expression in adenovirally transformed cells.

Vassen, R.T.M.J., Houweling, A., Bernards, R., Jochemsen, A.G., Offringa, R., van der Eb, A.J., Dept. of Medical Biochemistry, Sylvius Laboratories, State University of Leiden, The Netherlands: Regulation of MHC expression in adenovirally transformed cells.

Cook, J.L.,[1] Lewis, A.M., Jr.,[2] [1]National Jewish Hospital and Research Center, Denver, Colorado; [2]NIAID, NCI, National Institutes of Health, Bethesda, Maryland: Differential hosts' inflammatory-cell killing of hamster cells infected with or transformed by nononcogenic or highly oncogenic adenoviral species—Implications for T antigen function.

Simanis, V., Lane, D.P., CRC Eukaryotic Molecular Genetics Group, Dept. of Biochemistry, Imperial College of Science and Technology, London, England: Cloning of cellular sequences to which SV40 large T antigen is bound in the chromatin of SV40-transformed cells.

Glaichenhaus, N.,[1] Masiakowski, P.,[2] Mougneau, E.,[1] Lawrence, J.-J.,[3] Cuzin, F.,[1] [1]INSERM, Centre de Biochimie, Université de Nice; [2]Laboratoire de Génétique Moléculaire des Eucaryotes, Faculté de Médecine, Strasbourg; [3]Centre d'Etudes Nucléaires, Grenoble, France: Differential expression of cellular genes in the G2 and G1 phases of the cell cycles, modified in cells expressing the large T protein of polyoma virus.

Singh, K., Saragosti, S., Carey, M., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: Enhanced levels of polymerase III transcripts derived from the B2 repetitive element in SV40 transformed mouse cell lines.

Van Dyke. T.,[1] Levine, A.J.,[1] Brinster, R.,[2] Chen, H.Y.,[2] Messing, A.,[2] Palmiter, R.D.,[3] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia: [3]Howard Hughes Medical Institute, University of Washington, Seattle: Transgenic mice containing the SV40 early region genes develop tumors of the choroid plexus.

Garrels, J., Franza, B.R., Jr., Cold Spring Harbor Laboratory, New York: A two-dimensional gel database for proteins of normal and virally transformed rat cells.

SESSION 12 ADENOVIRUSES: REPLICATION

Chairperson: **B. Stillman,** Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Nagata, K., Guggenheimer, R.A., Kenny, M., Field, J., Lindenbaum, J., Murakami, Y., Gronostajski, R.M., Hurwitz, J., Dept. of Developmental Biology, Albert Einstein College of Medicine, Bronx, New York: Enzymatic DNA replication of adenoviral DNA.

Rawlins, D.R.,[1] Rosenfeld, P.J.,[1] Wides, R.J.,[1] Challberg, M.D.,[2] Kelly, T.J., Jr.,[1] [1]Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Dept. of Microbiology and Molecular Biology, Tufts University School of Medicine, Boston, Massachusetts: Sequence analysis of the adenoviral origin of replication.

Gronostajski, R.M., Nagata, K., Adhya, S., Guggenheimer, R.A., Hurwitz, J., Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Characterization of the HeLa cell DNA sequences homologous to the replication origin of Ad5.

Leegwater, P.A.J., de Vries, E., van Driel. W., van Wijnen, A., Kwant, M.M., van der Vliet, P.C., Laboratory for Physiological Chemistry, University of Utrecht, The Netherlands: Replication of adenoviral DNA with purified proteins—Origin sequences and stimulation by RNA.

Schneider, R., Dörper, T., Gröger, W., Winnacker, E.-L., Institut für Biochemie, Universität München, Federal Republic of Germany: Structure and function of the adenoviral replicon.

Hay, R.T., Medical Research Council Virology Unit, Glasgow, Scotland: The initiation of adenoviral DNA replication in vivo.

Bernstein, J., Porter, J., Challberg, M.D., Dept. of Molecular Biology and Microbiology, Tufts Medical School, Boston, Massachusetts: Nucleotide sequence requirements for the replication of plasmid DNA in Ad5-infected HeLa cells.

Rekosh, D., Mertz, L., Brewster, J., Prestine, L., Dept. of Biochemistry, State University of New York, Buffalo: Cloning and expression in *E. coli* of the gene encoding the adenoviral DNA polymerase.

Chow, K.-C., Pearson, G.D., Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Adenoviral infection elevates HeLa topoisomerase activity.

Graham, F.L., Depts. of Biology and Pathology, McMaster University, Hamilton, Canada: Covalently closed circles of human adenoviral DNA are infectious.

Phage and Bacterial Regulatory Mechanisms

August 21–August 26

ARRANGED BY

Sankar Adhya, National Cancer Institute
Harrison Echols, University of California, Berkeley

289 participants

The study of bacteriophage stands as a cornerstone of our present knowledge of the biological universe, and investigating gene structure and behavior in phage continues to be enormously satisfying and rewarding. What emerged at this year's phage meeting was testament to this fact. It was apparent from the presentations that both genetic and biochemical problems are being studied at a molecular detail that was only a dream in the last decade. Because the regulatory mechanisms of phage are now known to be so intricately related to host regulatory machinery and because host genes and proteins are also being studied at a

similar level of sophistication, it was thus proper that the meeting had wider participation.

In the new format the sessions were arranged not according to organism, as has been the usual practice, but by biochemical processes; e.g., recombination, replication, and transcription initiation and termination. Of course, it was difficult to draw a line between these processes, which are so interdependent. Each of the sessions was chaired by one of the veterans, whose direct participation in the discussions, instant analysis of the situation, and summary of the take-home lessons showed that a chairperson can make a significant difference.

Finally, the meeting brought back the "nonphage" prokaryotic molecular biologists to Cold Spring Harbor and provided a forum in which they and the phage biologists can interact, sharing problems as well as excitement with each other.

This meeting was supported in part by the Robertson Research Fund.

SESSION 1 BACTERIOPHAGE Mu

Goosen, N., van Heuvel, M., Moolenaar, G.F., van de Putte, P., Laboratory of Molecular Genetics, State University of Leiden, The Netherlands: The regulation of early and repressor transcriptions of bacteriophage Mu.

Schumann, W., Institut für Organ Chemie und Biochemie, Technische Hochschule Darnstadt, Federal Republic of Germany: Regulation of the early functions of temperate *E. coli* phage Mu.

Priess, H., Schmidt, C., Li, G.-Di., Kamp, D., Max-Planck-Institut, Martinsried, Federal Republic of Germany: Structure and some aspects of regulation of the early region of phage Mu.

Hattman, S., Ives, J., Dept. of Biology, University of Rochester, New York: Regulation of the methylation-sensitive DNA modification function of bacteriophage Mu.

Kahmann, R., Heisig, P., Seiler, A., Max-Planck-Institut, Berlin, Federal Republic of Germany: The *mom* gene of bacteriophage Mu—A complex regulatory cascade to control a lethal function.

Kahmann, R., Rudt, F., Mertens, G., Max-Planck-Institut, Berlin, Federal Republic of Germany: Site-specific recombination in bacteriophage Mu—G inversion in vitro.

Grundy, F.J., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: The role of invertible G-segment genes in the determination of phage Mu tail fiber structure and host specificity.

Castilho, B., Casadaban, M., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: The specificity of bacteriophage Mu insertion.

Craigie, R., Mizuuchi, M., Mizuuchi, K., NCI, National Institutes of Health, Bethesda, Maryland: Site-specific interactions between the bacteriophage Mu A protein and the Mu ends.

Desmet, L.,[1] Toussaint, A.,[1] Pato, M.,[2] Waggoner, B.,[2] [1]Université libre de Bruxelles, Laboratoire de Génétique, Belgium; [2]National Jewish Hospital and Research Center, Denver, Colorado: Requirement of primase for phage Mu replication and transposition.

Pato, M., Reich, C., Waggoner, B., National Jewish Hospital and Research Center, Denver, Colorado: Replicative transposition of bacteriophage Mu DNA.

Harshey, R.M., Scripps Clinic and Research Foundation, Dept. of Molecular Biology, La Jolla, California: Nonreplicative DNA transposition—Integration of infecting bacteriophage Mu.

Burlingame, R., Lynn, D.L., Obukowicz, M.G., Howe, M.M., Properties of phages with *cis*-dominant point mutations affecting Mu replication.

Glasgow, A.C., Miller, J.L., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: Mu-specific sites and functions involved in growth inhibition of λ::mini-Mu.

Shore, S.H., Ross, W., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: *E. coli* DNA gyrase mutations that inhibit the growth of bacteriophage Mu.

Yoshida, R.K., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: Analysis of the block to phage Mu development in *E. coli himA* mutant strains.

SESSION 2 DNA REPLICATION AND REPAIR

Dodson, M.,[1] Echols, H.,[1] Roberts, J.D.,[2] McMacken, R.,[2] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland: Specialized nucleoprotein structures at the replication origin of bacteriophage λ.

Zylicz, M., Yamamoto, T., Sell, S., Georgopoulos, C., Dept. of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: In vitro λ*dv* DNA replication dependent on the *E. coli dnaJ* and *dnaK* proteins.

Wickner, S., NCI, National Institutes of Health, Bethesda, Maryland: Limits of λ origin DNA required for the initiation of replication in vitro.

Macdonald, P., Mosig, G., Dept. of

Molecular Biology, Vanderbilt University, Nashville, Tennessee: Signals in a DNA replication origin of phage T4.

Hinton, D., Nossal, N., NCI, National Institutes of Health, Bethesda, Maryland: Cloning of T4 DNA encoding the T4 primase proteins, genes *41* and *61*, and a DNA-binding protein of 46 kD.

Selick, H.E., Nakanishi, M., Kreuzer, K.N., Alberts, B.M., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Molecular dissection of potential T4 replication genes.

Kreuzer, K.N., Alberts, B.M., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Molecular cloning and analysis of phage T4 replication origins.

Fulford, W., Model, P., Rockefeller University, New York, New York: Gene x of bacteriophage fl is required for phage DNA synthesis—Mutagenesis of in-frame overlapping genes.

Horiuchi, K., Rockefeller University, New York, New York: Dissection of functional domains of DNA replication origin in bacteriophage fl.

Scheuermann, R.H., Young, K., Echols, H., Dept. of Molecular Biology, University of California, Berkeley: The role of the ε-subunit of *E. coli* DNA polymerase III in the fidelity of DNA replication.

Panayotatos, N., Fontaine, A., Dept. of Molecular Biology, Biogen S.A., Switzerland: Expression of an endonuclease in *E. coli* damages the DNA and induces the SOS response.

Ennis, D.G.,[1] Fisher, B.L.,[1] Mount, D.W.,[1,2] Depts. of [1]Molecular and Cellular Biology, [2]Biochemistry, University of Arizona, Tucson: Direct involvement of *recA* protein in *umuCD* or *pkm*101-mediated inducible mutagenesis.

Bhatia, K., Martin, S., Lee, E., Hays, J., Dept. of Chemistry, University of Maryland Baltimore County, Catonsville: *uvr*, *phr*, and *sos* function in the dark repair of nonreplicating *uv*-irradiated phage λ DNA.

Wong, A., Lemmermann, L., Maurer, R., Dept. of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio: Requirement for *dnaC* proteins in *S. typhimurium* harboring a strong suppressor mutation in *dnaB*.

Engstrom, J., Maurer, R., Dept. of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio: Mutations in the *dnaA-dnaN* region in *S. typhimurium* suppress *dnaZ*(ts).

Pruss, G.J., Drlica, K., Dept. of Biology, Unviersity of Rochester, New York: Extremely high levels of negative supercoiling in *supX* mutants.

S. Adhya

Günthert, U., Max-Planck-Institut, Berlin, Federal Republic of Germany: The DNA methyltransferase gene of the *B. subtilis* phage SPR.

Kellman, S.I., Margolin, P., Public Health Research Institute of the City of New York, New York: Tolerance of *S. typhimurium* to the absence of DNA topoisomerase I and the effect on prophage induction.

SESSION 3 PHAGE MORPHOGENESIS

Feiss, M., Sippy, J., Miller, G., Dept. of Microbiology, University of Iowa, Iowa City: Terminase readthrough during sequential packaging of bacteriophage λ chromosomes.

Gold, M., Parris, W., Dept. of Medical Genetics, University of Toronto, Canada: A bacterial protein requirement for bacteriophage λ terminase activity in vitro.

Rosenberg, S.M., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: λ monomers cannot be packaged in vitro.

Stahl, F.W.,[1] Stahl, M.M.,[1] Kobayashi, I.,[2] Thaler, D.,[1] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]Dept. of Bacteriology, University of Tokyo Faculty of Medicine, Japan: Recombination and packaging of phage λ.

Russel, M., Model, P., Rockefeller University, New York, New York: Thioredoxin is required for filamentous phage assembly.

Smith, G.P., Division of Biological Sciences, University of Missouri, Columbia: Filamentous phage morphogenesis—Which genes are really essential?

Wyckoff, E., Casjens, S., Dept. of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: Expression of hybrid scaffolding protein—β-Galactosidase gene is regulated by unassembled scaffolding protein.

Berget, P.B., Kylberg, R.W., Rangwala, S.H., Plishker, M.F., Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: In vitro analysis of the sequential assembly of proteins of the bacteriophage T4 baseplate.

Davis, N., Model, P., Rockefeller University, New York, New York: Analysis of the membrane anchoring requirements of a model integral membrane protein.

Kurnit, D.,[1] Neve, R.,[1] Lagos, R.,[2] Lee, S.-J.,[2] Goldstein, R.,[2] [1]Genetics Division, Children's Hospital, [2]Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Plasmid cloning vector P4 *vir*1 *sid am*1—*supF* dosage mediates capsid size.

SESSION 4 TRANSPOSITION

Dalyrmple, B., Mollet, B., Iida, S., Arber, W., Dept. of Microbiology, Biozentrum of the University of Basel, Switzerland: Structure and functions of the prokaryotic *is* elements IS*26* and IS*30*.

Shen, M., Raleigh, E., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Analysis of unselected IS*10* rearrangements.

Roberts, D., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Effects of adenine methylation on activity of IS*10*.

Raleigh, E.A., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Rate of transcription and translation of IS*10*-right.

Kittle, J.D., Jr., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Pairing between IS*10*'s p_{IN} and p_{OUT} transcripts in vitro.

Morisato, D., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Physical analysis of Tn*10* transposition.

Davis, M.A., Simons, R.W., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: IS*10* protects itself at two levels from fortuitous activation by external promoters.

Bender, J., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Tn*10* transposition products contain information from both strands of donor transposon molecule.

Mazodier, P., Giraud, E., Gasser, F., Dépt. de Biochimie et Génétique Moléculaire, Institut Pasteur, Paris, France: Genetic organization, DNA sequence, and expression of the three resistance genes of the central region of Tn*5*.

Nag, D.K., Dasgupta, U., Berg, D.E., Dept. of Microbiology, Washington University, St. Louis, Missouri: IS*50*-mediated inverse transposition—Specificity and precision.

Amundsen, S.K.,[1] Ennis, D.G.,[2] Smith, G.R.,[1] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]University of Arizona, Tucson: Analysis of factors influencing inversion of a λ DNA segment flanked by inverted repeats.

Lichens, A., Syvanen, M., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Isolation of a novel bacteriophage λ plasmid that contains the right stem of Tn*5*.

Selwin Thomas, P., Shanmugasundaram, S., Lakshmanan, M., School of Biological Sciences, Madurai Kamaraj University, India: Transposon mutagenesis in *A. chroococcum* using Mud(Ap^R/lac).

Cheng, K.C.,[1,2] Smith, G.R.,[1] [1]Fred Hutchinson Cancer Research Center, [2]Dept. of Pathology, University of Washington, Seattle: Recombinational hotspot activity of Chi-like seqeunces.

Murphy, K.,[1] Casey, L.,[1] Yannoutsos, N.,[1] Poteete, T.,[1] Hendrix, R.,[2] [1]University of Massachusetts Medical School, Worcester; [2]University of Pittsburgh, Pennsylvania: Functional domains of P22 Erf protein.

SESSION 5 REGULATION I

Ebright, R.H.,[1] Miller, J.,[2] [1]Harvard Medical School, Boston, Massachusetts; [2]University of California, Los Angeles: Putative contact between Gln18 of *lac* repressor and base pair 7 of *lac* operator.

Majumdar, A., Polymeropoulos, M., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Interaction between repressor and operators of *gal* operon of *E. coli*.

Garges, S., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Analysis of mutations in *crp* gene of *E. coli*.

Ebright, R.H.,[1,2] Krakow, J.,[3] Kunkel, T.,[4] Cossart, P.,[2] Gicquel-Sanzey, B.,[2] Beckwith, J.,[1] Buc, H.,[2] [1]Harvard Medical School, Boston, Massachusetts; [2]Institute Pasteur, Paris, France; [3]Hunter College, New York, New York: [4]National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina: Ligand binding, conformation, and altered DNA-sequence recognition properties of CAP(Glu181→Leu) and CAP(Glu181→Val).

Okamoto, K., Pondo, C., Freundlich, M., Dept. of Biochemistry, State University of New York, Stony Brook: LexA protein represses transcription of the *crp* gene.

Wertman, K.F., Mount, D.W., Dept. of Molecular and Cellular Biology, University of Arizona, Tucson: Identification of the nucleotide sequence determinants of site-specific binding of *lexA* protein to DNA.

Martin, K., Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Repression-negative mutations of the *araBAD* operon lie in multiple regions.

Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Addition of DNA helical turns between the $araO_2$ operator and *araBAD* promoter cyclically hinders repression.

Hendrickson, W., Brunelle, A., Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Ara C protein of *E. coli* contacts three turns of the DNA helix.

Peterson, M.L., Reznikoff, W.S., Dept. of Biochemistry, University of Wisconsin, Madison: Overlapping and polymerase-binding sites within the *lac* promoter.

Gussin, G.N., Matz, K., Hwang, J.J., Zuber, M., Court, D.L., University of Iowa, Iowa City, and NCI, Frederick Cancer Research Facility, Frederick, Maryland: Altered spacing in the p_{RE} promoter reverses the effect of λcII protein.

Hoopes, B.C., McClure, W.R., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Activation of bacte-

riophage λ promoters by cII protein.

Wulff, D., Bogorad, L., Mahoney, M., Dept. of Biological Sciences, State University of New York, Albany: Mutations in phage λ partially restoring activity to a p_{RE}-promoter mutant.

Keilty, S., Ho, Y.-S., Sathe, G., Rosenberg, M., Dept. of Molecular Genetics, Smith, Kline and French Laboratories, Swedeland, Pennsylvania: Transcription of the λ p_{RE}- promoter – Absence of "– 35" region sequence specificity.

Basu, S., Maitra, U., Albert Einstein College of Medicine, Bronx, New York: Studies on the interactions of T3 RNA polymerase with its cognate promoter.

D. Ennis, H. Echols

SESSION 6 POSTER SESSION

Hasan, N., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Structure of the coliphage λ *nut*L antiterminator module.

MacHattie, L.A., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Expression of IS-interrupted coding sequence restored by plasmid dimerization.

Daniels, D., Isackson, P., Bertrand, K., Dept. of Microbiology and Molecular Genetics, University of California, Irvine: Interactions between the divergent overlapping promoters for Tn*10 tetA* and *tetR*.

Miller G., Christiansen, S., Feiss, M., Dept. of Microbiology, University of Iowa, Iowa City: Spacing and sequence changes in the *Xmn*I symmetry segment of cos*λ*.

Weisemann, J., Weinstock, G., NCI, Frederick Cancer Research Facility, Frederick, Maryland: A positive selection for *recA* mutations using *lacZ* protein fusions – Isolation of mutations affecting *recA* expression.

Chan, P.T.,[1] Ohmori, H.,[2] Tomizawa, J.-I.,[2] Lebowitz, J.,[1] [1]Dept. of Microbiology, University of Alabama, Birmingham; [2]NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence and gene organization of ColE1 DNA.

Lambert, P.F., Reznikoff, W.S., Dept. of Biochemistry, University of Wisconsin, Madison: DNA requirements at the bacteriophage G4 origin of complementary strand DNA replication.

Sisk, W., Court, D., NCI, Frederick Cancer Research Facility, Frederick, Maryland: A plasmid vector for identifying and expressing open reading frames utilizing the λ p_L promoter.

Harbrecht, D.F., Newman, D., Anderson, D., Salstrom, J.S., Molecular Genetics, Incorporated, Minnetonka, Minnesota: *E. coli* vectors for the expression of cloned genes under the control of p_L promoter and *cro* ribosome-binding site of phage λ.

Yin, J.C.P., Krebs, M.P., Reznikoff, W.S.. Dept. of Biochemistry, University of Wisconsin, Madison: Identification of the translational initiation sites for the right repeat proteins of Tn5.

Yu, X.M., Reznikoff, W.S., Dept. of Biochemistry, University of Wisconsin, Madison: Deletion analysis of the CAP-cAMP-binding site of the *E. coli* lactose promoter.

Watson, L.A., Cheung, A., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: An unlinked gene *glnR* enhances expression of the glutaminyl-tRNA synthetase gene, *glnS*, in *E. coli*.

Twitchell, D., Poteete, A., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Relationships among the lysis functions of P22, λ, and T4.

Trojanowska, M., Alford, C., Dawson, M., Karam, J., Dept. of Biochemistry, Medical University of South Carolina, Charleston: Control of expression of the T4 *regA* gene.

Shanblatt, S.H., Revzin, A., Dept. of Biochemistry, Michigan State University, East Lansing: Activation of transcription at the *E. coli* galactose operon by two CAP molecules.

Robinson, A.C., Dept. of Molecular Biology, University of Edinburgh, Scotland: DNA sequence of *ftsQ* and *ftsA* – Essential cell division genes of *E. coli*.

Cram, D., Ray, A., Skurray, R., Dept. of Microbiology, Monash University, Clayton, Australia: Cloning and analysis of F plasmid *pif* region specifying abortive infection of phage T7.

Podhajska, A.J., Hasan, N., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Regulation of cloned gene expression by in vivo structural rearrangements in the plasmid.

Podhajska, A.J., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: An *N*-unresponsive leftward terminator region in coliphage λ.

Peltz, S., Brown, A.L., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Loss of *boxA* results in the terminator-specific thermosensitivity of the cloned *nut*L antiterminator of coliphage λ.

Parker, R., Dept. of Chemistry, Indiana University, Bloomington: Regulation of transcription from dual-promoter regions in ColE1 DNA.

Newman, E.B., Ahmed, D., Dumont, D., Miller, B., Ramotar, D., Walker, C., Dept. of Biological Sciences, Concordia University, Montreal, Canada: Multiple controls on L-serine deaminase activity in *E. coli* K-12—Why so much fuss for a nonessential activity?

McFall, E.,[1] Palchaudhuri, S.,[2] Bornstein, S.,[1] [1]Dept. of Microbiology, New York University School of Medicine, New York; [2]Dept. of Microbiology, Wayne State University School of Medicine, Detroit, Michigan: Organization and minimum size of the *E. coli* K-12 D-serine deaminase control region.

McCormick, J.R., Zengel, J.M., Lindahl, L., Dept. of Biology, University of Rochester, New York: Further definition of ribosome-binding sites in *E. coli*.

Marsh, L., Walker, G.C., Dept.of Biology, Massachusetts Institute of Technology, Cambridge: *UmuDC*-induced cold sensitivity in *E. coli*.

K. Horiuchi

Marinus, M.G.,[1] Carraway, M.,[1] Frey, A.,[1] Youderian, P.,[2] [1]University of Massachusetts Medical School, Worcester; [2]University of Southern California, Los Angeles: Mutation spectrum in a *dam* mutant of *E. coli*.

Ludtke, D.,[1] Silhavy, T.,[1] Torriani, A.,[2] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of *phoM* and the identification of its gene product in *E. coli*.

Liao, S. M., McClure, W.R., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Properties of the small antisense RNA promoter (Psar) of bacteriophage P22.

LaRossa, R., Smulski, D., Central Research and Development Department, E.I. du Pont de Nemours and Company, Wilmington, Delaware: Inverse escape synthesis mediated by Mud(Ap,*lac*)-I prophage induction.

Koop, A.H., Hartley, M., Bourgeois, S., Regulatory Biology Laboratory, Salk Institute, San Diego, California: Construction of single-copy ampicillin-resistance plasmids for cloning and promoter analysis.

Kestler, H., Zengel, J.M., Lindahl, L., Dept. of Biology, University of Rochester, New York: A novel regulation of r-protein synthesis from the α operon of *E. coli*.

Jakubowski, H., Goldman. E., Dept. of Microbiology, University of Medicine and Dentistry of New Jersey Medical School, Newark: Quantities of individual aminoacyl-tRNA families and their turnover in *E. coli*.

Hoopes, R.R., Jr., Minkley, E.G., Jr., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Activation of the transfer operon of *E. coli* F plasmid.

Hinton, D.,[1] Musso, R.,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biology, University of South Carolina, Columbia: In vitro transcription from a promoter in the insertion sequence IS2 correlates with expression of a 14-kD IS2 protein.

Kalty, K., Goldman, E., Dept. of Microbiology, New Jersey Medical School, Newark: Pleiotropic suppression obtained by cloning in pBR322 restriction fragments from total DNA of Su+6 *E. coli*.

Friedman, S.,[1] Hays, J.,[2] Zagursky, R.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick; [2]Dept. of Chemistry, University of Maryland, Baltimore County, Catonsville: Specific inhibition of *E. coli recBC* nuclease activity by *λgam* clones.

Garcíarrubio, A., León, P., Rocha, M., Vázquez, M., Covarrubias, A.A., Nitrogen Fixation Research Center, National University of México, Cuernavaca, Morelos: Two functional promoters for the expression of the glutamine synthetase gene of *E. coli*.

Colasanti, J., Denhardt, D.T., Cancer Research Laboratory, University of Western Ontario, London, Canada: Effects of the cloned φX174 *A** gene on *E. coli* metabolism.

Christie, G.E.,[1] Ljungquist, E.,[2] Calendar, R.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Microbial Genetics, Karolinska Institute, Stockholm, Sweden: Sequence of the P2 *ogr* gene, a regulator of P2 late gene transcription.

Cohen, G., Dept. of Microbiology, Tel Aviv University, Ramat-Aviv, Israel: Early lytic replication of bacteriophage P1.

Brooks, J.E.,[1] Howard, K.A.,[1] Theriault, G.,[2] Roy, P.,[2] Gingeras, T.R.,[3] [1]New England Biological Laboratories, Beverly, Massachusetts; [2]Dept. of Biochemistry, Université Laval, Quebec, Canada; [3]Salk Institute for Biotechnology and Industrial Associates, La Jolla, California: Characterization of *Pae*R7, a restriction-modification system cloned from *P. aeruginosa*.

Bialkowska-Hobrzanska, H., Denhardt, D.T., Cancer Research Laboratory, University of Western Ontario, London, Canada: Use of in vitro gene fusions to study the *rep* gene of *E. coli* K-12: Analysis of transcription regulation.

Belfort, M., Pedersen-Lane, J., Center for Laboratories and Research, New York State Department of Health, Albany: A genetic system for studying the *thyA* gene and analyzing *E. coli* thymidylate synthase.

Zylicz, M., Sell, S., Yamamoto, T., Georgopoulos, C., Dept. of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: Host and phage protein interactions involved in λ replication.

Thylén, C., Dept. of Pharmaceutical Microbiology, Biomedical Center, Uppsala, Sweden: Expression of the T4 gene for β-glucosyltransferase, cloned on a plasmid vector, is dependent on the allelic state of the *rho* gene of the host.

Rothstein, D.M., Bertonis, J.M., Biogen Research Corp., Cambridge, Massachusetts: Efficiency of transcription of the plasminogen activator gene in *E. coli*.

SESSION 7 REGULATION II

Hayes, C., Tuer, R., Hayes, S., Dept. of Microbiology, University of Saskatshewan, Saskatoon, Canada: Nonimmune exclusion phenotype exhibited by defective λ lysogens.

Christie, G.E.,[1] Dale E.,[1] Calendar, R.,[1] Lin, C.,[2] Six, E.,[2] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Microbiology, University of Iowa, Iowa City: Comparison of the late gene promoters of coliphage P2 and satellite phage P4.

Christie, G.E., Pfaff, S., Calendar, R., Dept. of Molecular Biology, University of California, Berkeley: In vivo induction of P2 late gene expression from *galK* fusion plasmids.

Alano, P., Dehò, G., Ghisotti, D., Sironi, G., Zangrossi, S., Dept. di Biologia, Universita de Milano, Italy: Alternative regulatory states for the intracellular propagation of the genetic element P4.

Kim, S.K., Lagos, R., Goldstein, R., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Plasmid P4 – An integrated mastercopy is required for stable plasmid maintenance.

Lozano, P., Lagos, R., Agarwal, M., Lee, S.-J., Kim, S., Goldstein, R., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Differential gene expression by phasmid P4 in the plasmid and phage stages.

Horabin, J.I., Webster, R.E., Dept. of Biochemistry, Duke University, Durham, North Carolina: Regulation of gene I expression of the filamentous bacteriophage fl.

Greene, J.R., Geiduschek, E.P., Dept. of Biology, University of California, San Diego: A bacteriophage SPO1-coded type-II DNA-binding protein shows site-selectivity.

Markiewicz, P.,[1] Haynes, L.,[1] Malone, C.,[1] Chase, J.,[2] Rothman-Denes, L.B.,[1] [1]Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois; [2]Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: N4 virion RNA polymerase – Template and host-factor requirements for transcription.

Jin, D.J., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Search for structural and functional domains in the β-subunit of RNA polymerase in *E. coli* by studying rifampicin-resistant mutations.

Guidi-Rontani, C.,[1] Spassky, A.,[2] Depts. of [1]Biochimie et Génétique Moleculaire, [2]Biologie Moléculaire, Institut Pasteur, Paris, France: Mutation of the β-subunit of *E. coli* RNA polymerase that affects expression of the catabolite-sensitive operons.

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 positive control of gene expression.

Franco, R., Drlica, K., Dept. of Biology, University of Rochester, New York: Supercoiling and gyrase gene expression in *E. coli*.

Daniel, J., Danchin, A., Dept. de Biochimie et Génétique Moléculaire, Institut Pasteur, Paris, France: On the mediators of catabolite repression in *E. coli*.

SESSION 8 REGULATION III

Arps, P.J.,[1] Marvel, C.C.,[2] Rubin, B.C.,[1] Winkler, M.E.,[1] [1]Northwestern University Medical School, Chicago, Illinois; [2]Naval Biomedical Research Laboratory of the University of California, Berkeley: The *hisT* gene is part of a multigene operon in *E. coli* K-12.

Nieuwkoop, A.J., Bender, R.A., Division of Biological Sciences, University of Michigan, Ann Arbor: "Double negative" control of the *huhUH* operon in *K. aerogenes* by CAP-cAMP.

Reitzer, L., Magasanik, B., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The three promoters of the *glnALG* operon.

Lupski, J., Nesin, M., Svec, P., Godson, G.N., Dept. of Biochemistry, New York University Medical Center, New York: Regulation of the *rpsU-dnaG-rpoD* macromolecular synthesis operon of *E. coli* K-12.

Haughn, G.W.,[1] Squires, C.H.,[2] DeFelice, M.,[3] Calvo, J.M.,[1] [1]Cornell University, Ithaca, New York; [2]Synergen, Boulder, Colorado; [3] CNR, Naples, Italy: Transcriptional initiation of the *ilvIH* operon of *E. coli*.

Springer, M.,[1] Plumbridge, J.A.,[1] Fayat, G.,[2] Mayaux, J.F.,[2] Blanquet, S.,[2] Grunberg-Manago, M.,[1] [1]Institut de Biologie Physico-chimique, Paris, [2]Laboratoire de Biochimie, Ecole Polytechnique, Paris, France: Different control mechanisms for the expression of two aminoacyl-tRNA synthetases in *E. coli*.

Gourse, R., Takebe, Y., Nomura, M., Institute for Enzyme Research, University of Wisconsin, Madison: Feedback regulation of rRNA and tRNA transcription in *E. coli*.

Imamoto, F., Ishii, S., Maekawa, T., Nagase, T., Dept. of Molecular Ge-

netics, Riken, Wako, Japan: Structure and expression of the *nusA* and *nusB* genes in *E. coli*.

Albin, R., Silverman, P., Albert Einstein College of Medicine, Bronx, New York: The c*pxA* locus of *E. coli* K-12 is a single gene.

Guiso, N., Roy, A., Danchin, A., Ullmann, A., Institut Pasteur, Paris, France: Structure and regulation of adenylate cyclase in *E. coli*.

LaRossa, R.A., Smulski, D.R., Van Dyk, T.K., Central Research and Development Department, E.I. du Pont de Nemours and Company, Wilmington, Delaware: Sulfometuron methyl—A new probe of the *ilv* regulon.

Kusta, S., Hirschman, J., Meeks, J.C., Dept. of Bacteriology, University of California, Davis: Covalent modification of bacterial glutamine synthetase—Physiological significance.

Chapon, C.,[1] Michaelis, S.,[1,2] Willemot, K.,[3] Cornelis, P.,[3] Raibaud, O.,[1] Schwartz, M.,[1] [1]Institut Pasteur, Paris, France; [2]University of California, San Francisco; [3]Université de Louvain, Belgium: Pullulanase, an extracellular product of a gene in the maltose regulon of *K. pneumoniae*.

Gilson, E., Bedouelle, H., Charbit, A., Dassa, E., Duplay, P., Rousset, J.P., Hofnung, Institut Pasteur, INSERM, CNRS, Paris, France: The m*alB* region in *E. coli* K-12—Structure and expression.

Stern, M., Ferro-Luzzi Ames, G., Dept. of Biochemistry, University of California, Berkeley: Repetitive extragenic palindromic (REP) sequences—A major component of the bacterial chromosome.

SESSION 9 REGULATION IV AND TRICKS

Bardwell, J.C.A., Craig, E.A., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Isolation of an *E. coli* heat-inducible gene homologous to the *Drosophila* 83,000-m.w. heat-shock protein gene.

Grossman, A.D.,[1] Zhou, Y.,[1] Heilig, J.,[2] Christie, G.,[2] Calendar, R.,[1] Gross, C.,[1] [1]Dept. of Bacteriology, University of Wisconsin, Madison; [2]Dept. of Molecular Biology, University of California, Berkeley: Extragenic suppressors of a ts mutation in the σ subunit of *E. coli* RNA polymerase.

Grossman, A.D., Erickson, J.W., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: The *htpR*-gene product of *E. coli* is a σ factor for heat-shock promoters.

Paek, K.-H., Walker, G.C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A defect in induction of heat-shock proteins at high temperature in *xthA* mutants.

Winter, R.B., Gold, L., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: The maturation (A_2) protein from the RNA bacteriophage QB induces the synthesis of some *E. coli* heat-shock proteins.

Zengel, J.M., Dept. of Biology, University of Rochester, New York: The synthesis of ribosomal proteins in *E. coli* is inhibited during the heat-shock response.

Christman, M.F., Morgan, R.W., Jacobson, F.S., Ames, B.N., Dept. of Biochemistry, University of California, Berkeley: Regulation of cellular defenses against oxidative stress.

Gaitanaris, G.A., Gottesman, M.E., McCormick, M., Howard, B.H., NCI, National Institutes of Health, Bethesda, Maryland: Reconstitution of an operon from overlapping fragments.

Backman, K., O'Connor, M.J., Maruya, A., Erfle, M., BioTechnica International, Inc., Cambridge, Massachusetts: Regulation of gene expression by in vivo rearrangement of DNA sequences.

Refolo, L.M., Clarke, M.A., Conley, M.P., Jacobsen, J.S., Humayun, M.Z., Dept. of Microbiology, University of Medicine and Dentistry of New Jersey Medical School, Newark: Nucleotide sequence context effects in spontaneous frameshift mutagenesis of M13 cloning vectors.

Mott, J.E.,[1] Grant, R.A.,[1] Ho, Y.-S.,[2] Platt, T.,[1] [1]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut; [2]Smith, Kline and French Laboratories, Philadelphia, Pennsylvania: Maximizing gene expression from plasmid vectors containing the λ p_L promoter—Strategies for overproducing transcription termination factor *rho*.

Raibaud, O., Mock, M., Schwartz, M., Institut Pasteur, Paris, France: A technique for integrating any DNA fragment into the chromosome of *E. coli*.

Shub, D.A., Casna, N.J., Dept. of Biological Sciences, State University of New York, Albany: Expression of β-galactosidase fusion proteins in bacteriophage T4.

Groisman, E., Casadaban, M., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: In vivo cloning of DNA from enterobacteriaceae with mini-Mu bacteriophage containing plasmid replicons.

Weinstock, G., Bremer, E., NCI, Frederick Cancer Research Facility, Frederick, Maryland: λ *plac*Mu phages.

Van Gijsegem, F., Toussaint, A., Schoonejans, E., Piecq, M., Laboratoire de Génétique, Université libre de Bruxelles, Belgium: In vivo cloning of pectinase and cellulase genes of *E. chrysanthemi* using RP4::mini-Mu plasmids.

SESSION 10 REGULATION V

Schauer, A.T., Eades, L.J., Bigelow, B.D., Friedman, D.I., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Host elements of the λ *N* antitermination reaction—Studies of the *nusA* and *nusE* genes.

Franklin, N.C., Howard Hughes Medical Institute, Dept. of Biology, University of Utah, Salt Lake City: Relationships deduced from

completed DNA sequences of phages λ, φ21, and P22 in early expressed genome regions relevant to the *N* transcription-antitermination function.

Zuber, M., Court, D., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Transcription termination and antitermination at the t_{R1} terminator of bacteriophage λ.

Brown, A.L., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Effect of site-specific mutations on the antitermination activity of the *boxA-nutR* sequence of coliphage λ.

Hasan, N., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Translation of the *nutL* site and its effect on the *N* mediated antitermination function of phage λ.

Roberts, J.W., Grayhack, E.J., Yang, X., Goliger, J.A., Section of Biochemistry, Molecular, and Cell Biology, Cornell University, Ithaca, New York: Mechanism of late gene regulation by phage λ gene *Q*—Activity of an antiterminator in vitro.

Wolska, K., University of Connecticut, Farmington: An analysis of *E. coli* "*nus*" function by in vitro complementation.

Ghosh, B., University of Connecticut, Farmington: Formation of the antitermination apparatus—Role of *nusA* and *nusB* proteins.

Sparkowski, J., University of Connecticut, Farmington: The "*ran*" alleles of RNA polymerase—A class of *rpoB* mutations that restore antitermination in the *nusA*1 strain.

Somasekhar, G., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Structure and function of deletion mutants in the *Q*-utilization (*gut*) site required for transcription antitermination in coliphage λ.

Holben, W.E., Morgan, E.A., Dept. of Biology, State University of New York, Buffalo: Antitermination of transcription from an *E. coli* rRNA promoter.

Kuroda, M.I., Yanofsky, C., Dept. of Biological Sciences, Stanford University, California: Nuclease cleavage analysis of the transcript secondary structures that form in the *trp* leader transcript.

Engelberg-Kulka, H., Kopelowitz, J., Schoulaker-Schwarz, R., Dept. of Molecular Biology, Hebrew University Hadassah Medical School, Jerusalem, Israel: UGA readthrough—A new regulatory mechanism involved in attenuation of the tryptophan operon of *E. coli*.

Lynn, S.P., Donohue, T.J., Kasper, L.M., Gardner, J.F., Dept. of Microbiology, University of Illinois, Urbana: Effects of mutations in the RNA stem and polyuridine tract on transcription termination and pausing in the threonine operon of *E. coli*.

Postle, K., Godd, R.F., Dept. of Microbiology and Molecular Genetics, University of California, Irvine: The *tonB* terminator also terminates transcription from an opposing gene.

McKenney, K., NCI, National Institutes of Health, Bethesda, Maryland: Analysis of the "simple" λ t_0 transcription-termination site.

Galloway, J.L., Platt, T., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: ρ-Dependent transcription termination of the *E. coli* tryptophan operon.

SESSION 11 POST-TRANSCRIPTION

Shimamoto, N., Ikushima, N., Horie, K., Utiyama, H., Life Science Group, Hiroshima University, Japan: Regulation of free form of single-stranded DNA-binding protein in *E. coli*.

Wu, T.,[1] Liao, S.,[2] McClure, W.R.,[2] Susskind, M.M.,[1] [1]Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester; [2]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Negative control of P22 antirepressor synthesis by a small antisense RNA.

Young, F.S., Reid, J., Genentech, Inc., South San Francisco, California: Stabilization of cloned mRNAs by sequences from the *E. coli* lipoprotein gene.

Berkhout, B., Kastelein, R.A., van Duin, J., Dept. of Biochemistry, University of Leiden, The Netherlands: Translational interference at overlapping reading frames in mRNA.

Davidson, A., Gold, M., Dept. of Medical Genetics, University of Toronto, Canada: Regulation and structure of the bacteriophage λ terminase genes.

Sampson, L., Casjens, S., Dept. of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: Translation initiation controls the relative rates of expression of the λ late genes.

Belfort, M., Chu, F., Maley, G., Ehrenman, K., Pedersen-Lane, J., Maley, F., Center for Laboratories and Research, New York State Department of Health, Albany: Expression of the interrupted T4 thymidylate synthase gene.

Gottesman, S., Torres-Cabassa, A., Maurizi, M., Trisler, P., NCI, National Institutes of Health, Bethesda, Maryland: Genetics of protein degradation in *E. coli*—*lon* mutations.

Gimble, F.S., Sauer, R.T., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: λ repressor mutants having a noninducible (*ind*$^-$) phenotype.

Froshauer, S., Beckwith, J., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Analysis of the localization and the regulation of the *malF*-gene product.

Cheng, H., MacFarlane, L., Hoyt, M.A., Echols, H., Dept. of Molecular Biology, University of California, Berkeley: The regulatory function of *hflA*—Controls on the cII protein of phage λ and on bacterial proteins.

Manning, F.,[1] Rosas, D.,[1] Christiansen, G.,[2] Svanborg-Eden, C.,[3] Wilkinson, B.,[4] Rubin, B.,[5] Hanson, P.,[6] O'Brien, T.,[4] Goldstein, R.,[1] [1]Harvard University, Cam-

bridge, Massachusetts; [2]University of Aarhus, Denmark; [3]Goteborg University, Sweden; [4]Brigham and Women's Hospital, Boston, Massachusetts; [5]Massachusetts General Hospital, Boston; [6]Cambridge Research Laboratory, Massachusetts: The *pap* operon of urogenic *E. coli*—Cloning, expression, function, and epidemiology.

SESSION 12 PLASMIDS AND EXCLUSION

Chattoraj, D., Abeles, A., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Control of P1 plasmid replication.

Austin, S., Abeles, A., Frederick Cancer Research Facility, Frederick, Maryland: The partition functions of P1, P7, and F mini-plasmids.

Fowler, T., Thompson, R., Institute of Virology, University of Glasgow, Scotland: Control points in F plasmid conjugation.

Lupski, J., Ozaki, L., Projan, S., Godson, G.N., Dept. of Biochemistry, New York University Medical Center, New York: A temperature-dependent pBR322 copy-number mutant due to a Tn*5* position effect.

Novick, R.P., Highlander, S.K., Projan, S.J., Kumar, C.C., Carleton, S., Dept. of Plasmid Biology, Public Health Research Institute of the City of New York, New York: Replication of plasmid pT181 is regulated by two countertranscripts.

Sun, T.P., Webster, R.E., Dept. of Biochemistry, Duke University, Durham, North Carolina: Bacterial mutants defective in phage fl infection.

Molineux, I.J., Schmitt, M., Kearney, C., Condreay, J.P., Dept. of Microbiology, University of Texas, Austin: A basis for F-mediated restriction of phage T7.

Bross, P., Bürger, B., Bussmann, K., Rasched, I., Fakultat für Biologie, Universität Konstanz, Federal Republic of Germany: Analysis of the effects of sequences of the fd-phage gene III on the host conjugative system.

Modern Approaches to Vaccines

September 12–September 16

ARRANGED BY

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

229 participants

This second meeting on Modern Approaches to Vaccines included presentations that described the identification, molecular cloning, and sequence analysis of genes coding for antigens that are specific for each stage of the life cycle of the malaria parasite. Many problems remain to be solved in the development of a malaria vaccine, but it should be noted that several encouraging findings were described. Progress was also reported in the construction of viable bacterial deletion mutants that lack the sequences for exotoxin. Other encouraging events in the development of modern bacterial vaccines included the synthesis of antigenic peptides that correspond to active regions of bacterial toxins or adhesion proteins.

The meeting was also concerned with the rapidly moving area of viral vaccines. Significant progress was reported for candidate vaccinia-viral gene recombinant vaccines as well as viral host range and reassortant vaccines. In addition, a number of presentations described advances in the current understanding of protective antigenic sites on viral surface proteins. Finally, the molecular basis for attenuation of one of the major licensed live-virus vaccines (i.e., poliovirus vaccine) now appears to be coming into focus.

At the conclusion of the conference the organizers and members of the Cold Spring Harbor Laboratory staff decided that further annual meetings would be

held during the next 5 years in order to bring together scientists from different disciplines who might interact to accelerate progress in vaccine development.

Funding for this meeting was provided in part by Johnson & Johnson Biotechnology Center, Inc.; Wellcome Biotechnology Ltd.; and the following divisions of the National Institutes of Health: Fogarty International Center and the National Institute of Allergy and Infectious Diseases.

SESSION 1 CHEMISTRY OF MALARIA SURFACE ANTIGENS

Chairperson: V. Nussenzweig, New York University Medical Center, New York

Miller, L.H., Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland: An overview on research toward a vaccine against malaria.

Nussenzweig, V., New York University Medical Center, New York: Presence of multiple epitopes on the CS protein of the monkey malaria parasite *P. knowlesi*.

Dame, J.B.,[1] McCutchan, T.F.,[1] Hockmeyer, W.T.,[2] Miller, L.H.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Immunology, Walter Reed Army Institute of Research, Washington, D.C.: Structure of the gene encoding the immunodominant surface antigen of the sporozoite of the human malaria parasite *P. falciparum*.

Cowman, A.F., Saint, R.B., Coppel, R.L., Brown, G.V., Favaloro Dahl, J., Langford, C.J., Crewther, P., Stahl, H.-D., Bianco, E.A., Mitchell, G.F., Kemp, D.J., Anders, R.F., Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia: Repeat structures in protein antigens of asexual erythrocyte stages of *P. falciparum*.

Cohen, S.,[1] Deans, J.A.,[1] Mitchell, G.H.,[1] Thomas, A.W.,[1] Waters, A.P.,[1] Alderson, T.,[2] [1]Dept. of Chemical Pathology, Guy's Hospital Medical School, London; [2]Laboratory of Molecular Biology, Cambridge, England: Putative protective antigens of the erythrocyte stage of *P. knowlesi*.

McGarvey, M.J., Perrin. L., Mach, B., Dept. of Microbiology, University of Geneva Medical School, Switzerland: Cloning and expression in *E. coli* of genes corresponding to specific merozoite proteins expressed late in the asexual blood cycle of *P. falciparum*.

Holder, A.A., Odink, K.G., Lockyer, M.J., Freeman, R.R., Dept. of Molecular Biology, Wellcome Research Laboratories, Beckenham, England: A major surface antigen of *P. falciparum* merozoites—Studies on the protein and its gene.

SESSION 2 SURFACE ANTIGENS OF MALARIA, TRYPANOSOMES, AND SCHISTOSOMES

Chairperson: L.H. Miller, National Institutes of Health, Bethesda, Maryland

Kumar, N., Graves, P.M., Rener, J., Kaushal, D.C., Grotendorst, C., Miller, L.H., Carter, R., Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland: Target antigens of transmission-blocking immunity of malaria parasites.

Langsley, G.,[1] Mercereau, O.,[1] Konen, M.,[2] Scherf, A.,[2] Pereira da Silva, L.,[1] Muller-Hill, B.,[2] [1]Institut Pasteur, Paris, France; [2]Institut für Genetik, Cologne, Federal Republic of Germany: Identification of *P. falciparum* antigens by differential antibody screening of a genomic expression library.

Wahlgren, M.,[1,2] Perlmann, H.,[1] Wåhlin, B.,[1] Berzins, K.,[1] Carlsson, J.,[1] Björkman, A.,[2] McNicols, L.A.,[3] Dame, J.B.,[3] McCutchan, T.F.,[3] Perlmann, P.,[1] [1]Dept. of Immunology, University of Stockholm; [2]Dept. of Infectious Diseases, Karolinska Institutet, Roslagstulls Hospital, Stockholm, Sweden; [3]NIAID, National Institutes of Health, Bethesda, Maryland: Pf 155, a putative vaccine candidate for protection against asexual stages of *P. falciparum* malaria.

Cross, G.A.M., Rockefeller University, New York, New York: Variant surface antigens of *T. brucei*.

Boothroyd, J.C., Campbell, D.A., Sutton, R.E., Dept. of Medical Microbiology, Stanford University School of Medicine, California: Expression of surface-antigen genes in *T. brucei* involves a novel system of discontinous transcription.

Nogueira, N., Lizardi, P., Lerner, T., Di Giovanni, L., Gonzalez, A., Rockefeller University, New York, New York: Expression in *E. coli* of cDNA clones encoding surface antigens of *T. cruzi*—Insect and mammalian stages.

Snary, D., Wellcome Research Laboratories, Beckenham, England: Cell-surface glycoproteins on *T. cruzi*.

L. Miller

Sher, A.,[1] James, S.,[2] Correa-Oliveria, R.,[1] Lanar, D.,[1] Pearce, E.,[1] [1]Laboratory of Parasitic Diseases, NIAID, Bethesda, Maryland; [2]Dept. of Medicine, George Washington University School of Medicine, Washington, D.C.: Immunologic and immunochemical analysis of an attenuated vaccine against experimental schistosomiasis.

SESSION 3 NEW DIRECTIONS AND PRIORITIES; BACTERIAL VACCINES

Chairperson: M. So, Research Institute of Scripps Clinic, La Jolla, California

Warren, K., Rockefeller University, New York, New York: The great neglected diseases of mankind.

Beale, A.J., Wellcome Biotechnology Ltd., Beckenham, England: Perspective of a traditional manufacturer.

Petricciani, J.C., Center for Drugs and Biologics, Food and Drug Administration, Bethesda, Maryland: Summary of a workshop on the use of abnormal cells to produce new products.

Gotschlich, E.C., Blake, M.S., Laboratory of Bacteriology and Immunology, Rockefeller University, New York, New York: Structural and functional properties of gonococcal porins.

So, M., Hagblom, P., Segal, E., Deal, C., Getzoff, E., Trainer, J., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Genetic and structural studies of *N. gonorrheae* pilus antigenic variation.

Rothbard, J.B., Schoolnik, G.K., Depts. of Medicine and Medical Microbiology, Stanford University School of Medicine, California: Approaches toward a gonococcal vaccine.

Houghten, R.A.,[1] Klipstein, F.A.,[2] Lerner, R.A.,[1] Wright, P.E.,[1] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California; [2]Dept. of Medicine, University of Rochester, New York: Synthetic vaccine against *E. coli* heat stable enterotoxin.

SESSION 4 BACTERIAL VACCINES AND IMMUNOLOGY

Chairperson: M. Oldstone, Research Institute of Scripps Clinic, La Jolla, California

Miller, T.,[1] Auerbach, J.,[2] Peetz, R.,[1] Reed, P.,[2] Kost, T.,[1] Brown, A.,[1] Rosenberg, M.,[2] [1]Norden Laboratories, Lincoln, Nebraska; [2]Dept. of Molecular Genetics, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania: High-level expression of *E. coli* heat-labile toxin by a λ lysogen.

Fields, P., Heffron, F., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Genetic analysis of intracellular parasitism and virulence in *S. typhimurium*.

Mekalanos, J.,[1] Goldberg, I.,[1] Miller, V.,[1] Pearson, G.,[1] Swartz, D.,[1] Taylor, R.,[1] Harford, N.,[2] Groyne, F.,[2] Simoen, E.,[2] deWilde, M.,[2] [1]Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; [2]Molecular Genetics Division, Smith Kline-RIT, Rixensart, Belgium: Genetic construction of cholera vaccine prototypes.

E. Norrby, R. Lerner

Kaper, J.B., Levine, M.M., Lockman, H.A., Baldini, M.M., Black, R.E., Clements, M.L., Center for Vaccine Development, University of Maryland School of Medicine, Baltimore: Development and testing of a recombinant live oral colera vaccine.

Reiser, J., Friedman, R.L., Germanier, R., Swiss Serum and Vaccine Institute, Berne, Switzerland: Molecular cloning of *B. pertussis* toxin and filamentous HA genes.

Ladant, D.,[1] Brézin, C.,[2] Guiso, N.,[1] Michelson, S.,[3] Alonso, J.M.,[2] Bârzu, O.,[1] Ullmann, A.,[1] [1]Unité de Biochimie des Régulations Cellulaires, [2]Unité d'Ecologie Bacterienne, [3]Unité de Virologie Médicale, Institut Pasteur, Paris, France: Purification and characterization of extracellular adenylate cyclase of *B. pertussis*.

Mallett, C.P., Everhart, D.L., Dept. of Microbiology, New York University Dental Center, New York: Mono-

clonal antibody analysis of a ribosome-derived immunogen from *S. mutans*.

Ahmed, R., Southern, P., Oldstone, M.B.A., Dept. of Immunology, Research Institute of Scripps Clinic, La Jolla, California: Viral genes, cytotoxic T lymphocytes and immunity.

SESSION 5 LESSONS FROM SMALLPOX ERADICATION AND USE OF VACCINIA RECOMBINANTS

Chairperson: **F. Fenner,** John Curtin School of Medical Research, Australian National University, Canberra, Australia

Fenner, F., John Curtin School of Medical Research, Australian National University, Canberra: Lessons from the smallpox eradication campaign.

Paoletti, E., Perkus, M., Piccini, A., Wos, S., Lipinskas, B.R., Center for Laboratories and Research, New York State Department of Health, Albany, New York: Genetically engineered poxviruses.

Koprowski, H., Wiktor, T.J., Reagan, K., Macfarlan, R., Dietzschold, B., Wistar Institute, Philadelphia, Pennsylvania: New generation of rabies vaccines—Rabies glycoprotein gene recombinants, anti-idiotypic antibodies, and synthetic peptides.

Buller, R.M.L.,[1] Smith, G.L.,[1] Cremer, K.,[2] Notkins, A.L.,[2] Moss, B.,[1] [1]NIAID, [2]NIDR, National Institutes of Health, Bethesda, Maryland: Infectious vaccinia virus TK⁻ hybrid recombinants that express foreign genes are less virulent than wild-type virus in BALB/cByJ mice.

Cremer, K.,[1] Mackett, M.,[2] Wohlenberg, C.,[1] Notkins, A.L.,[1] Moss, B.,[2] [1]NIDR, [2]NIAID, National Institutes of Health, Bethesda, Maryland: Vaccinia virus recombinant that expresses the HSV-1 glycoprotein-D gene protects mice against lethal challenge by HSV-1 or HSV-2 and the establishment of a latent ganglionic infection by HSV-1.

Small, P.A., Jr.,[2] Smith, G.L.,[1] Moss, B.,[2] [1]Dept. of Immunology and Medical Microbiology, University of Florida, Gainesville; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Intranasal vaccination with recombinant vaccinia containing influenza HA prevents both influenza viral pneumonia and nasal infection—Intradermal vaccination prevents only viral pneumonia.

Geysen, H.M.,[1] Mason, T.J.,[1] Rodda, S.,[1] Meloen, R.H.,[2] Barteling, S.J.,[2] [1]Commonwealth Serum Laboratories, Parkville, Australia; [2]Central Veterinary Institute, Lelystad, The Netherlands: Amino acid composition of antigenic determinants—Implications for antigen processing by the immune system of animals.

Shih, M.-F.,[1] Tiollais, P.,[2] Roizman, B.,[1] [1]Kovler Viral Oncology Laboratories, University of Chicago, Illinois; [2]INSERM, Institut Pasteur, Paris, France: Herpes simplex virus as a vector for eukaryotic viral genes.

SESSION 6 POSTER SESSION

Amann, E., Bröker, M., Wurm, F., Research Laboratories of Behringwerke, Marburg, Federal Republic of Germany: Expression of HSV-1 glycoprotein-C antigens in *E. coli*.

Audibert, F.,[1] Jolivet, M.,[1] Gras-Masse, H.,[2] Tartar, A.,[2] Chedid, L.,[1] [1]CNRS, Institut Pasteur, Paris; [2]Chimie Organique, Faculté de Pharmacie, Lille, France: Construction of antimicrobial polyvalent synthetic vaccines active in saline.

Britton, P., Garwes, D.J., Bountiff, L., Millson, G.C., AFRC Institute for Research on Animal Diseases, Compton, England: Identification of the immunogenic antigen of porcine transmissible gastroenteritis virus.

Crainic, R., Blondel, B., Coste, M., Horaud, F., Virologie Médicale, Institut Pasteur, Paris, France: In-vitro-derived neurovirulant revertants of attenuated Sabin type-1 poliovirus.

Deufel, T., Vissing, H., Kofod, H., Lernmark, Å., Hagedorn Research Laboratory, Gentofte, Denmark: Antibodies against synthetic peptides recognize an HLA-class-II-positive subpopulation of human peripheral blood cells.

Fox, G.M., Hu, S., Bruszewski, J., Langley, D., Amgen, Inc., Thousand Oaks, California: Immunological response to porcine parvovirus proteins cloned and expressed in *E. coli*.

Guesdon, J.L.,[1] Bouges-Bocquet, B.,[2] Debarbouillé, M.,[3] Hofnung, M.,[2] [1]Unité d'Immunocytochimie, [2]Unité de Programmation Moléculaire et Toxicologie Génétique, [3]Unité de Virologie Moléculaire, Institute Pasteur, Paris, France: An enzymoimmunoassay allowing detection of *E. coli* cells expressing a given antigenic determinant at their surfaces.

Hackett, C.J., Hurwitz, J.L., Moller, C., Dietzschold, B., Heber-Katz, E., Gerhard, W., Wistar Institute, Philadelphia, Pennsylvania: Helper-T-cell (T_H) determinants of influenza HA—Immunization of mice with a synthetic decapeptide

F. Brown

M.A. Epstein, F. Prince

of an immunodominant region elicits T_H cells that recognize whole virus.

Hu, S.-L., Potts, D.E., Molecular Genetics, Inc., Minnetonka, Minnesota: Expression of antigenic determinants of bovine adenovirus hexon in *E. coli*.

Hu, S., Bruszewski, J., Smalling, R., Browne, J.K., Amgen, Inc., Thousand Oaks, California: Studies of TGEV spike protein gp195 expressed in *E. coli* and by a TGE-vaccinia virus.

Hudecz, F.,[1] Gönczöl, E.,[1] Pereira, L.,[2] Dietzschold, B.,[1] Plotkin, S.,[1] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Viral and Rickettsial Disease Laboratory, Dept. of Health Services, Berkeley, California: Immune responses to envelope proteins of HCMV.

Keller, P.M., Ellis, R.W., Lowe, R.S., Zivin, R.A., Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania: Genetic mapping of the major glycoprotein gene gC of varicella-zoster virus.

Kennedy, R.C., Henkel, R.D., Frenchiek, P.J., Eichberg, J.W., Dreesman, G.R., Dept. of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas: Use of anti-idiotype antibodies to modulate the immune response in HBV chronic carrier chimpanzees.

Kieny, M.-P.,[1] Lathe, R.,[1] Drillien, R.,[2] Wiktor, T.,[3] Koprowski, H.,[3] Lecocq, J.-P.,[1] [1]Transgène S.A.; [2]Institut de Virologie, Strasbourg, France; [3]Wistar Institute, Philadelphia, Pennsylvania: Effective immunization against rabies using a vaccinia-rabies recombinant virus expressing the surface glycoprotein.

Lenstra, J.A., van der Zeijst, B.A.M., Niesters, H.G.M., de Groot, R.J., ter Haar, R.J., Jacobs, C.E., Rottier, P.J.M., Spaan, W.J.M., Horzinek, M.C., Institute of Virology, State University, Utrecht, The Netherlands: Toward synthetic vaccines against coronaviruses.

Levings, R.L., National Veterinary Services Laboratories, Ames, Iowa: Identification of a neutralization epitope and a complementary diagnostic antigen of some inactivated bovine herpesvirus vaccines.

Manavathu, E., Hasnain, S.E., Liu, A.P., Bouma, C., Zwaagstra, J., Leung, W.C., Dept. of Medicine, University of Alberta, Edmonton, Canada: Construction of HSV-1 and HSV-2 glycoproteins, their hybrids, and subfragments in yeast expression vector.

Mazigh, D.A., Chalvignac, M.A., Quilicci, M.L., Mollaret, H.H., Institut Pasteur, Ecologie Bactérienne, Paris, France: A 36,000-D protein coded by the 47-MD virulence-associated plasmid of *Y. enterocolitica* implicated in the immunogenicity against *Y. pertis*.

Minor, P.D.,[1] Evans, D.M.A.,[1] Schild, G.C.,[1] Almond, J.W.,[2] [1]NIBSC, London; [2]University of Leicester, England: Antigenic sites in the neutralization of poliovirus.

Morrissey, P.,[1] Russel, R.,[2] Novotny, P.,[1] Dougan, G.,[1] [1]Wellcome Biotechnology Ltd., Beckenham, [2]Royal College of Surgeons, Downe, England: Molecular approaches to the development of safe, acellular bacterial vaccines.

Neurath, A.R.,[1] Kent, S.B.H.,[2] Strick, N.,[1] [1]Lindsley F. Kimball Research Institute of the New York Blood Center, New York; [2]California Institute of Technology, Pasadena: Immune response to pre-*S*-gene-coded determinants of HBV.

Parry, N.R.,[1] Ouldridge, E.J.,[1] Barnett, P.V.,[1] Rowlands, D.J.,[1] Brown, F.,[1] Bittle, J.L.,[2] Houghten, R.A.,[2] Lerner, R.A.,[2] [1]Wellcome Biotechnology Ltd., Pirbright, England; [2]Research Institute of Scripps Clinic, La Jolla, California: Identification of neutralizing epitopes of FMDV.

Petteway, S.R., Jr.,[1] Ivanoff, L.A.,[1] Ray, J.,[2] Korant, B.D.,[1] [1]Dept. of Central Research & Development, E.I. du Pont de Nemours, Wilmington; [2]Dept. of Biomedical Products, E.I. du Pont de Nemours, Glasgow, Delaware: Expression of rubella and poliovirus proteins in *E. coli*.

Robertson, J.S.,[1] Naeve, C.W.,[2] Webster, R.G.,[2] Bootman, J.S.,[1] Newman, R.,[1] Schild, G.C.,[1] [1]National Institute for Biological Standards and Control, London, England; [2]St. Jude Children's Research Hospital, Memphis, Tennessee: Molecular changes in the HA of influenza virus associated with adaptation of the virus to growth in eggs.

Siddiqui, A., Bulla, G., Dept. of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver: Expression of hepatitis B surface antigen in mammalian cells.

Southern, P., Singh, M., Oldstone, M.B.A., Buchmeier, M.J., Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Application of synthetic peptides to gene mapping and protein characterization with lymphocytic choriomeningitis virus.

Timmins, J.G., Rea, T.J., Post, L.E., Molecular Biology Unit, Upjohn Company, Kalamazoo, Michigan: Cloning, sequencing, and bacterial expression of the excreted glycoprotein of PRV.

Weijer, W.J.,[1] Kerling, K.E.T.,[2] Hoogerhout, P.,[2] Bloemhoff, W.,[2] Geerligs, H.,[1] Wilterdink, J.B.,[1] Popken-Boer, T.,[1] Welling-Wester, S.,[1] Welling, G.W.,[1] [1]Laboratorium voor Medische Microbiologie, Groningen; [2]Laboratorium voor Organische Chemie, Leiden. The Netherlands: Synthetic peptides as basis for a herpesvirus vaccine.

Whetstone, C.A., National Veterinary Services Laboratories, Ames, Iowa: Mechanisms of adenovirus neutralization as defined with monoclonal antibodies.

Wright, S.,[1,2] Bennett, D.,[1] [1]Viral Oncology Laboratory, VA Medical Center; [2]Depts. of Medicine and Cellular, Viral, and Molecular Biology, University of Utah School of Medicine, Salt Lake City, Utah: Potential avian RNA tumor virus vaccine.

SESSION 7 MAJOR ANTIGENIC SITES ON PROTECTIVE VIRAL ANTIGENS

Chairperson: R. Lerner, Research Institute of Scripps Clinic, La Jolla, California

Kent, S.B.H.,[1] Neurath, A.R.,[2] Strick, N.,[2] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Lindsley F. Kimball Research Institute of the New York Blood Center, New York: Location and chemical synthesis of a pre-S-gene-coded immunodominant epitope of HBV.

Jameson, B.A.,[1] Bonin, J.,[1] Murray, M.,[1] Kew, O.,[2] Wimmer, E.,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Molecular Virology Branch, Center for Infectious Diseases, Atlanta, Georgia: Studies of poliovirus neutralization.

Francis, M.J.,[1] Fry, C.M.,[1] Rowlands, D.J.,[1] Brown, F.,[1] Bittle, J.L.,[2] Houghten, R.A.,[2] Lerner, R.A.,[2] [1]Wellcome Biotechnology Ltd., Beckenham, England; [2]Scripps Clinic, La Jolla, California: Priming with peptides of FMDV.

Emini, E.A.,[1] Boger, J.,[2] Hughs, J.V.,[1] Mitra, S.W.,[3] Linemayer, D.L.,[3] Depts. of [1]Virus and Cell Biology, [2]Medicinal Chemistry, Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania; [3]Dept. of Biochemical Genetics, Merck, Sharp & Dohme Laboratories, Rahway, New Jersey: Priming of an anti-HAV antibody response by poliovirus-specific synthetic peptides—Localization of potential HAV-neutralizing antigenic sites.

Nunberg, J.H.,[1] Gilbert, J.H.,[1] Rodgers, G.,[1] Snead, R.M.,[1] Nitecki, D.,[1] Winston, S.,[2] [1]Cetus Corporation, Emeryville, California; [2]Syngene Products and Research, Fort Collins, Colorado: Localization of a determinant of virus neutralization of FeLV envelope protein gp70.

Dietzschold, B.,[1] Heber-Katz, E.,[1] Hudecz, F.,[1] Hollosi, M.,[2] Fasman, G.,[2] Eisenberg, R.J.,[3] Cohen, G.H.,[3] [1]Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania; [2]Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts; [3]University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania: Structure-function relationship in immunogenic synthetic HSV peptides.

Gerin, J.L.,[1] Purcell, R.H.,[2] Lerner, R.A.,[3] [1]Georgetown University, Rockville; [2]NIAID, National Institutes of Health, Bethesda, Maryland; [3]Research Institute of Scripps Clinic, La Jolla, California: A search for protective epitopes of the hepatitis B surface antigen.

SESSION 8 MAJOR ANTIGENIC SITES ON PROTECTIVE VIRAL ANTIGENS AND MOLECULAR BASIS FOR ATTENUATION OF POLIOVIRUSES

Chairperson: F. Brown, Animal Virus Research Institute, Pirbright, England.

Diamond, D.C., Jameson, B.A., Wimmer, E., Dept. of Microbiology, State University of New York, Stony Brook: Antibody-resistant variants of poliovirus type 1—Capsid structure, antibody binding, and neutralization are complex phenomena.

Baroudy, B.M.,[1] Ticehurst, J.R.,[1] Miele, T.,[1] Maizel, J.V.,[2] Purcell, R.H.,[1] Feinstone, S.M.,[1] [1]NIAID, [2]NICHD, National Institutes of Health, Bethesda, Maryland: Sequence analysis of HAV cDNA coding for the capsid region and its application to vaccine development.

Hughes, J., Bennett, C., Stanton, L., Linemeyer, D., Mitra, S., Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania: HAV structural proteins—Sequencing and ability to induce virus-neutralizing antibody responses.

Patzer, E.,[1] Gregory, T.,[2] Nakamura, G.,[1] Simonsen, C.,[3] Hershberg, R.,[2] Levinson, A.,[3] Depts. of [1]Vaccine Development, [2]Process Development, [3]Molecular Biology, Genetech, Inc., South San Francisco, California: Immunogenic potency of recombinant HBV from cell culture.

Valenzuela, P., Coit, D., Kuo, G., Medina-Selby, M.A., Graves, P.V., Chiron Research Laboratories, Chiron Corporation, Emeryville, California: Synthesis and assembly in yeast of modified HBsAg particles.

Almond, J.W.,[1] Westrop, G.D.,[1] Stanway, G.,[1] Cann, A.J.,[1] Minor, P.D.,[2] Evans, D.M.A.,[1] Schild, G.C.,[2] [1]Dept. of Microbiology, University of Leicester, [2]NIBSC, London, England: Molecular basis of attenuation in the Sabin type-3 poliovirus vaccine.

Omata, T.,[1] Kohara, M.,[2] Abe, S.,[2] Itoh, H.,[2] Komatsu, T.,[3] Arita, M.,[3] Semler, B.L.,[5] Wimmer, E.,[5] Kuge, S.,[2] Kameda, A.,[4] Nomoto, A.,[4] [1]Dept.of Public Health, School of Pharmaceutical Sciences, Kitasato University, [2]Japan Poliomyelitis

B. Chanock, R.M. Chanock, M.B.A. Oldstone

Research Institute, [3]Dept. of Enteroviruses, National Institute of Health, [4]Dept. of Microbiology, Faculty of Medicine, University of Tokyo, Japan; [5]Dept. of Microbiology, School of Medicine, State University of New York, Stony Brook: Construction of recombinant viruses between Mahoney and Sabin strains of type-1 poliovirus and their biological characteristics.

SESSION 9 GLYCOPROTEIN ANTIGENS AND VIRULENCE OF ENVELOPED VIRUSES

Chairperson: R. Chanock, National Institutes of Health, Bethesda, Maryland

Epstein, J., Qi, Y., Quinnan, G., Center for Drugs and Biologics, Food and Drug Administration, Bethesda, Maryland: Immunogenic structural proteins of CMV.

Collins, P.L.,[1] Wertz, G.W.,[2] [1]Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [2]University of North Carolina School of Medicine, Chapel Hill: Nucleotide sequences of six mRNAs of human RS virus.

Paterson, R.G., Hiebert, S.W., Harris, T.J.R., Lamb, R.A., Dept. of Biochemistry, Northwestern University, Evanston, Illinois: Fusion protein and hemagglutinin-neuraminidase protein of the paramyxovirus SV5—Nucleotide sequence derived from mRNAs.

Shaw, M.W., Choppin, P.W., Rockefeller University, New York, New York: Studies on the synthesis and antigenicity of the NB glycoprotein of influenza-B virus.

Epstein, M.A., Dept. of Pathology, University of Bristol Medical School, England: Prevention of EBV-associated malignant diseases.

Miller, G.,[1,2,3] Heston, L.,[2] Rabson, M.,[2] Countryman, J.,[3] Jenson, H.,[1] Depts. of [1]Pediatrics, [2]Epidemiology and Public Health, [3]Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: EBVs that do not immortalize lymphocytes nor remain latent. tent.

Maassab, H.F., DeBorde, D.C., Donabedian, A.M., Smitka, C.W., School of Public Health, University of Michigan, Ann Arbor: Development of cold-adapted "master" strains for type-B influenza virus vaccines.

SESSION 10 MOLECULAR BASIS FOR ATTENUATION OF VIRUSES

Chairperson: R. Purcell, National Institutes of Health, Bethesda, Maryland

Cox, N.J.,[1] Naeve, C.W.,[2] Kitame, F.,[3] Kendal, A.P.,[1] [1]WHO Collaborating Center for Influenza, Centers for Disease Control, Atlanta, Georgia; [2]Dept. of Virology, St. Jude Children's Hospital for Research, Memphis, Tennessee; [3]Dept. of Bacteriology, Yamagata University, Japan: Sequence changes in genes of the attenuated influenza A vaccine donor strain, A/Ann Arbor/6/60.

Buckler-White, A.,[1] Naeve, C.,[2] Murphy, B.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]St. Jude Children's Hospital, Memphis, Tennessee: Characterization of the genes involved in host range restriction of avian influenza A viruses in primates.

Thompson, R.L.,[1,2] Stevens, J.G.,[1] Wagner, E.K.,[2] Devi, G.B.,[1] [1]University of California School of Medicine, Los Angeles; [2]Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Genetic control of herpes simplex virus neurovirulence.

Kapikian, A.Z.,[1] Midthun, K.,[1] Levine, M.M.,[2] Clements, M.L.,[2] Dolin, R.,[3] Wright, P.F.,[4] Belshe, R.B.,[5] Anderson, E.L.,[5] Hoshino, Y.,[1] Flores, J.,[1] Wyatt, R.G.,[1] Askaa, J.,[1] Glass, R.I.,[1] Nakagomi, O.,[1] Nakogomi, T.,[1] Potash, L.,[6] Chanock, R.M.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]University of Maryland, Baltimore; [3]University of Rochester, New York; [4]Vanderbilt University, Nashville, Tennessee; [5]Marshall University, Huntington, West Virginia; [6]Flow Labs Inc., McLean, Virginia: Rhesus rotavirus strain MMU 18006—Candidate vaccine for humans.

Vesikari, T.,[1] Isolauri, E.,[1] Delem, A.,[2] D'Hondt, E.,[2] André, F.E.,[2] [1]Dept. of Clinical Sciences, University of Tampere, Finland; [2]Biological Division, Smith Kline-RIT, Rixensart, Belgium: Protection of infants against human rotavirus diarrhea after vaccination with live attenuated bovine (NCDV) rotavirus strain RIT 4237.

Summary: **E. Norrby,** Karolinska Institute, Stockholm, Sweden

Growth Factors and Transformation

September 19–September 23

ARRANGED BY

James Feramisco, Cold Spring Harbor Laboratory
Brad Ozanne, University of Dallas, Texas
Charles Stiles, Dana-Farber Cancer Institute

208 participants

The rapidly accumulating evidence of the intimate connection between cellular growth factors and transformation provided the impetus as well as the focus for organizing the second in the series of meetings devoted to the cancer cell. Investigators from various disciplines, including cell biologists, molecular biologists, and tumor virologists, came together to present their latest findings in these converging fields. Topics covered at the meeting included oncogenes, kinases, lymphokines, PDGF, EGF, TGF, insulin, and a concluding session that explored promising lines of future research. Among the highlights of the conference were: (1) the correlation of the expression of the c-*fos* and c-*myc* proto-oncogenes with the stimulation of cells by growth factors; (2) the involvement of the *ras* oncogene with adenylate cyclase in yeast; and (3) the demonstration that microinjection of the oncogenic form of *ras* protein into normal cells causes rapid proliferation of these cells without the need for growth factors.

This second Cancer Cells meeting was an exciting demonstration of the fact that this area of research has reached a detailed molecular level. The first proposals of the molecular mechanisms of the oncogenes *sis*, *erb-B*, and *ras* in cell proliferation have been stated, and it is clear that further research in the areas of growth factors and oncogenes will yield tremendous new information in the molecular biology of cancer. This series of meetings will certainly serve as a mainstay for the presentation of this work.

Funding for this meeting was supplied in part by the Cold Spring Harbor Cancer Center Grant from the National Cancer Institute, National Institutes of Health.

Introduction: **C. Stiles,** Dana-Farber Cancer Institute, Boston, Massachusetts

SESSION 1 ONCOGENES

Chairperson: S. Martin, University of California, Berkeley, California

Foulkes, J.G., Baltimore, D., Whitehead Institute for Biomedical Research. Cambridge, Massachusetts: Characterization of a tyrosyl-protein kinase encoded by Ab-MLV.

Feramisco, J.R.,[1] Fasano, O.,[1] Goldfarb, M.,[1] Wigler, M.,[1] Katama, T.,[1] Gross, M.,[2] Yokoyama, S.,[2] Rosenberg, M.,[2] Sweet, R.W.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Molecular Genetics, Smith, Kline and French Laboratories, Swedeland, Pennsylvania: Microinjection of the oncogene form of the human Ha-*ras* (T24) protein results in rapid proliferation of quiescent cells.

Cooper, G.M., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Structure and function of *ras* and *Blym* oncogenes.

Wigler, M.,[1] Kataoka, T.,[1] Powers, S.,[1] Toda, T.,[1] Fasano, O.,[1] Strathern, J.,[2] Broach, J.,[3] [1]Cold Spring Harbor Laboratory, New York; [2]Frederick Cancer Research Facility, Frederick, Maryland; [3]Dept. of Molecular Biology, Princeton University, New Jersey: Structure, genetics, and activation of yeast and mammalian *ras* genes.

Ruley, E.,[1] Moomaw, J.,[1] Garrels, J.,[1] Furth, M.,[2] Franza, R.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Cellular immortality is not sufficient for oncogenic transformation by the T24 Ha-*ras* oncogene.

Verma, I.M.,[1] Meijlink, F.,[1] Mitchell, R.,[1] Zokas, L.,[1] Schreiber, R.,[2] Curran, T.,[1] Miller, A.D.,[1] Van Bev-

C.D. Stiles

eren, C.,[1] [1]Molecular Biology and Virology Laboratory, Salk Institute, San Diego; [2]Scripps Clinic and Research Foundation, La Jolla, California: Oncogene *fos*—Structure and expression.

Ferguson, B.,[1] Krippl, B.,[2] Westphal, H.,[2] Jones, N.,[3] Rosenberg, M.,[1] [1]Dept. of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania; [2]NICHD, National Institutes of Health, Bethesda, Maryland; [3]Purdue University, West Lafayette, Indiana: Functional characterization of human adenovirus E1A proteins synthesized and purified from *E. coli*.

Lavu, S., Mushinski, J.F., Shen-ong, G.L.C., Potter, M., Reddy, E.P., NCI, National Institutes of Health, Bethesda, Maryland: Structural organization of the mouse *c-myb* locus and the mechanism of its rearrangement in mouse plasmacytoid lymphosarcomas.

SESSION 2 KINASES

Chairperson: J. Feramisco, Cold Spring Harbor Laboratory, New York

Cooper, J.A., Hunter, T., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Endogenous substrates for tyrosine protein kinases in growth factor-treated cells

J. Feramisco

Frackelton, A.R., Jr., Roger Williams General Hospital and Brown University, Providence, Rhode Island: Use of a monoclonal antibody to phosphotyrosine to characterize phosphotyrosyl proteins in cells transformed by Ab-MLV.

Rosner, M.R., McCaffrey, P.G., Friedman, B.-A., Massachusetts Institute of Technology, Cambridge: Modulation of growth factor action by tumor promoters and C kinase.

Parker, P.,[1] Downward, J.,[1] Earp, H.S.,[1] Gullick, W.,[1] Waterfield, M.,[1] Seeberg, P.,[2] Ullrich, A.,[2] [1]Imperial Cancer Research Fund Laboratories, London, England; [2]Genentech, Incorporated, South San Francisco, California: The structure and function of EGF receptor and its interaction with protein kinases A and C.

Macara, I.G.,[1] Marinetti, G.,[2] Livingston, J.N.,[3] Balduzzi, P.C.,[4] Depts. of [1]Radiation Biology and Biophysics, [2]Biochemistry, [3]Medicine, [4]Microbiology, University of Rochester School of Medicine and Dentistry, New York: Lipid phosphorylating activity associated with tyrosine kinases—A possible role for phosphatidylinositol turnover in transformation.

Goldberg, A.R.,[1] Wong, R.W.,[1] Tse-Dinh, Y.-C.,[2] [1]Rockefeller University, New York, New York; [2]Dept. of Central Research and Development, E.I. du Pont de Nemours and Company, Wilmington, Delaware: Properties of the major species of tyrosine protein kinase in rat liver—Effects of DNA topoisomerase activity.

Blenis, J., Sugimoto, Y., Biemann, H.-P., Erikson, R.L., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Control of cell proliferation and transformation by the RSV transforming gene product.

SESSION 3 POSTER SESSION

Bonin, P.D., Tebaldi, A.F., D'Alessandro, J.S., Singh, J.P., Collaborative Research Incorporated, Lexington, Massachusetts: Isolation of three physicochemically distinct IL-2 activities from human lymphocyte conditioned media.

Chen, M.-J., Weiss, R., Sathe, G., Henner, J., Young, P., Rosenberg, M., Dept. of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania: Expression of human *c-sis* gene in normal and tumor cells.

Dani, C., Blanchard, J.M., Piechaczyk, M., El Sabouty, S., Marty, L., Jeanteur, P., Université de Sciences et Techniques de Languedoc, Centre Paul Lamarque, Montpellier, France: Unstability of *c-myc* mRNA in normal and transformed human cells.

Dunn, A.R.,[1] Metcalf, D.,[2] Burgess, A.W.,[1] Gough, N.,[1] [1]Ludwig Institute for Cancer Research; [2]Walter and Eliza Hall Institute for Medical Research, Victoria, Australia: Molecular characterization of murine hematopoietic growth regulators.

Fine, L.G., Badie-Dezfooly, B., Lowe, A., Dept. of Medicine, University of California School of Medicine, Los Angeles: Induction of hypertrophy in a primary culture of renal proximal tubular cells by stimulation of Na^+-H^+ antiport.

Frick, K.K.,[1] Doherty, P.,[2] Scher, C.D.,[1] Liau, G.,[2] Ganguly, K.,[1] Gottesman, M.M.,[2] [1]Children's Hospital of Philadelphia, Pennsylvania; [2]NCI, National Institutes of Health, Bethesda, Maryland: PDGF, TPA, and retroviruses increase the accumulation of MEP RNA transcripts.

Poster Session

Gross, M.,[1] Yokoyama, S.,[1] Sathe, G.,[1] Rosenberg, M.,[1] Sweet, R.,[1] Fasano, O.,[2] Goldfarb, M.,[2] Wigler, M.,[2] Kamata, T.,[2] Feramisco, J.,[2] [1]Smith Kline and French Laboratories, Philadelphia, Pennsylvania; [2]Cold Spring Harbor Laboratory, New York: Purification and characterization of mammalian *ras* proteins produced in *E. coli*.

Haskell, J., Nissley, P., Rechler, M., Sasaki, N., Greenstein, L., Lee, L., National Institutes of Health, Bethesda, Maryland: Phosphorylation of the type-II insulinlike growth factor receptor in cultured cells.

Heath, J.K.,[1] Isacke, C.M.,[2] [1]Dept. of Zoology, University of Oxford, England; [2]Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: PC13 embryonal/carcinoma-derived growth factor.

Holmgren, A., Dept. of Chemistry, Karolinska Institutet, Stockholm, Sweden: Functions of thioredoxin in insulin and IGFs degradation, receptor modulation, and growth factor action.

Jonak, G.J., Anton, E.D., Fahey, D., Friedland, B., Cheng, Y.-S.E., Knight, E., Jr., Dept. of Central Research and Development, E.I. du Pont de Nemours and Company, Wilmington, Delaware: Interferon-mediated regulation of the *c-myc* oncogene expression in human cells and its relation to the inhibition of cell growth.

Kruijer, W., Cooper, J., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Induction of the *c-fos* gene by PDGF and FGF in quiescent fibroblasts.

Lanahan, A.,[1] Strazdis, J.,[2] Johnson, D.E.,[1] Kucherlapati, R.,[3] Bothwell, M.,[1] [1]Dept. of Molecular Biology, Princeton University, New Jersey; [2]Dept. of Biological Sciences, Oakland University, Rochester, Michigan; [3]Center for Genetics, University of Illinois, College of Medicine, Chicago: Transfer of the human EGF receptor gene in mouse L cells.

Lehrman, S.R.,[1] Felix, A.M.,[1] Heimer, E.,[1] Boublik, M.,[2] [1]Hoffman-La Roche Incorporated; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Synthesis and conformational analysis of a 37-amino-acid peptide form the amino terminus of p21.

Linzer, D., Lee, S.-J., Nathans, D., Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland: Expression of a growth-related member of the prolactin-growth hormone family.

Macdonald-Bravo, H., Bravo, R., European Molecular Biology Laboratory, Heidelberg, Federal Repubic of Germany: Growth factors induce the expression of the nuclear protein "cyclin" in quiescent 3T3 cells.

Magun, B.E., Finch, J.S., Dept. of Anatomy and Cell Biology, Oregon Health Sciences University, Portland: Effect of methylamine on induction of VL30 gene activity by EGF.

Marchildon, G.A.,[1] Casnellie, J.E.,[2] Walsh, K.A.,[1] Krebs, E.G.,[1] [1]University of Washington, Seattle; [2]University of Rochester, New York: The LSTRA tyrosine protein kinase is myristilated.

Moolenaar, W., Defize, L., van der Saag, P., de Laat, S., Hubrecht Laboratory, Utrecht, The Netherlands: Cytosolic free Ca^{++}, Na^{+}/H^{+} exchange and tyrosine phosphorylation in the action of growth factors.

Mowat, M., Bernstein, A., Mak, T.W., Benchimol, S., Ontario Cancer Research Institute and University of Toronto, Canada: Heterogeneity in cellular p53 antigen expression and tumorigenicity in F-MuLV induced cell lines.

Newman, M.J.,[1] Lane, E.A.,[1] Racker, E.,[2] [1]Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts; [2]Section of Biochemistry, Cornell University, Ithaca, New York: Isolation of a partially transformed derivative of normal rat kidney cells—Anchorage-independent growth induced by growth factors.

Norris, J.S.,[1] Syms, A.J.,[2] Smith, R.G.,[2] [1]Depts. of Medicine and Physiology, University of Arkansas School of Medical Sciences, Little Rock; [2]Depts, of Urology and Cell Biology, Baylor College of Medicine, Houston, Texas: Glucocorticoids inhibit production of *c-sis*, *c-myc* poly(A)$^{+}$ RNA and inhibit growth.

Patch, C.T., Akagi, K., Lewis, A.M., Levine, A.S., National Institutes of Health, Bethesda, Maryland: Differences in the mitogenic activity of hamster cells transformed by adenovirus 2 or SV40.

Piasecki, A., Paul, D., Institute of Toxicology, Hamburg University Medical School, Hamburg, Federal Republic of Germany: Growth control of rat hepatocytes by hormones + rat platelet mitogens + selection of phenotypic hepatocyte variants from DEN-treated rats in primary cultures.

Quarless, S.A., Dept. of Biological Sciences, Massachusetts Institute of Technology, Cambridge: Properties of a novel coupled deoxyribonuclease-protein kinase activity from HeLa cells.

Radding, W., Dept. of Cell Biology, New York University Medical School, New York, New York: A hypothesis about the purpose of autophosphorylation.

Rittling, S.R., Hirschhorn, R.R., Gibson, C.W., Aller, P., Yuan, Z.-A., Calabretta, B., Kaczmarek, L., Baserga, R., Fells Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania: Cell cycle-specific genes may be essential for cell cycle control.

Ross, A.H.,[1] Bothwell, M.,[2] Grob, P.,[2] Marano, N.,[2] Chao, M.,[3] Ernst, C.S.,[4] Elder, D.E.,[4] Koprowski, H.,[1] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Dept. of Biochemistry, Princeton University, New Jersey; [3] Dept. of Cell Biology, Cornell Medical School, New York, New York; [4]Dept. of Pathology, Hospital of the University of Pennsylvania, Philadelphia: Characterization of the nerve growth factor receptor using monoclonal antibodies.

Scheurich, P., Ucer, U., Pfizenmaier, K., Max-Planck-Society, Göttingen, Federal Republic of Germany: Influence of γ-IFN on human T cell proliferation via regulation of IL-2 receptor expression.

Seifert, R.A.,[1] Ross, R.,[1,2] Bowen-Pope, D.F.,[1] Depts. of [1]Pathology, [2]Biochemistry, University of Washington, Seattle: Demonstration of "cryptic" receptors for PDGF in transformed cells.

Simmen, F.A.,[1] Gope, M.L.,[1] Schultz, T.Z.,[1] Carpenter, G.,[2] Wright, D.A.,[3] O'Malley, B.W.,[1] [1]Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas; [2]Dept. of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee; [2]Dept. of Genetics, M.D. Anderson Hospital and Tumor Institute, Houston, Texas: Human EGF receptor proteins – Analysis of mRNA by translation in *Xenopus* oocytes and cDNA cloning.

Stacey, D.W.,[1] Kung, H.-F.,[2] [1]Roche Institute of Molecular Biology, [2]Dept. of Molecular Genetics, Roche Research Center, Nutley, New Jersey: Translation of NIH-3T3 cells by microinjected Ha-*ras* p21 protein.

Stoppelli, M.P., Verde, P., Locatelli, E., Blasi, F., International Institute Genetics and Biophysics, Naples, Italy: Induction of urokinase mRNA growth factors – Similarities with c-*myc* induction.

Tamanoi, F., Rao, M., Samiy, S., Walsh, M., Cold Spring Harbor Laboratory, New York: Enzymatic properties of yeast *RAS* proteins.

Tempst, P.,[1] Barth, R.,[1] Fors, L.,[1] Winoto, A.,[1] Aebersold, R.,[1] Woo, D.,[2] Antoniades, H.,[3] Hood, L.,[1] [1]California Institute of Technology, Pasadena; [2]University of California, Los Angeles; [3] Harvard School of Public Health, Boston, Massachusetts: Structural studies of the PDGF (*c-sis*) gene, the PDGF-receptor protein, and their possible involvement in the formation of specific tumors.

Wang, J.L., Hsu, Y.-M., Dept. of Biochemistry, Michigan State University, East Lansing: Isolation and characterization of a growth regulatory factor from 3T3 cells.

Willey, J.,[1] Moser, C.,[1] Moody, T.,[2] Harris, C.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biochemistry, George Washington University, Washington, D.C.: Characterization of receptors for the carboxy-terminal tetradecapeptide of gastrin-releasing peptide in normal human bronchial epithelial cells.

Yoakum, G.,[1] Lechner, J.,[1] Gabrielson, E.,[1] Korba, B.,[1] Malan-Shibley, L.,[1] Willey, J.,[1] Valerio, M.,[2] Shamsuddin, A.,[3] Trump, B.,[3] Harris, C.,[1] [1]NCI, National Institutes of Health, Bethesda; [2]Litton Bionetics, Rockville; [3]Dept. of Pathology, University of Maryland School of Medicine, Baltimore: Transformation of human bronchial epithelial cells transfected by H-*ras* oncogene.

SESSION 4 LYMPHOKINES

Chairperson: K.A. Smith, Dartmouth Medical School, Hanover, New Hampshire

Smith K.A., Dartmouth Medical School, Hanover, New Hampshire: Interleukin 2-receptor regulation of T-cell proliferation.

Lane, M.A., Stephens, H.A.F., Doherty, K.M., Tobin, M.B., Dept. of Pathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: TLym-I, a stage-specific transforming gene from T-cell lymphomas.

Ihle, J.N., Keller, J., Rein, A., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Regulation of the proliferation of normal and transformed cells by IL-3.

Waldmann, T.A., Leonard, W.J., Depper, J.M., Greene, W.C., NCI, National Institutes of Health, Bethesda, Maryland: The structure, function, and expression of the lymphocyte receptor for IL-2.

Wong, G.G., Wang, E.A., Kaufman, R.J., Clark, S.C., Genetics Institute, Boston, Massachusetts: Molecular cloning of a human GM-CSF cDNA.

SESSION 5 PDGF

Chairperson: H. Antoniades, Harvard School of Public Health, Boston, Massachusetts

Antoniades, H.N., Pantazis, P., Owen, A.J., Center for Blood Research, Harvard School of Public Health, Boston, Massachusetts: PDGF and cell transformation.

Pantazis, P.,[1] Morgan, D.A.,[2] Brodsky, I.,[2] Antoniades, H.N.,[1] [1]Center for Blood Research, Harvard School of Public Health, Boston, Massachusetts; [2]Dept. of Hematology and Oncology, Hahnemann University, Philadelphia, Pennsylvania: PDGF polypeptides in human megakaryocytelike cell lines.

Aaronson, S.A., Gazit, A., Igarashi, H., Chiu, I.-M., Tronick, S.R., Robbins, K.C., NCI, National Institutes of Health, Bethesda, Maryland: Activation of a normal human gene coding for a growth factor to one with transforming properties.

Deuel, T.F., Tong, B.D. Huang, J.S., Jewish Hospital at Washington Universtiy Medical Center, St.

B. Ozanne, C.S. Richards

Louis, Missouri: PDGF – Roles in growth of normal and SSV-transformed cells.

Williams, L.T.,[1] Garrett, J.S.,[1] Coughlin, S.R.,[1] Wang, J.Y.H.,[2] Frackelton, A.R.,[1] Daniel, T.O.,[1] [1]Howard Hughes Medical Institute, University of California, San Francisco; [2]Dept. of Biology, University of California, San Diego: Blockade of autocrine stimulation in SSV-transformed cells reverses downregulation of PDGF receptors.

Wang, J.Y.J.,[1] Williams, L.T.,[2] [1]Dept. of Biology, University of California, San Diego; [2]Howard Hughes Medical Institute, University of Califonia, San Francisco: Biological activity of *v-sis*-encoded protein produced in bacteria.

Bowen-Pope, D.F., DiCorleto, P.E., Harlan, J.M., Reidy, M.A., Ross, R., Schwartz, S.M., Seifert, R.A., Vogel, A., Walker, L.N., Dept. of Pathology, University of Washington, Seattle: Nonplatelet sources of PDGF – Possible roles in transformation, normal development, and response to injury.

Pledger, W.J., Herman, B., University of North Carolina, Chapel Hill: PDGF-stimulated cellular events initiate proliferation.

Armelin, M.C.S.,[1] Armelin, H.,[1] Cochran, B.H.,[2] Stiles, C.D.,[2] [1]Dept. of Bioquimica, Universidade de Sao Paulo, Brazil; [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Intracellular mediators of the mitogenic response to PDGF.

SESSION 6 EGF

Chairperson: **B. Ozanne,** University of Texas, Dallas, Texas

Ullrich, A.,[1] Coussens, L.,[1] Hayflick, J.S.,[1] Dull, T.J.,[1] Gray, A.,[1] Tam, A.W.,[1] Lee, J.,[1] Yarden, Y.,[2] Libermann, T.A.,[2] Schlessinger, J.,[2] Downward, J.,[3] Mayes, E.L.V.,[3] Whittle, N.,[3] Waterfield, M.D.,[3] Seeburg, P.H.,[1] [1]Dept. of Molecular Biology, Genentech, Incorporated, South San Francisco, California; [2]Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel; [3]Protein Chemistry Laboratory, Imperial Cancer Research Fund, London. England: Structure and expression of the human EGF receptor.

Gill, G.N., Dept. of Medicine, University of California School of Medicine San Diego, La Jolla: Characterization of the EGF receptor.

Merlino, G.T.,[1] Xu, Y.-H.,[1] Ishii, S.,[1] Clark, A.,[1] Wilson, R.,[2] Ma, D.P.,[2] Roe, B.,[2] Knutsen, T.,[1] Whang-Peng, J.,[1] Pastan, I.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Chemistry, University of Oklahoma, Norman: Cloning and characterization of EGF receptor gene sequences in A431 human carcinoma cells.

Gilmore, T., DeClue, J., Martin, G.S., Dept. of Zoology, University of California, Berkeley: Tyrosine phosphorylation induced by the *v-erb-B* protein in vivo and in vitro.

Novak-Hofer, I., Martin Perez, J., Siegmann, M., Thomas, G., Friedrich Miescher-Institut, Basel, Switzerland: EGF-mediated phosphorylation of 40S ribosomal protein S6 phosphorylation in Swiss mouse 3T3 cells.

Hendler, F.,[1] Shum, A.,[1] Richards, C.S.,[1] Cassells, D.,[1] Gusterson, B.,[2] Ozanne, B.,[1] [1]Depts. of Internal Medicine and Microbiology, Universiy of Texas Health Science Center, Dallas; [2]Ludwig Institute, Sutton, England: Evidence for increased EGF receptor in epidermoid malignancies.

SESSION 7 TGF

Chairperson: **G. Todaro,** Oncogen, Seattle, Washington

Assoian, R., Roberts, A., Anzano, M., Frolik. C., Wakefield, L., Sporn, M., NCI, National Institutes of Health, Bethesda. Maryland: Structure and action of transforming growth factor-β.

Moses, H.L., Shipley, G.D., Tucker. R.F., Dept. of Cell Biology. Mayo Foundation and Medical School, Rochester, Minnesota: Type-β transforming growth factor is a growth stimulator and a growth inhibitor.

Massagué, J., Dept. of Biochemistry, University of Massachusetts Medical Center, Worcester: Identification of membrane receptors for type-β transforming growth factor in target cells.

Derynck, R.,[1] Roberts, A.B.,[3] Winkler, M.E.,[2] Chen, E.Y.,[1] Goeddel, D.V.,[1] Depts. of [1]Molecular Biology, [2]Protein Biochemistry, Genentech, Incorporated, South San

Francisco, California; [2]NCI, National Institutes of Health, Bethesda, Maryland: Human transforming growth factor -α – Precursor structure and heterologous expression.

Adkins, B.,[1,2] Leutz, A.,[1] Graf, T.,[1] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]Dept. of Pathology, Stanford University, California: Viruses carrying *v-src*-related oncogenes induce autocrine growth in transformed chicken myeloid cells.

Sager, R., Craig, R., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Genetic suppression of tumorigenicity in hybrids from fusions of normal × EJ-transformed CHEF/18 cells.

SESSION 8 INSULIN

Chairperson: O. Rosen, Albert Einstein College of Medicine, Bronx, New York

Czech, M.P., Mottola, C., Oka, Y., Yu, K.-T., Davis, R., MacDonald, R.G., Dept. of Biochemistry, University of Massachusetts Medical School, Worcester: Mechanisms that regulate the receptors for insulin and the insulinlike growth factors.

Roth, R.A.,[1] Morgan, D.O.,[1] Masarachia, R.A.,[2] [1]Dept. of Pharmacology, Stanford University School of Medicine, California; [2]Dept. of Biochemistry, North Texas State University, Denton: Monoclonal antibodies as probes of the structure and function of the insulin receptor.

Rosen, O.M., Ganguly, S., Herrera, R., Petruzzelli, L.M., Stadtmauer, L., Memorial Sloan-Kettering Cancer Center, New York, New York: The insulin-dependent tyrosine protein kinase of human placenta.

Fujita-Yamaguchi, Y., Kathuria, S., Beckman Research Institute of the City of Hope, Duarte, California: Relationship between insulin receptor structure and its protein kinase activity.

Bruni, C.B.,[1] Frunzio, R.,[1] Whitfield, H.J.,[2] Rechler, M.M.,[2] [1]Centro di Endocrinologia ed Oncologia Sperimentale, CNR, Naples, Italy; [2]NIADDKD, National Institutes of Health, Bethesda, Maryland: Structure and expression of rat insulinlike growth factor-II gene.

Jacobs, S., Wellcome Research Laboratories, Research Triangle Park, North Carolina: Phorbol ester-stimulated phosphorylation of receptors for insulin and IGF-I.

SESSION 9 FUTURES

Chairperson: A. Pardee, Dana-Farber Cancer Institute, Boston, Massachusetts

Pardee, A.B., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: New directions – Molecular basis of growth regulation.

A. Pardee

Cunningham, D., Thompson, J., Hiramoto, S., Van Nostrand, K., Dept. of Microbiology and Molecular Genetics, University of California, Irvine: Thrombin-stimulated cell division.

Pouyssegur, J., Chambard, J.-C., Franchi, A., L'Allemain, G., Paris, S., Van Obberghen-Schilling, E., Centre de Biochimie, CNRS, Nice, France: Growth factor activation of the Na^+/H^+ antiporter controls growth of fibroblasts by regulating intracellular pH.

Jakobovits, A., Martin, G.R., Dept. of Anatomy, University of California, San Francisco: Embryonal carcinoma-derived growth factors – Specific growth-promoting and differentiation-inhibiting activities.

Villereal, M., Owen, N., Vicentini, L., Mix-Muldoon, L., Jamieson, G., Dept. of Pharmacology and Physiological Science, University of Chicago, Illinois: Mechanism for growth factor-induced increase of $Na+/H+$ exchange and in $Ca++$ activity in cultured human fibroblasts.

O'Brian, C., Columbia University College of Physicians & Surgeons, New York, New York: Studies on PKC and their relevance to tumor promotion.

Maness, P.F., Dept. of Biochemistry, University of North Carolina School of Medicine, Chapel Hill: Expression of pp60c-*src* inthe developing nervous system.

Chen, L.B., Weiss, M.J., Davis, S., Shepherd, E.L., Walker, E.S., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: The use of rhodamine-123 to probe mitochondrial bioenergetics in living cells.

BANBURY CENTER

Recto: Detail of Sammis Hall guest house at Banbury Center.

BANBURY CENTER DIRECTOR'S REPORT

This was the seventh consecutive year of operation of the Banbury Center of Cold Spring Harbor Laboratory. Throughout this period, there has been a continual expansion of the Center's conference programs and publications, particularly in the realms of health risk assessment and carcinogenesis, as well as in the impact upon these areas of recent developments in molecular biology. That this program has been able to flourish during a period when governmental support has become increasingly scarce and problematic is due, to a large extent, to the response the Center has been fortunate in eliciting from private sector sources. A particularly important step in 1984 was the partial consolidation of such support under the Cold Spring Harbor Laboratory Corporate Sponsor Program. This and the ongoing essential contributions from Core Supporters of the Banbury Center and specific corporate contributions toward particular programs remain crucial for the continuance and further development of the Banbury program.

It is perhaps fitting that the 1984 schedule began with the first meeting of the newly initiated Corporate Sponsor Program. This, the first of three such Sponsor meetings, was on Site-directed Mutagenesis and Protein Structure and Function. The meeting was almost prototypic of those to be held within this program, which enables the development of small scientific conferences in the rapidly developing field of molecular biology, especially in the area of recombinant DNA methodologies and their applications. These emphases were reflected in the remaining two Sponsor meetings of the year, a June conference on Yeast Expression Vectors and a November meeting on Transport and Secretion of Proteins.

Robertson House provides housing and dining accommodations at Banbury Center

Sammis Hall, guest house

The Sponsor Program thus acted as a rich complement to the ongoing Banbury concern with human health risk assessment in addition to the Center's increasing interest in newly emerging areas in the biological sciences that may particularly bear social or regulatory implications. The first major conference of the year in these areas was an April conference on the Biological Mechanisms of Dioxin Action. Organized jointly by Dr. Alan Poland (McArdle Laboratory of the University of Wisconsin) and Dr. Renate Kimbrough (Centers for Disease Control), this conference focused on the specific mechanisms by which the dioxins and closely related halogenated aromatic hydrocarbons bring about their broad range of biological effects. These compounds represent potentially far-reaching human health consequences and are among the most highly publicized and incitive of environmental pollutants. Bringing epidemiological and public health points of view together with major cellular and molecular research approaches, in the nonadversarial yet highly interactive setting of a small Banbury conference, was the impetus for this important conference. The papers and discussions of this meeting will appear early in 1985 as volume 18 in the Banbury Reports series.

The second major risk assessment conference of the year, to be published in 1985 as Banbury Report 19, was a mid-May conference on Risk Quantitation and Regulatory Policy. Organized by Dean Richard Merrill (University of Virginia School of Law) and Dr. David Hoel (Director of the Biometry and Risk Assessment Program of the National Institute of Environmental Health Sciences), this conference brought together legal, regulatory, and research representatives to consider the most efficacious ways to make the evolving science of risk assessment accessible and amenable to incorporation into regulatory policy formulations. The papers and discussions of this meeting proved a most useful compendium of centralized information and ideas in an area that has otherwise become a frequent source of public consternation and institutional conflict.

The final 1984 conference, also to be published in the Banbury series, was an October meeting organized by Dr. Frank Costantini (Columbia University) and Dr. Rudolph Jaenisch (now of the Whitehead Institute) on the Genetic Manipulation

Robertson House Library

of the Mammalian Ovum and Early Embryo. This relatively new and rapidly advancing research area has already received considerable public attention. Yet this attention has rarely focused on the new abilities that such advances have been bringing to current approaches toward an understanding of gene regulation and the genetic control of development. Given the nature and potentially far-reaching impact of this field, a present-state assessment and consideration of its likely future developments seemed especially appropriate at this particular time.

The 1984 Banbury program was rounded out by a full complement of summer courses and a series of smaller workshops held throughout the year. Among these smaller workshops was one held in early spring on the large T antigen of the SV40 oncogenic virus, followed by a National Institute of Mental Health Workshop on Single Unit Activity and Behavior, an Alfred P. Sloan Foundation Computational Neuroscience Workshop, and a late October workshop on the Structure and Function of Gap Junctions. The meeting year concluded at the end of November with a further Journalists' Workshop in the series held under the auspices of an ongoing grant from the Alfred P. Sloan Foundation. As previously mentioned, public confusion and concern often arises over issues of environmental health risk assessment. This is especially true in areas of occupational and environmental carcinogenesis. This year's journalists' workshop topic was thus "Assessing Risk Assessment". The workshop again proved to be both an intense and enjoyable few days, leading to considerably greater journalistic sophistication concerning the strengths and weaknesses in the quantitation of risk.

Robertson House hall

1984 Support

As noted earlier, the ability to carry on such an overall program is due largely to support that the Banbury Center has been fortunate to receive from private sector sources. The donation to Cold Spring Harbor Laboratory of the Banbury estate by Charles S. Robertson generously included an endowment for the maintenance of the grounds and of Robertson House. Funding of actual programs,

Banbury Center Meeting House

however, as well as maintenance and operation of the Meeting House and of Sammis Hall remain the responsibility of the Banbury Center itself. It is thus with considerable gratitude that I take this opportunity to thank the Corporate Sponsors and Core Supporters of the Center. The names of these companies are listed separately in this report. It is also with great thanks that I acknowledge the support of specific programs from the following sources: Biological Mechanisms of Dioxin Action was supported by contributions from Hoffmann-La Roche Inc., Monsanto Company, the Dow Chemical Company, and Diamond Shamrock Corporation; Risk Quantitation and Regulatory Policy was supported in part by a grant from the U.S. Department of Energy, together with contributions from the Monsanto Company and National Distillers and Chemical Corporation; and Genetic Manipulation of the Mammalian Ovum and Early Embryo was held under grants from the March of Dimes Birth Defects Foundation, the Fogarty International Center, and the National Institute of Child Health and Human Development.

Michael Shodell

SV40 Large T Antigen

March 8–March 11

ARRANGED BY

E. Harlow, Cold Spring Harbor Laboratory, New York

SESSION 1 MUTANTS

Chairperson: P. Tegtmeyer, State University of New York, Stony Brook

C. Cole, Dartmouth Medical School, Hanover, New Hampshire
D. Kalderon, MRC National Institute for Medical Research, London, England
R. Lanford, Baylor College of Medicine, Houston, Texas
M. Manos, Cold Spring Harbor Laboratory, New York
K. Peden, Johns Hopkins University, Baltimore, Maryland
T. Shenk, State University of New York, Stony Brook
A.E. Smith, MRC National Institute for Medical Research, London, England

SESSION 2 TRANSFORMATION

Chairperson: C. Prives, Columbia University, New York, New York

R. Baserga, Temple University Medical School, Philadelphia, Pennsylvania
F. Birg, INSERM, Marseille, France
M. Botchan, University of California, Berkeley
J. Feunteun, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France
J. Pipas, University of Pittsburgh, Pennsylvania
R. Pollack, Columbia University, New York, New York
P. Rigby, Imperial College, London, England
L. Sompayrac, University of Colorado, Boulder

SESSION 3 SURFACE T

Chairperson: E. Harlow, Cold Spring Harbor Laboratory, New York

J. Butel, Baylor College of Medicine, Houston, Texas
W. Deppert, Universität Ulm, Federal Republic of Germany
L. Gooding, Emory University, Atlanta, Georgia
B. Knowles, Wistar Institute, Philadelphia, Pennsylvania
S. Tevethia, Pennsylvania State University, Hershey

SESSION 4 EXPRESSION SYSTEMS

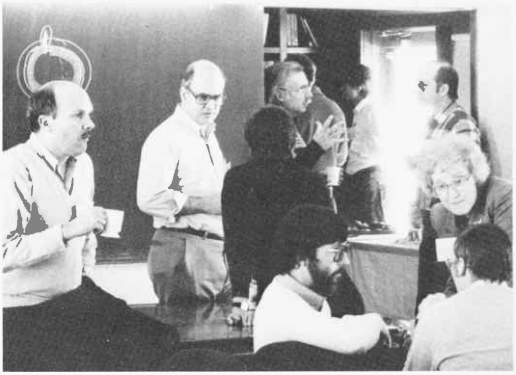
Chairperson: E. Harlow, Cold Spring Harbor Laboratory, New York

Y. Gluzman, Cold Spring Harbor Laboratory, New York
T. Grodzicker, Cold Spring Harbor Laboratory, New York
D. Rio, University of California, Berkeley

SESSION 5 DNA BINDING AND REGULATION

Chairperson: T. Shenk, State University of New York, Stony Brook

J.C. Alwine, University of Pennsylvania, Philadelphia
J. Brady, Laboratory of Molecular Virology, National Institutes of Health, Bethesda, Maryland
R. Dixon, Merck Sharp & Dohme, West Point, Pennsylvania
E. Fanning, Ludwig Maximillian University, Munich, Federal Republic of Germany
D.P. Lane, Imperial College, London, England
D.M. Livingston, Sidney Farber Cancer Institute, Boston, Massachusetts
J.L. Manley, Columbia University, New York, New York
D. Rio, University of California, Berkeley
J. Stringer, University of Cincinnati, Ohio
P. Tegtmeyer, State University of New York, Stony Brook



SESSION 6 MODIFICATIONS WORKSHOP

Chairperson: D.M. Livingston, Sidney Farber Cancer Institute, Boston, Massachusetts

R.B. Carroll, New York University Medical Center, New York
W. Deppert, Universität Ulm, Federal Republic of Germany
R. Henning, Universität Ulm, Federal Republic of Germany
E. May, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France
D. Simmons, University of Delaware, Newark
G. Walter, University of California, San Diego, La Jolla

SESSION 7 ANTIBODIES WORKSHOP

Chairperson: D.P. Lane, Imperial College, London, England

L. Gooding, Emory University, Atlanta, Georgia
E. Gurney, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France
E. Paucha, MRC National Institute for Medical Research, London, England

SESSION 8 SEQUENCES WORKSHOP

Chairperson: S. Weissman, Yale University, New Haven, Connecticut

Site-directed Mutagenesis and Protein Structure and Function

March 18–March 21

ARRANGED BY

R.T. Sauer, Massachusetts Institute of Technology, Cambridge

SESSION 1 ALTERATIONS OF ENZYMATIC PROPERTIES I

Chairperson: **M. Smith,** University of British Columbia, Vancouver, Canada

M. Smith, University of British Columbia, Vancouver, Canada: Oligonucleotide mutagenesis and the study of heme proteins.

S.J. Benkovic, Pennsylvania State University, Hershey: Strategies for mutagenesis: Preliminary results on dihydrofolate reductase.

S.A. Benner, Harvard University, Cambridge, Massachusetts: Synthetic genes for fundamental studies in protein chemistry.

G.A. Petsko, Massachusetts Institute of Technology, Cambridge: Site-directed alteration of enzymatic properties.

M.L. Sinnott, University of Bristol, England: The catalytic consequences of experimental evaluation of the *ebg* gene of *E. coli.*

J.N. Abelson, California Institute of Technology, Pasadena.

J. Kraut, University of California, San Diego, La Jolla: Site-directed mutagenesis of *E. coli* dihydrofolate reductase.

W.J. Rutter, University of California, San Francisco: Altering trypsin proteolytic specificity.

SESSION 2 ALTERATIONS OF ENZYMATIC PROPERTIES II

Chairperson: **J.R. Knowles,** Harvard University, Cambridge, Massachusetts

J.R. Knowles, Harvard University, Cambridge, Massachusetts: Regional mutagenesis in the signal codons of the β-lactamase gene.

A.R. Fersht, Imperial College of Science and Technology, London, England: Structure and activity of tyrosyl-tRNA synthetase.

M. Zoller, Cold Spring Harbor Laboratory, New York: Specific DNA/protein interactions in yeast. Point mutations in the DNA recognition sequence of the "HO" endonuclease.

P.R. Schimmel, Massachusetts Institute of Technology, Cambridge: Domain structure of amino acyl tRNA synthetase.

R. Wetzel, Genentech, Inc., South San Francisco, California: Thermostability of phage T4 lysozyme.

J.H. Richards, California Institute of Technology, Pasadena: β-lactamase: Mutagenic strategies and properties of mutants—Processing, secretion, stability, catalysis.

J.A. Gerlt, Yale University, New Haven, Connecticut: Genetic studies of nucleotidyl transferases.

D. Shortle, State University of New York, Stony Brook: Physical chemical genetics of staphylococcal nuclease.

SESSION 3 NONENZYME STRUCTURE/ACTIVITY CORRELATIONS

Chairperson: **H.O. Smith,** Johns Hopkins University School of Medicine, Baltimore, Maryland

P.B. Berget, University of Texas Medical School, Houston: Structure/function relationships in the bacteriophage P22 tail protein.

J.M. Pipas, University of Pittsburgh, Pennsylvania: The large tumor antigen of simian virus 40—Genetic analysis of a viral oncogene.

G.K. Ackers, Johns Hopkins University, Baltimore, Maryland: Hemoglobin mutants and cooperative energies.

P.A. Youderian, University of Southern California, Los Angeles: Altering DNA-binding specificities of repressor proteins.

R.T. Sauer, Massachusetts Institute of Technology, Cambridge: Using mutants to probe the stability and activities of DNA-binding proteins.

R.H. Ebright, Harvard Medical School, Boston, Massachusetts: Mutations that alter the DNA sequence specificity of the catabolite gene activator protein of *E. coli.*

M. Ptashne, Harvard University, Cambridge, Massachusetts: Repressors and *cro* proteins—Structure and function.

S.C. Harrison, Harvard University, Cambridge, Massachusetts: Structural organization of spherical plant viruses.

SESSION 4 PROTEIN FOLDING DYNAMICS AND STABILITY

Chairperson: R.L. Baldwin, Stanford University Medical Center, California

R. Matthews, Stanford University Medical Center, California: Protein alteration and folding of dihydrofolate reductase.

B.T. Nall, University of Texas Medical School, Houston: Folding of yeast cytochrome c.

I.B. Kingston, MRC Laboratory of Molecular Biology, Cambridge, England.

M. Karplus, Harvard University, Cambridge, Massachusetts: Protein folding: Some model studies.

M. Levitt, Weizmann Institute of Science, Rehovot, Israel: Computer modeling of mutant proteins.

J.B. Matthew, Genex Corporation, Gaithersburg, Maryland: Electrostatic contribution to protein stability and macromolecular assembly.

G. Rose, Pennsylvania State University, Hershey: Toward a taxonomy of protein structure.

H.A. Scheraga, Cornell University, Ithaca, New York: Molecular recognition in proteins.

SESSION 5 NOVEL PEPTIDES

Chairperson: M.F. Perutz, MRC Laboratory of Molecular Biology, Cambridge, England

M. Eigen, Max-Planck-Institut für biophysikalische Chemie, Gottingen, Federal Republic of Germany: Evolving systems.

E.T. Kaiser, Rockefeller University, New York, New York: Amphiphilic secondary structures—The design of biologically active peptides.

C.O. Pabo, Johns Hopkins Medical School, Baltimore, Maryland: Designing novel peptides.

J.S. Richardson, Duke University, Durham, North Carolina: Design criteria for inventing a protein.

SESSION 6 HEMOGLOBIN AND PROTEIN EVOLUTION

Chairperson: M.F. Perutz, MRC Laboratory of Molecular Biology, Cambridge, England

M.F. Perutz, MRC Laboratory of Molecular Biology, Cambridge, England: The structural basis of protein evolution.

R.F. Doolittle, University of California, San Diego, La Jolla: Evolving proteins with novel functions.

Biological Mechanisms of Dioxin Action

April 1–April 4

ARRANGED BY

A. Poland, University of Wisconsin, Madison
R.D. Kimbrough, Centers for Disease Control, Atlanta, Georgia

SESSION 1 CHEMISTRY AND GENERAL PATHOLOGY I

Chairperson: R.D. Kimbrough, Centers for Disease Control, Atlanta, Georgia

D. Firestone, U.S. Food and Drug Administration, Washington, D.C.: Chlorinated aromatic compounds and related dioxins and furans—Production, uses, and environmental exposure.

C. Rappe, University of Umea, Sweden: Chemistry and analysis of polychlorinated dioxins and dibenzofurans in biological samples.

E.E. McConnell, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Clinicopathologic concepts of dibenzo-*p*-dioxin intoxication.

SESSION 2 CHEMISTRY AND GENERAL PATHOLOGY II

Chairperson: J.A. Moore, Environmental Protection Agency, Washington, D.C.

H. Poiger, Federal Institute of Technology and the University of Zurich, Switzerland: The metabolism of TCDD in the dog and rat.

R.A. Neal, Chemical Industry Institute of Toxicology, Research Triangle

Park, North Carolina: Metabolism of TCCD intoxication.

R.M. Pratt, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Mechanism of TCDD-induced cleft palate in the mouse.

R.J. Kociba, Dow Chemical USA, Midland, Michigan: Evaluation of the carcinogenic and mutagenic potential of TCDD and other chlorinated dioxins.

SESSION 3 RECEPTOR BINDING

Chairperson: A. Poland, University of Wisconsin, Madison

D.R. Koop, University of Michigan Medical School, Ann Arbor: Identity of cytochromes P-450 induced by diverse xenobiotics.

B.A. Taylor, Jackson Laboratory, Bar Harbor, Maine: The aryl hydrocarbon hydroxylase inducibility locus (*Ah*) of the mouse, a genetic perspective.

A. Poland, University of Wisconsin, Madison: Reflections on the mechanisms of action of halogenated aromatic hydrocarbons.

J.A. Goldstein, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Regulation of a multigene family of P-450 isozymes by TCDD and related compounds.

S.H. Safe, Texas A&M University, College Station: Binding to the TCDD receptor and AHH/EROD induction – In vitro QSAR.

SESSION 4 ENZYME INDUCTION

Chairperson: J.A. Goldstein, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

J.-A. Gustafsson, Huddinge University Hospital, Sweden: Physicochemical characteristics of the TCDD receptor.

T.A. Gasiewicz, University of Rochester Medical Center, New York: Evidence for a homologous nature of *Ah* receptors among various mammalian species.

A. Schecter, State University of New York, Binghamton: Ultrastructural alterations of liver mitochondria in response to dioxins, furans, PCBs, and biphenylenes.

J.P. Whitlock, Jr., Stanford University School of Medicine, California: TCDD regulates cytochrome P_1-450 gene expression.

H.J. Eisen, National Institute of Child Health and Human Development, Bethesda, Maryland: The nuclear TCDD *Ah* receptor complex and the induction of cytochrome P_1-450 mRNA.

SESSION 5 BIOCHEMICAL CHANGES IN LIVER

Chairperson: J.B. Greig, MRC Toxicology Unit, Carshalton, England

S. Sassa, Rockefeller University, New York, New York: Inhibition of uroporphyrinogen decarboxylase activity in polyhalogenated aromated hydrocarbon poisoning.

G.D. Sweeney, McMaster University, Hamilton, Canada: Mechanisms underlying the hepatotoxicity of TCDD.

S.J. Stohs, University of Nebraska Medical Center, Omaha: Induction of lipid peroxidation and inhibition of glutathione peroxidase by TCDD.

A.B. Rifkind, Cornell University Medical Center, New York, New York: The chick embryo as a model for PCB and dioxin toxicity – Evidence of cardiotoxicity and increased prostaglandin synthesis.

F. Matsumura, Michigan State University, East Lansing: Toxicological significance of pleiotropic changes of plasma membrane functions, particularly that of EGF receptor caused by TCDD.

SESSION 6 LIPID METABOLISM AND WASTING DISEASE

Chairperson: R.E. Peterson, University of Wisconsin, Madison

R.E. Peterson, University of Wisconsin, Madison: The wasting syndrome in TCDD toxicity – Basic features and their interpretation.

S.D. Aust, Michigan State University, East Lansing: On the mechanism of anorexia and toxicity of TCDD and related compounds.

C.M. Schiller, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Metabolic impairment associated with a low dose of TCDD in adult male Fischer rats.

T. Thunberg, Karolinska Institute, Stockholm, Sweden: Effect of TCDD on vitamin A and its relation to TCDD toxicity.

K.K. Rozman, University of Kansas Medical Center, Kansas City: Role of thyroid hormones and brown adipose tissue in the toxicity of TCDD.

SESSION 7 SKIN AND IN VITRO RESPONSES

Chairperson: E.E. McConnell, National Insitute of Environmental Health Sciences, Research Triangle Park, North Carolina

R.D. Kimbrough, Centers for Disease Control, Atlanta, Georgia: Skin lesions in animals and humans: A brief overview.

W.F. Greenlee, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: Studies on the mechanisms of toxicity of TCDD to human epidermis.

R.H. Rice, Harvard School of Public Health, Boston, Massachusetts: Response of malignant epidermal keratinocytes to TCDD.

J. Knutson, University of Wisconsin, Madison: XB cells – An in vitro model for the differentiation and proliferation response to TCDD.

J.B. Greig, MRC Toxicology Unit, Carshalton, England: Differences between skin and liver toxicity of TCDD in mice.

SESSION 8 IMMUNOLOGICAL MECHANISMS

Chairperson: J.G. Vos, National Institute of Public Health, Bilthoven, The Netherlands

J.G. Vos, National Institute of Public Health, Bilthoven, The Netherlands: Dioxin-induced thymic atrophy and suppression of thymus-dependent immunity.

M.I. Luster, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: In vivo and in vitro effects of TCDD on stem cell and B cell differentiation.

G.D. Sweeney, McMaster University, Hamilton, Canada: Dose response, time-course, and mechanism for suppression of cytotoxic T cell generation by TCDD.

W.F. Greenlee, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: A proposed model for the actions of TCDD on epidermal and thymic epithelial target cells.

SESSION 9 EPIDEMIOLOGY

Chairperson: N. Nelson, New York University Medical Center, New York

H. Falk, Centers for Disease Control, Atlanta, Georgia: A pilot epidemiologic study of health effects due to TCDD contamination in Missouri.

M.A. Fingerhut, National Institute for Occupational Safety and Health, Cincinnati, Ohio: An evaluation of reports of dioxin exposure and soft tissue sarcoma pathology in U.S. chemical workers.

G.D. Lathrop, Brooks Air Force Base, San Antonio, Texas: An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides.

Risk Quantitation and Regulatory Policy

May 13–May 16

ARRANGED BY

D. Hoel, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
R.A. Merrill, University of Virginia, Charlottesville
M.C. Pike, Imperial Cancer Research Fund, Oxford, England

SESSION 1 REGULATORY PROGRAMS UTILIZING RISK ASSESSMENT

Chairperson: L.B. Lave, Carnegie-Mellon University, Pittsburgh, Pennsylvania

L.B. Lave, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Quantitative risk assessment in regulation.

P.B. Hutt, Covington & Burling, Washington, D.C.: Use of quantitative risk assessment in regulatory decision making under federal health and safety statutes.

M.R. Taylor, King & Spalding, Washington, D.C.: The use of risk assessment in food safety decision making: The scope of FDA's discretion and the safeguards against abuse.

R.A. Merrill, University of Virginia School of Law, Charlottesville: The significance of risk quantitation in health and environmental regulation.

SESSION 2 EPIDEMIOLOGY IN RISK ESTIMATION I

Chairperson: M.C. Pike, Imperial Cancer Research Fund, Oxford, England

M.C. Pike, Imperial Cancer Research Fund, Oxford, England: Epidemiology and risk assessment: Estimation of GI cancer risk from asbestos in drinking water.

P. Landrigan, National Institute for Occupational Safety and Health, Cincinnati, Ohio: Approaches to the evaluation of dose in occupational epidemiology.

SESSION 3 EPIDEMIOLOGY IN RISK ESTIMATION II

Chairperson: M.C. Pike, Imperial Cancer Research Fund, Oxford, England

J.M. Kaldor, International Agency for Research on Cancer, Lyon, France: The use of epidemiological data for the assessment of human cancer risk.

J. Peto, Institute of Cancer Research, Sutton, England: Limitations in the quantitation of carcinogenic risk – The asbestos data base.

SESSION 4 MODELING AND EXTRAPOLATION

Chairperson: R. Wilson, Harvard University, Cambridge, Massachusetts

M.D. Hogan. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Risk estimation models in epidemiology studies.

J. Van Ryzin, Columbia University School of Public Health, New York. New York: Consequences of non-linear kinetic dose response models on carcinogenic risk assessment.

R. Wilson, Harvard University, Cambridge, Massachusetts: Expression of uncertainty in risk assessment.

SESSION 5 MUTAGENIC RISK IN HUMAN POPULATIONS

Chairperson: F.P. Perera, Columbia University School of Public Health, New York, New York

M.F. Lyon, MRC Radiobiology Unit, Harwell, England: Attempts to estimate genetic risks caused by mutagens to later generations.

J.V. Neel, University of Michigan Medical School, Ann Arbor: How do we get better human genetic risk data, and how then do we use the data?

SESSION 6 TOXICOLOGY AND BIOLOGICAL MECHANISMS I

Chairperson: I.B. Weinstein, College of Physicians & Surgeons, Columbia University, New York, New York

I.F.H. Purchase, Imperial Chemical Industries PLC, Macclesfield, England: The toxicologist's contribution to risk quantitation.

R.L. Dedrick, National Institutes of Health, Bethesda, Maryland: Application of model systems in pharmacokinetics.

J.R. Gillette, National Institutes of Health, Bethesda, Maryland: Biological variation: The unsolvable problem in quantitative extrapolations from laboratory animals and other surrogate systems to human populations.

F.P. Perera, Columbia University School of Public Health, New York, New York: Methods of measuring biologically effective doses of carcinogenic substances—Monitoring using DNA adducts.

SESSION 7 TOXICOLOGY AND BIOLOGICAL MECHANISMS II

Chairperson: B.D. Goldstein, Environmental Protection Agency, Washington, D.C.

I.B. Weinstein, College of Physicians & Surgeons, Columbia University, New York, New York: Relevance of mechanisms of action of tumor promoters to risk assessment.

R.H. Reitz, Dow Chemical Company, Midland, Michigan: Mechanistic considerations in the formulation of carcinogenic risk estimations.

SESSION 8 SPECIFIC CASE HISTORIES

Chairperson: P.W. Preuss, Consumer Product Safety Commission, Bethesda, Maryland

J.A. Swenberg, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: A scientific approach to formaldehyde risk assessment.

M. Cohn, Consumer Product Safety Commission, Bethesda, Maryland: Risk assessment and the formaldehyde data base.

M. Corn, Johns Hopkins University, Baltimore, Maryland: Human exposure estimates in hazardous waste site risk assessment.

B.D. Goldstein, Environmental Protection Agency, Washington, D.C.: Risk assessment and risk management of benzene by the EPA.

SESSION 9 RISK QUANTITATION AND THE DYNAMICS OF POLICY FORMULATION

Chairperson: R.S. Merrill, University of Virginia School of Law, Charlottesville

R.E. Albert, New York University Medical Center, New York: Issues of concern in the revision of the U.S. EPA's guidelines for carcinogen risk assessment.

P.W. Preuss, Consumer Product Safety Commission, Bethesda, Maryland: The changing role of risk assessment in Federal regulation.

Yeast Expression Vectors

June 21–June 24

ARRANGED BY

J. Strathern, Cold Spring Harbor Laboratory, New York
J.R. Broach, State University of New York, Stony Brook

SESSION 1 VECTORS

M.V. Olson, Washington University School of Medicine, St. Louis, Missouri

R. Rothstein, New Jersey Medical School, Newark

S. Weisbrod, Cold Spring Harbor Laboratory, New York

K. Bloom, University of North Carolina, Chapel Hill

R.W. Davis, Stanford University Medical Center, California

C.P. Hollenberg, Universitat Dusseldorf, Federal Republic of Germany

V.A. Zakian, Fred Hutchinson Cancer Research Center, Seattle, Washington

SESSION 2 PROMOTERS I

L.P. Guarente, Massachusetts Institute of Technology, Cambridge
A. Hinnen, Ciba-Geigy AG, Basel, Switzerland
F. Sherman, University of Rochester School of Medicine, New York
J. Hopper, Pennsylvania State University, Hershey
J.R. Broach, State University of New York, Stony Brook
S. Fields, University of California, San Francisco, School of Medicine

SESSION 3 PROMOTERS II

P. Silver, Harvard University, Cambridge, Massachusetts
A. Kingsman, University of Oxford, England
G.R. Fink, Massachusetts Institute of Technology, Cambridge
K. Nasmyth, Laboratory of Molecular Biology, Cambridge, England
J. Abraham, California Biotechnology, Inc., Palo Alto

SESSION 4 PROTEIN LOCALIZATION AND SECRETION

J. Thorner, University of California, Berkeley
R.W. Schekman, University of California, Berkeley
S. Emr, California Institute of Technology, Pasadena
G. Sprague, Jr., University of Oregon, Eugene
L. Hereford, Dana-Farber Cancer Institute, Boston, Massachusetts
K. Bostian, Brown University, Providence, Rhode Island

SESSION 5 EXPRESSION SYSTEMS

R.A. Hitzeman, Genentech, Inc., South San Francisco, California
G.A. Bitter, Applied Molecular Genetics, Inc., Thousand Oaks, California
M. Duncan, Collaborative Research, Inc., Lexington, Massachusetts
G. Ammerer, ZymoGenetics, Inc., Seattle, Washington

U.S. Environmental Protection Agency Workshop on "Possible Short-term Evolutionary Consequences of Biotechnology"

August 28–August 31

ARRANGED BY

J.R. Fowle III, U.S. Environmental Protection Agency, Washington, D.C.

Chairperson: P. Regal, University of Minnesota, Minneapolis

PARTICIPANTS

D. Archer, Food and Drug Administration, Washington, D.C.
F. Betz, U.S. Environmental Protection Agency, Washington, D.C.
R. Bierbaum, Office of Technology Assessment, Washington, D.C.
A.W. Bourquin, U.S. Environmental Protection Agency, Gulf Breeze, Florida
J. Brown, University of Arizona, Tucson
E. Carlson, State University of New York, Stony Brook
R. Colwell, University of California, Berkeley
M.A. Danello, Food and Drug Administration, Washington, D.C.
J.R. Fowle III, U.S. Environmental Protection Agency, Washington, D.C.

M. Gough, Office of Technology Assessment, Washington, D.C.
L.L. Greenlee, Agrigenetics Corp., Boulder, Colorado
F. Harris, National Science Foundation, Washington, D.C.
A. Hollander, U.S. Environmental Protection Agency, Washington, D.C.
D. Kamely, U.S. Environmental Protection Agency, Washington, D.C.
D. Kaplan, Dow Chemical Company, Midland, Michigan
R. Lensky, University of Massachusetts, Amherst
B.R. Levin, University of Massachusetts, Amherst
M. Levin, House Subcommittee on Investigations and Oversight, U.S. Congress, Washington, D.C.
M. Lloyd, University of Chicago, Illinois
D. MacKenzie, Louisiana State University, Baton Rouge
B. McClintock, Cold Spring Harbor Laboratory, New York
A. McDaniels, U.S. Environmental Protection Agency, Washington, D.C.
E. Milewski, National Institutes of Health, Washington, D.C.
S. Panem, U.S. Environmental Protection Agency, Washington, D.C.
J. Rissler, U.S. Environmental Protection Agency, Washington, D.C.
A. Rose, Department of Health and Human Services, Washington, D.C.
D. Simberloff, Florida State University, Tallahassee
Z. Vaituzis, U.S. Environmental Protection Agency, Washington, D.C.

National Institute of Mental Health Workshop on "Single Unit Activity and Behavior"

September 9–September 11

ARRANGED BY

R. Schoenfeld, National Institute of Mental Health, Rockville, Maryland

Chairpersons: **B.S. Bunney,** Yale University School of Medicine, New Haven, Connecticut
B.L. Jacobs, Princeton University, New Jersey

PARTICIPANTS

N. Bernick, National Institute of Mental Health, Rockville, Maryland
M.R. DeLong, Johns Hopkins Hospital, Baltimore, Maryland
P.S. Goldman-Rakic, Yale University School of Medicine, New Haven, Connecticut
M. Konishi, California Institute of Technology, Pasadena
S.H. Koslow, National Institute of Mental Health, Rockville, Maryland
I. Kupfermann, College of Physicians & Surgeons, Columbia University, New York, New York
J.B. Ranck, State University of New York, Downstate Medical Center, Brooklyn
R. Schoenfeld, National Institute of Mental Health, Rockville, Maryland
R.F. Thompson, Stanford University, California
T.N. Wiesel, Rockfeller University, New York, New York
D.J. Woodward, University of Texas Health Science Center, Dallas

Alfred P. Sloan Foundation Computational Neuroscience Workshop

September 28–September 30

ARRANGED BY

E. Wanner, Alfred P. Sloan Foundation, New York, New York

SESSION 1 VISION

Organizers: **T. Poggio,** Massachusetts Institute of Technology, Cambridge
T. Sejnowski, Johns Hopkins University, Baltimore, Maryland

PARTICIPANTS

D. Ballard, University of Rochester, New York
H.B. Barlow, Cambridge University, England
C. Gilbert, Rockefeller University, New York, New York
D.A. Glaser, University of California, Berkeley
E. Hildreth, Massachusetts Institute of Technology, Cambridge
A.J. Movshon, New York University, New York
G.F. Poggio, Johns Hopkins University School of Medicine, Baltimore, Maryland
T. Poggio, Massachusetts Institute of Technology, Cambridge
T. Sejnowski, Johns Hopkins University, Baltimore, Maryland
C.F. Stevens, Yale University School of Medicine, New Haven, Connecticut
V. Torre, Universita di Genova, Italy
S. Ullman, Massachusetts Institute of Technology, Cambridge

SESSION 2 VESTIBULO-OCULAR PROCESSING

Organizer: **D.A. Robinson,** Johns Hopkins Hospital, Baltimore, Maryland

PARTICIPANTS

A.F. Fuchs, University of Washington, Seattle
H. Galiana, McGill University, Montreal, Canada
E.L. Keller, Medical Research Institute of San Francisco at Pacific Medical Center, California
G.E. Loeb, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland
L.M. Optican, National Eye Institute, Bethesda, Maryland
D.A. Robinson, Johns Hopkins Hospital, Baltimore, Maryland

SESSION 3 MOTOR CONTROL

Organizers: **E. Bizzi,** Massachusetts Institute of Technology, Cambridge
J.M. Hollerbach, Massachusetts Institute of Technology, Cambridge

PARTICIPANTS

E. Bizzi, Massachusetts Institute of Technology, Cambridge
E.V. Evarts, National Institutes of Health, Bethesda, Maryland
L. Finkel, Rockefeller University, New York, New York
C. Ghez, College of Physicians & Surgeons, Columbia University, New York, New York
N. Hogan, Massachusetts Institute of Technology, Cambridge
J.M. Hollerbach, Massachusetts Institute of Technology, Cambridge
J.C. Houk, Northwestern University, Chicago, Illinois
R.W. Mann, Massachusetts Institute of Technology, Cambridge
M.H. Raibert, Carnegie-Mellon University, Pittsburgh, Pennsylvania

Genetic Manipulation of the Mammalian Ovum and Early Embryo

October 7–October 10

ARRANGED BY

F. Costantini, Columbia University, New York, New York
R. Jaenisch, Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany

SESSION 1 DEVELOPMENTAL GENETICS

Chairperson: L. Silver, Princeton University, New Jersey

L. Silver, Princeton University, New Jersey: The origin and evolution of mouse *t* haplotypes.

V.E. Chapman, Roswell Park Memorial Institute, Buffalo, New York: X-chromosome regulation in female mammals.

S.M. Tilghman, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Tissue-specific expression of cloned AFP genes in cells and mice.

M.J. Evans, University of Cambridge, England: EK cell contribution to chimaeric mice: From tissue culture to sperm.

D. Solter, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Capacity of nuclei from preimplantation mouse embryos to support normal development.

A. Surani, Institute of Animal Physiology, Cambridge, England: Regulation of embryogenesis by maternal and paternal genomes in the mouse.

K. Willison, Chester Beatly Laboratory, London, England: Haploid gene expression and the mouse *t* complex.

SESSION 2 ENDOGENOUS VIRUSES AND VIRAL VECTORS

Chairperson: P.W.J. Rigby, Imperial College of Science and Technology, London, England

N.A. Jenkins, University of Cincinnati College of Medicine, Ohio: Instability of ecotropic proviruses in RF/J-derived hybrid mice.

N.G. Copeland, University of Cincinnati College of Medicine, Ohio: Molecular genetic approaches to the study of murine lymphoid cell neoplasms.

R. Jaenisch, Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany: Recombinant viral probes of gene regulation and methylation patterns in the mouse.

E.F. Wagner, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Introducing and expressing genes in stem cells and mice.

SESSION 3 INTRODUCTION OF CLONED GENES INTO THE MAMMALIAN GERM LINE I

Chairperson: F.H. Ruddle, Yale University, New Haven, Connecticut

R.L. Brinster, University of Pennsylvania, Philadelphia: Introduction of SV40 genes into mice.

R.D. Palmiter, University of Washington, Seattle: Expression of growth-hormone genes in mice.

D. Hanahan, Cold Spring Harbor Laboratory, New York: Expression of insulin-SV40 T-antigen hybrid genes in transgenic mice.

T.A. Stewart, Genentech, Inc., South San Francisco, California: Transgenic mice carrying MTV/*myc* fusion genes develop mammary adenocarcinomas.

R. Evans, Salk Institute, San Diego, California: Novel tissue-specific expression of growth-hormone genes in transgenic animals.

SESSION 4 INTRODUCTION OF CLONED GENES INTO THE MAMMALIAN GERM LINE II

Chairperson: A.J. Levine, Princeton University, New Jersey

F. Costantini, Columbia University, New York, New York: Regulated expression of a foreign β-globin gene in transgenic mice.

D. Baltimore, Massachusetts Institute of Technology, Cambridge: Tissue-specific expression of immunoglobulin genes in transgenic mice and cultured cells.

U. Storb, University of Washington, Seattle: Expression of a microinjected immunoglobulin κ gene in transgenic mice.

C.T. Caskey, Baylor College of Medicine, Houston, Texas: Elevated expression of human HPRT in CNS of transgenic mice.

D.C. Kraemer, Texas A&M University, College Station: Gene transfer in cattle and sheep.

C. Polge, Institute of Animal Physiology, Cambridge, England: Gene transfer approaches in farm animals.

SESSION 5 NONMAMMALIAN SYSTEMS

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

E. Davidson, California Institute of Technology, Pasadena: Transfer and expression of cloned sequences in the sea urchin embryo.

J. Newport, University of California, San Diego: Nuclear formation in the *Xenopus* embryo.

D.T. Stinchcomb, Harvard University, Cambridge, Massachusetts: DNA transformation of *C. elegans;* extrachromosomal arrays are replicated, segregated, and expressed.

T. Maniatis, Harvard University, Cambridge, Massachusetts: Analysis of *Drosophila* alcohol dehydrogenase gene expression by P-element transformation.

Gap Junctions

October 21–October 24

ARRANGED BY

M.V.L. Bennett, Kennedy Center, Bronx, New York

SESSION 1 STRUCTURE

Chairperson: M.V.L. Bennett, Kennedy Center, Bronx, New York

L. Makowski, College of Physicians & Surgeons, Columbia University, New York, New York: Structural domains in gap junctions and implications for gating.

G. Zampighi, University of California, Los Angeles, School of Medicine: Structure of lens and liver junctional proteins.

R. Hanna, State University of New York College of Environmental Science and Forestry, Syracuse, New York: Fast-frozen gap junctions.

S.B. Yancey, and J.-P. Revel, California Institute of Technology, Pasadena: Organization of junctional proteins in the membrane as deduced from sequence data.

E. Page, University of Chicago, Illinois: Structure and protein composition of cardiac gap junctions.

SESSION 2 BIOCHEMISTRY

Chairperson: J.-P. Revel, California Institute of Technology, Pasadena

E.L. Hertzberg, Baylor College of Medicine, Houston, Texas: Tissues and species specificity of gap junctional proteins.

K. Willecke, Universitat Essen, Federal Republic of Germany: Immunochemical characterization of gap junction protein from different mammalian tissues.

M. Finbow, Beatson Institute for Cancer Research, Glasgow, Scotland: Tissue, species, and phylogenetic variation of gap junctional proteins.

R.G. Johnson, University of Minnesota, St. Paul: Lens junctions—Antibodies to and phosphorylation of MP26.

D. Paul, Harvard University Medical School, Boston, Massachusetts: Proteolytic cleavage of junctional proteins from lens and liver.

SESSION 3 BIOPHYSICS

Chairperson: R. Llinas, New York University Medical Center, New York

P. Brink, State University of New York, Stony Brook: Solvent effects on junctional permeability.

D.C. Spray, Kennedy Center, Bronx, New York: Gating by voltage and chemical agents.

F. Ramon, Centro de Investigacion y de Estudios Avanzados Del IPN, Mexico City, Mexico: Physiological control mechanisms.

J. Wojtchak, Rockefeller University, New York, New York: Electrical uncoupling induced by general anesthetics—A calcium independent process?

J.E. Hall, University of California, Irvine: Channel reconstitution from junctional proteins.

C. Peracchia, University of Rochester Medical School, New York: Phosphorylation and reconstitution of lens junctions.

SESSION 4 CONTROL OF FORMATION

Chairperson A. Warner, University College London, England

J. Sheridan, University of Minnesota, Minneapolis: Altered junctional permeability between virally transformed cells.

J.D. Pitts, Beatson Institute for Cancer Research, Glasgow, Scotland: Tissue specificity.

W. Coles, McMaster University, Hamilton, Canada: Alterations in coupling in uterine muscle.

J. Kessler, Kennedy Center, Bronx, New York: Coupling between cultured vertebrate neurons.

S.B. Kater, University of Iowa, Iowa City: Specificity of coupling at molluscan neurons.

SESSION 5 ROLE IN INTERCELLULAR COMMUNICATION AND DEVELOPMENT

Chairperson: J.D. Pitts, Beatson Institute for Cancer Research, Glasgow, Scotland

C.W. Lo, University of Pennsylvania, Philadelphia: Compartmentalization in embryos.

S. Caveney, University of Western Ontario, London, Canada: Control of molecular movement within and between developmental compartments.

A. Warner, University College London, England: Antibodies to gap junction proteins—Probes for studying development.

W.J. Larsen, University of Cincinnati College of Medicine, Ohio: Relationships of gap junction modulation to cell and tissue function.

W. Beers, New York University, New York: Gap junctions and ovulation.

O.H. Petersen, University of Liverpool, England: Communication in secretory epithelia.

SESSION 6 ELECTROTONIC SYNAPSES

Chairperson: D.C. Spray, Kennedy Center, Bronx, New York

F.E. Dudek, Tulane University School of Medicine, New Orleans, Louisiana: Electrical interactions and synchronization of hippocampal neurons—Electrotonic coupling vs. field effects.

R. Llinas, New York University Medical Center, New York: Functional significance of coupling in the inferior olive.

M.V.L. Bennett, Kennedy Center, Bronx, New York: Interactions among chemical and electrotonic synapses.

C. Giaume, Institut Pasteur, Paris, France: The rectifying electrotonic synapse of crayfish.

H.M. Gerschenfeld and J. Neyton, École Normale Supérieure, Paris, France: Dopaminergic control of coupling between horizontal cells.

E. Lasater and J. Dowling, Harvard University, Cambridge, Massachusetts: Characteristics of coupling between pairs of cultured horizontal cells.

Transport and Secretion of Proteins

November 7–November 10

ARRANGED BY

M.-J. Gething, Cold Spring Harbor Laboratory, New York

SESSION 1 TRANSLOCATION OF PROTEINS ACROSS THE LIPID BILAYER

Chairperson: G. Blobel, Rockefeller University, New York, New York

P. Walter, University of California, San Francisco: Targeting of nascent secretory proteins to the endoplasmic reticulum membrane.

D. Meyer, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Factors mediating protein translocation in the endoplasmic reticulum—The docking protein and beyond.

R. Gilmore, Rockefeller University, New York, New York: Evidence for the existence of a signal receptor in the microsomal membrane.

S. Benson, Princeton University, New Jersey: Intragenic information required for the export of LamB to the outer membrane of *E. coli*.

W. Wickner, University of California, Los Angeles, School of Medicine—Mechanisms of bacterial cell-surface assembly.

S. Ferro-Novick, Harvard Medical School, Boston, Massachusetts: Genetic evidence for the coupling of the synthesis and secretion of proteins in *E. coli*.

M. Inouye, State University of New York, Stony Brook: Functional and structural analysis of the signal peptide.

C. Kaiser, Massachusetts Institute of Technology, Cambridge: Mutations in the signal sequence affecting the localization of invertase.

SESSION 2 TRANSPORT FROM THE ENDOPLASMIC RETICULUM TO THE PLASMA MEMBRANE

Chairperson: **H.F. Lodish,** Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

I. Schauer, University of California, Berkeley: Invertase sequence substitutions interfere with transport of active enzyme from the ER.

J. Sambrook, Cold Spring Harbor Laboratory, New York: How to get SV40 T antigen into membranes.

J. Rose, Salk Institute, San Diego, California: Redesigning transport signals in the VSV glycoprotein.

P. Berman, Genentech, Inc., South San Francisco, California: Processing and export of membrane-bound and secreted forms of cloned HSV-1 glycoprotein D in continuous cell lines.

M.-J. Gething, Cold Spring Harbor Laboratory, New York: Site-directed mutagenesis of the hemagglutinin of influenza virus—Effects on transport through the cell.

A. Brake, Chiron Corporation, Emeryville, California: Sequence of two genes encoding precursors to the yeast peptide mating pheromone a factor.

J. Thorner, University of California, Berkeley: Cell biology and enzymology of the specific endopeptidase required for processing of yeast precursor proteins at pairs of basic residues.

SESSION 3 STRUCTURES INVOLVED IN TRANSPORT AND ENDOCYTOSIS

Chairperson: **G.E. Palade,** Yale University School of Medicine, New Haven, Connecticut

P.W. Robbins, Massachusetts Institute of Technology, Cambridge: Protein glycosylation.

M.G. Farquahar, Yale University School of Medicine, New Haven, Connecticut: Receptor traffic to Golgi subcompartments.

W.S. Sly, St. Louis University School of Medicine, Missouri: Mannose-6-P receptor-mediated sorting and transport of lysosomal enzymes.

I. Mellman, Yale University School of Medicine, New Haven, Connecticut: Fc receptor transport, proton transport, and the control of intracellular membrane traffic.

G. Warren, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Reconstruction of an endocytic fusion event in a cell-free system.

S. Schmid, Stanford University School of Medicine, California: Enzymatic recycling of clathrin from coated vesicles.

P.C. Tai, Boston Biomedical Research Institute, Massachusetts: Clathrin in *B. subtilis*? Bacterial protein translocation.

SESSION 4 DIRECTED TRANSPORT I

Chairperson: **D. Sabatini,** New York University Medical Center, New York

D. Sabatini, New York University Medical Center, New York: Intracellular sorting and distinct recycling patterns of viral glycoproteins in polarized epithelial cells.

K. Mostov, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Transepithelial transport of IgA and IgM.

H.-P. Moore, University of California, San Francisco: Sorting and transport of proteins in regulated secretory cells.

J.-P. Kraehenbuhl, University of Lausanne, Switzerland: Biogenesis of (Na^+,K^+)-ATPase in ion-transporting epithelia.

D. Louvard, Institut Pasteur, Paris, France: Basolateral membrane protein markers are expressed at the surface of undifferentiated precursors of enterocytes in vitro, whereas transport of apical markers is abortive.

E. Rodriguez-Boulan, Cornell University Medical College, New York, New York: Vectorial exocytosis of plasma membrane glycoproteins in epithelial cells.

K. Simons, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Sorting of apical and basolateral proteins in MDCK cells.

SESSION 5 DIRECTED TRANSPORT II

Chairperson: **D. Botstein,** Massachusetts Institute of Technology, Cambridge

S.D. Emr, California Institute of Technology, Pasadena: Use of gene fusions to study protein traffic in yeast.

T.H. Stevens, University of Oregon, Eugene: Yeast mutants defective in the sorting of vacuolar proteins.

M.N. Hall, University of California, San Francisco: Studies on the mechanism of nuclear protein localization in yeast.

E.C. Hurt, University of Basel, Switzerland: Import of proteins into mitochondria.

J. Kaput, Rockefeller University, New York, New York: Translocation of nuclearly encoded mitochrondrial proteins translated from SP6-promoted transcripts.

M.G. Douglas, University of Texas Health Sciences Center, San Antonio: The biochemistry and genetics of protein import into mitochondria.

Journalists' Workshop on "Assessing Health Risk Assessment"

November 28–November 30

ARRANGED BY

M. Shodell, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

D.P. Rall, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: An introduction to the principles of risk assessment.

J.A. Swenberg, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: Models in biological risk assessment.

SESSION 2

I.C.T. Nisbet, Clement Associates, Inc., Arlington, Virginia: Assessing the models used for assessing risk.

F.P. Perera, Columbia University School of Public Health, New York, New York: New approaches in risk assesssment.

SESSION 3

R.E. Albert, New York University Medical Center, New York, New York: Risk assessment in the regulation of carcinogens.


EDUCATIONAL
ACTIVITIES

Recto: James laboratory, erected in 1929, houses the Laboratory's tumor virology program and a number of summer postgraduate research courses. The Annex (shown here), built in 1971, contains offices, a seminar room, and a library.

Postgraduate Courses

The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Immunoglobulins: Molecular Probes of the Nervous System

June 8–June 28

INSTRUCTORS

Hockfield, Susan, Ph.D., Cold Spring Harbor Laboratory, New York
Kintner, Chris, Ph.D., Medical Research Council, Kings College London, England
Silbertstein, Laura, Ph.D., Stanford Medical Center, California
Weber Eckard, M.D., Stanford Medical School, California

ASSISTANT

Evans, Chris, Ph.D., Stanford University, California

Over the last decade, antibodies have become powerful and popular reagents in neurobiological research. This course, intended for research scientists of all levels, was designed to provide an advanced understanding of the power and limitations of immunoglobulins both as biochemical and anatomical reagents. Through laboratory work and lectures, we explored immunochemical and immunohistochemical techniques in detail and the application of these techniques to current issues in neurobiology.

The laboratory work included: generation of monoclonal antibodies; preparation and characterization of antibodies to synthetic peptides and to complex antigens; solid-phase immunoassays; antibody characterization and purification; antigen purification using antibody affinity methods; Western blotting; antibody conjugation; biochemical characterization of neuropeptides using RIA; light and electron microscopic immunohistochemistry using peroxidase, fluorescence, and biotin-avidin techniques; and methods for the colocalization of multiple antibodies.

A series of lectures by invited speakers covered: structure and function of immunoglobulins; molecular genetics of antibody diversity; cellular regulation of the immune response; hybridoma technology; studies using synthetic peptides; immunological characterization of the acetylcholine receptor and the neuromuscular junction; and immunological characterization of cellular diversity in vertebrate and invertebrate nervous systems and in cell culture.

PARTICIPANTS

Drysdale, Rachel A., B.A., University of Wisconsin, Madison
Flanagan, Thomas R., Ph.D., Cold Spring Harbor Laboratory, New York
Kalil, Katherine, Ph.D., University of Wisconsin, Madison
Messing, Albee, Ph.D., University of Pennsylvania School of Medicine, Philadelphia
Milner, Teresa Ann, Ph.D., Cornell University School of Medicine, New York, New York
Ridgway, Richard L., Ph.D., Washington State University, Pullman
Simonds, William F., M.D., National Institutes of Health, Bethesda, Maryland
Thompson, Wesley J., Ph.D., University of Texas, Austin
Treiman, Marek, M.D., Rockefeller University, New York, New York
Wahle, Petra, Msc., Max-Planck-Institut für Biophysikalische Chemie, Frankfurt, Federal Republic of Germany

SEMINARS

Wilson, D., University of Pennsylvania. Cellular immunology.
Immanishi-Kari, T., Massachusetts Institute of Technology. Molecular immunology.
Davis, D., National Institutes of Health. 3-D structure of immunoglobulins.
Lindstrom, J., Salk Institute. Using monoclonal antibodies to study acetylcholine receptors.
Burden, S., Massachusetts Institute of Technology. Subsynaptic protein at nerve-muscle synapses.
Matthews, B., Harvard Medical School. Monoclonal antibodies used to study neurite-promoting factors.
Hendry, S., University of California, Irvine. Immunocytochemical studies of the mammalian cerebral cortex.
Jessel, T., Harvard University Medical School. Probes for mammalian dorsal root ganglion cells.
Pickel, V., Cornell University School of Medicine. Use of combined immunocytochemical techniques for the localization of more than one antigen.
Karten, H., State University of New York, Stony Brook. Immunochemical analysis of the retina.

Molecular Biology of Plants

June 8–June 28

INSTRUCTORS

Malmberg, Russell, Ph.D., Cold Spring Harbor Laboratory, New York
Messing, Joachim, Ph.D., University of Minnesota, St. Paul
Sussex, Ian, Ph.D., Yale University, New Haven, Connecticut

ASSISTANTS

Boylan, Margaret, Ph.D., Yale University, New Haven, Connecticut
McIndoo, Jean, B.S., Cold Spring Harbor Laboratory, New York
Zarowitz, Michael, Ph.D., University of Minnesota, St. Paul

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.

PARTICIPANTS

Avivi, Aaron A., Ph.D., Weizmann Institute of Science, Rehovot, Israel
Choi, Cha Yong, Ph.D., Seoul National University, Korea
DasSarma, Shiladitya, B.S., Massachusetts Institute of Technology, Cambridge
De Block, M., Ph.D., Rijksuniversiteit Gent, The Netherlands
Dilworth, Machi F., U.S. Department of Agriculture, Washington, D.C.
Dynan, William S., Ph.D., University of California, Berkeley
Hille, Jacques, Ph.D., University of Wageningen, The Netherlands
Jones, Davy, Ph.D., University of Kentucky, Lexington
Kakidani, Hitoshi, Ph.D., Sagami Chemical Research Center, Japan
Lammers, Peter J., Ph.D., University of Chicago, Illinois
Lee, Nancy, Ph.D., Interferon Sciences, Inc., New Brunswick, New Jersey
Mindrinos, Michael N., Ph.D., Boston College, Massachusetts
Peterson, Thomas A., B.S., University of California, Davis
Sagar, Anurag, M.S., Rockefeller University, New York, New York
Tavantzis, Stellos M., Ph.D., University of Maine, Orono
Thirion, J.-P., Ph.D., Centre Hospitalier Universitaire, Sherbrooke, Canada

SEMINARS

van Montagu, M., Rijksuniversiteit Gent. Agrobacterium and crown gall disease.
Sims, T., University of California, Los Angeles. Soybean embryonic genes.
Gengenbach, B., University of Minnesota. Embryogenesis in *Zea* tissue cultures.
———. Mutant selection in *Zea* tissue cultures.
Broglie, R., L. Hanley-Bowdoin, Rockefeller University. Nuclear and chloroplast gene regulation.
Walbot, V., Stanford University. Developmental genetics of maize.
Long, S., Stanford University. *Rhizobium*-legume developmental genetics.
Quail, P., University of Wisconsin. Phytochrome gene expression.
Lonsdale, D., Plant Breeding Institute. The mitochondrial genome.
Crouch, M., University of Indiana. Compatibility/incompatibility systems.
Phillips, R., University of Minnesota. Somaclonal variation.
———. Cytogenetics of plant cells in culture.
Ellingboe, A., University of Wisconsin. Genetics of plant-pathogen interactions.
Hanson, M., University of Virginia. Organelle genetics.
Ho, D., Washington University. Hormone response mutants.
Palukaitis, P., Cornell University. Plant viruses.
Miles, D., University of Missouri. Photosynthesis.
Dooner, H., Advanced Genetic Sciences. Controlling elements of maize.
Binns, A., University of Pennsylvania. Biology of transformed plants.

Molecular Cloning of Eukaryotic Genes

June 8–June 28

INSTRUCTORS

Bothwell, Al, Ph.D., Yale Medical School, New Haven, Connecticut
Alt, Fred, Ph.D., Columbia University College of Physicians & Surgeons, New York, New York
Mullins, James, Ph.D., Harvard School of Public Health, Boston, Massachusetts

ASSISTANTS

LeClair, Ken, B.A., Yale Medical School, New Haven, Connecticut
Yancopoulos, George, B.A., Columbia University, New York, New York
Bruck, Claudine, Ph.D., Harvard Medical School, Boston, Massachusetts

This laboratory and lecture course covered the principles of recombinant DNA technology and the application of these procedures to the study of eukaryotic genes. The isolation and characterization of lymphocyte-specific genes were emphasized. Among the topics covered were: construction of cDNA libraries in plasmid or bacteriophage λ vectors, construction of bacteriophage λ and cosmid libraries of high-molecular-weight eukaryotic DNA, screening DNA libraries with gene-specific hybridization probes and by recombination, purification, and characterization of recombinant clones using restriction endonuclease and blot hybridization analyses, and reintroduction and expression of cloned genes in heterologous systems. Strategies for isolating genes that encode rare mRNA sequences were discussed. Guest lecturers discussed the application of molecular cloning procedures to the study of specific eukaryotic gene systems.

PARTICIPANTS

Bignami, Margherita, Ph.D., Istituto Superiore Di Sanita, Rome, Italy
Binninger, Dorothea, M.S., Paul-Ehrlich-Institut, Frankfurt, Federal Republic of Germany
Cox, Diane W., Ph.D., Hospital for Sick Children, Toronto, Canada
Crittenden, Lyman B., Ph.D., USDA Regional Poultry Research Laboratory, East Lansing, Michigan
Foster, Patricia L., Ph.D., Boston University School of Public Health, Massachusetts
Iscove, Norman N., Basel Institute for Immunology, Switzerland
Jalkanen, Markku T., Ph.D., Stanford University Medical Center, California
Kasuga, Masato, M.D., University of Tokyo, Japan
Lever, Julia E., Ph.D., University of Texas Medical School, Houston
Nelson, David L., M.D., National Cancer Institute, Bethesda, Maryland
ole-MoiYoi, Onesmo, M.D., International Laboratories for Research on Animal Diseases, Nairobi, Kenya

Prusiner, Stanley B., M.D., University of California, San Francisco
Remold, Heinz G., Ph.D., Harvard Medical School, Boston, Massachusetts
Schuurmann, R.K.B., Ph.D., University Hospital Leiden, The Netherlands
Sidman, Charles L., Ph.D., Jackson Laboratory, Bar Harbor, Maine
Zingales, Bianca, D.Sc., Instituto De Quimica, Brazil

SEMINARS

Lehrach, H., EMBL. Molecular cloning of the T Locus.
Botstein, D., Massachusetts Institute of Technology. Reverse genetics: cloning of cytoskeletal genes.
Goff, S., Columbia University. In vitro mutagenesis of retroviral genes.
Reth, M., Columbia University. Antibodies and Western blotting.
Baltimore, D., Massachusetts Institute of Technology. Expression of Ig genes introduced into the germline.
Miyada, G., City of Hope Hospital. Use of synthetic oligonucleotides for cloning and expression of murine MHC genes.
Lacy, E., Memorial Sloan-Kettering Cancer Center. Gene transfer into mice.
Rubin, J., University of California, Berkeley.P-element-mediated gene transfer in *Drosophila*.
Schimke, B., Stanford University. Molecular mechanisms of gene amplification.
Maniatis, T., Harvard University. Globin gene transcription and RNA splicing.
Schwarzbauer, J., Massachusetts Institute of Technology.Transfer of genes into hematopoeitic stem cells.
Seed, B., Massachusetts General Hospital. Recovery and manipulation of recombinant DNA in vivo.
Gluzman, Y., Cold Spring Harbor Laboratory. Eukaryotic cloning vectors.
Hughes, S., Frederick Cancer Research Facility. Oncogenes.
Messing, J., University of Minnesota. M13 vectors.
McKnight, S., Hutchinson Cancer Research Institute. Regulation of transcription.
Wahl, G., Salk Institute. Gene transfer technology.
Hamyln, J., University of Virginia. Isolation of eukaryotic origins of replication.
Mullins, J., Harvard School of Public Health. FeLV, AIDS, and HTLV.
Ruley, E., Cold Spring Harbor Laboratory. Transformation and oncogenesis.
Wigler, M., Cold Spring Harbor Laboratory. Yeast *RAS* genes.
Szostak, J., Dana-Farber Cancer Institute. Replication and segregation of linear chromosomes in yeast.

Neurobiology of Human Disease

June 8–June 17

INSTRUCTORS

Breakfield, Xandra O., Ph.D., E.K. Shriver Center and Harvard Medical School, Boston, Massachusetts
Black, Ira B., M.D., Cornell University School of Medicine, New York, New York
Gusella, James, Ph.D., Massachusetts General Hospital, Boston

This intensive seminar course explored the molecular and cellular basis of abnormal neural function. It focused on basic scientific studies that have provided insight into the etiology and pathogenesis and neurologic and psychiatric diseases. Topics included: (1) molecular pathology of neurotransmitter derangement; (2) developmental plasticity and choice of neurotransmitter phenotype; (3) synthesis and regulation of neuropeptides; (4) cellular events in neural regeneration and brain transplantation; (5) neural pathways involved in pain syndromes; (6) genetic linkage analysis using DNA polymorphisms; (7) defects in DNA repair and activation of *onc* genes; (8) biochemistry of the lipidoses; (9) autoimmune diseases; (10) brain imaging and metabolism; (11) epilepsy and seizure disorders; (12) cell death in degenerative disorders; (13) viral infections of the nervous system; and (14) experimental models of learning and memory.

PARTICIPANTS

Arias, Clorinda, M.D., Universidad Nacional Autonoma de Mexico, Mexico City
Bellini, Fabrizio, Ph.D., Fidia Research Laboratories, Terme, Italy
Cambi, Franca, M.D., E.K. Shriver Center, Harvard Medical School, Boston, Massachusetts
Desan, Paul, Ph.D., Harvard Medical School, Boston, Massachusetts
Foxman, Brett T., M.D., Cornell Medical Center, New York, New York
Kagan, Bruce L., Ph.D., University of

California Neuropsychiatric Institute, Los Angeles
Kremer, Norbert E., Ph.D., State University of New York, Stony Brook
Kurlan, Roger M., M.D., University of Rochester Medical Center, New York
Maehlen, Jan, M.D., Institute of Neurophysiology, Oslo, Norway
Mangun, George R., B.S., University of California, San Diego
Moroi, Sayoko E., B.A., Medical Administration Center, Columbus, Ohio
Nakajima, Gene A., M.D., Johns Hopkins Medical School, Baltimore, Maryland
Oliver, Eugene J., Ph.D., National Institutes of Health, Bethesda, Maryland
Sanes, Dan H., M.A., Princeton University, New Jersey
Segal, Rosalind A., B.A., Rockefeller University, New York, New York
Sims, Stephen J., B.A., Downstate Medical Center, New York, New York
Singh, Dyal N.P., Ph.D., University of Alberta, Canada

SEMINARS

Gage, F., Lund University. Brain transplantation.
Aguayo, A., Montreal General Hospital. Neural regeneration.
Horwich, A., Yale University School of Medicine. Molecular genetic approaches to organic acidemias.
Sokoloff, L., National Institute of Mental Health. Brain metabolism and imaging.
Phelps, M., University of California Medical School, Los Angeles. Brain metabolism and imaging.
Coyle, J., Johns Hopkins University School of Medicine. Movement disorders and degenerative diseases: experimental models.
Wexler, N., Hereditary Disease Foundation. Huntington's disease in Venezuela.
Ginns, E., National Institutes of Health. Lipidoses.
Basbaume, A., University of California, San Francisco. Pain syndromes and neuropeptides.
Jessell, T., Harvard Medical School. Pain syndromes and neuropeptides.
Herbert, E., University of Oregon. Neuroendocrinology.
Lindstrom, J., Salk Institute. Autoimmune diseases: myasthenia gravis.
McNamara, J., Duke University Medical Center. Epilepsy and seizure disorders.
Crill, W., University of Washington School of Medicine. Epilepsy and seizure disorders.
Hawkins, R., Columbia University College of Physicians and Surgeons. Learning and memory: experimental models.
Mishkin, M., National Institute of Mental Health. Learning and memory: primate models.
Gazziniga, M., Cornell University Medical College. Cognitive disorders.
Haase, A., Veterans Administration Medical Center, San Francisco. Viral infections: latency and defective particles.
Fields, B., Harvard Medical School. Neurotropic viruses.

Advanced Bacterial Genetics

July 1–July 21

INSTRUCTORS

Bear, Susan, Ph.D., National Institutes of Health, Bethesda, Maryland
Berman, Michael, Ph.D., Frederick Cancer Research Facility, Frederick, Maryland
Enquist, Lynn, Ph.D., National Institutes of Health, Bethesda, Maryland
Silhavy, Thomas, Ph.D., Frederick Cancer Research Facility, Frederick, Maryland

ASSISTANTS

Trun, Nancy, Frederick Cancer Research Facility, Frederick. Maryland
Zagursky, Bob 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 region; however, the experimental techniques presented are sufficiently general to be applicable to any gene in *E. coli* for which there exists a mutation conferring a recognizable phenotype.

PARTICIPANTS

Bardwell, James, B.S., University of Wisconsin, Madison
Booth, Ian R., Ph.D., University of Aberdeen, Scotland
Edwards, Mary Francis, B.S., Stanford University, California
Gardiol, Alicia E., B.S., Michigan State University, East Lansing
Hsiung, Hansen M., Ph.D., Eli Lilly and Co., Indianapolis, Indiana
Jones, Helen M., B.S., University of California, Los Angeles
Kirschner, Richard J., Ph.D., Upjohn Co., Kalamazoo, Michigan
Niesel, David W., Ph.D., University of Texas Medical Branch, Galveston
Olmedo, Gabriela, B.S., Centro De Estudios Avanzados del I.P.N., Mexico City, Mexico
Sanfacon, Helene, B.Sc., Laval University, Quebec, Canada
Selvaraj, Gopalan, Ph.D., Carleton University, Ottawa, Canada
Seto-Young, Donna L.T., Ph.D., National Research Council of Canada, Ottawa
Skvirsky, Rachel C., Ph.D., Simmons College, Boston, Massachusetts
Stout, Valerie G., B.S., Kansas State University, Manhattan
Vary, Patricia S., Ph.D., Northern Illinois University, De Kalb
Welkos, Susan L., Ph.D., U.S. Army Medical Research Institute, Frederick, Maryland

SEMINARS

Austin, S., Frederick Cancer Research Facility. Stability and maintenance of bacterial plasmids.
Howe, M., University of Wisconsin. Bacteriophage Mu: properties and uses.
Friedman, D., University of Michigan. Protein and nucleic acid signals in transcription termination and antitermination.
Wolf, R., Jr., University of Maryland. Growth-rate-dependent regulation of an *E. coli* central metabolism gene, *gnd*.
Youdarian, P., University of Southern California. Genetic analysis of protein-DNA interactions.
Beckwith, J., Harvard Medical School. Genetic studies on the basis of the specificity of a DNA binding protein, CAP.
Roth, J., University of Utah. Selection and analysis of bacterial chromosome inversions.

Neurobiology of *Drosophila*

July 1–July 21

INSTRUCTORS

Jan, Lily Yeh, Ph.D., University of California, San Francisco
Jan, Yuh Nung, Ph.D., University of California, San Francisco
O'Farrell, Patrick H., Ph.D., University of California, San Francisco
Greenspan, Ralph J., Ph.D., Princeton University, New Jersey

ASSISTANT

Royden, Stanzi, University of California, San Francisco

This laboratory/lecture course was designed for people who may want to use *Drosophila* as an experimental system for studying function or development of the nervous system. One major aim was to introduce students to the various genetic, molecular, and physiological techniques that are currently available in *Drosophila* research and that make it distinctive.

The course began with a crash course on *Drosophila* genetics (a series of lectures supplemented with laboratory demonstrations and exercises) to familiarize the students with classical genetics of *Drosophila*, strategies of mutant isolation, methods used to analyze newly isolated mutations, and mosaic analysis. This was followed by workshops and laboratory projects on molecular genetics and

electrophysiology. In the area of molecular genetics topics covered were strategies and techniques for cloning genes, in situ hybridization to polytene chromosomes, PM hybrid dysgenesis, and DNA-mediated transformation, while the electrophysiology section covered neuromuscular transmission and voltage clamp analysis, as applied to mutants with altered ion channels. Additionally, a series of lectures by invited speakers illustrated the application of these techniques to current research. The research topics included: molecular and functional analysis of mutants with distinctive ion channels, establishment of neuronal pathways in the developing embryo as well as in imaginal discs, analysis of behavioral mutants, maternal and zygotic mutations that alter polarity and segmentation, homeotic mutations, and the use of DNA transformation for the study of gene expression.

PARTICIPANTS

Bredesen, Dale, M.D., San Francisco General Hospital, California
Burnell, Anne, M.Sc., St. Patrick's College, Kildare, Ireland
Caudy, Michael, B.S., University of California, Berkeley
DiNardo, Stephen, Ph.D., University of California, San Francisco
Hasan, Gaiti, Ph.D., Tata Institute of Fundamental Research, Bombay, India
Nash, Howard, Ph.D., National Institutes of Mental Health, Bethesda, Maryland
Pollock, John, Ph.D., Syracuse University, New York
Roof, Dorothy, Ph.D., Purdue University, Lafayette, Indiana
Shelton, David, B.A., University of California, San Francisco
Taylor, Barbara, B.A., University of California, San Diego

SEMINARS

Hall, J., Brandeis University. Mutations affecting sexual behavior and circadian rhythm.
Timpe, L., University of California, San Francisco. Voltage-clamp studies of *Drosophila* mutants.
Quinn, W., Princeton University. Learning mutants.
Pak, W., Purdue University. Visual mutants.
Spradling, A., Carnegie Institution of Washington. Hybrid dysgenesis and P-element transformation.
———. Use of transformation to dissect functional elements, chorion amplification.
Cherbas, P., R. Schultz, Harvard University. Complexities of eukaryotic transcription units and techniques for studying transcription.
———. Ecdysone-inducible transcription.
Levine, M., University of California, Berkeley. In situ localization of transcripts.
———. Engrailed and homeotic loci.
Pardue, M.L., Massachusetts Institute of Technology. Cytogenetics.
Bender, W., Harvard Medical School. Molecular biology of mutation.
———. Combining classical genetics with molecular biology, homeotic mutations.
Ghysen, A., University Libre de Bruxelles. Sensory projections in homeotic mutants.
Wyman, R., Yale University. Early embryogenesis, segmentation mutants.
Thomas, J., Stanford University. Neurogenesis in embryos.
Horvitz, R., Massachusetts Institute of Technology. Cell lineage in nematode.
Goodman, C., Stanford University. Pathway formation in grasshopper embryos.

Molecular Biology of the Nervous System

July 5–July 18

INSTRUCTORS

Kelly, Regis B., Ph.D., University of California, San Francisco
McKay, Ronald D., Ph.D., Cold Spring Harbor Laboratory, New York

The technologies of patch-clamping and immunochemistry have revolutionized molecular neurobiology. Increasingly, the tools of recombinant DNA technology and molecular genetics are being used to identify and characterize molecules involved in neuronal function and development. The aim of this lecture course was to familiarize the participants with recent developments in molecular biology and to discuss their application to neurobiological problems.

The following topics were discussed: patch-clamping living cells and channels in bilayers, gene cloning, DNA sequencing, regulation of gene expression, in vitro mutagenesis, expressing foreign genes in heterologous hosts, in situ hybridization, transposable elements and hybrid dysgenesis, in vitro immunization, anti-idiotype antibodies, T cell/B cell interaction, new optical and electron microscope techniques, the cytoskeleton, organelle sorting and movement, regulation of transmitter release, growth factors, cell lineages in development, assaying synaptogenesis in vitro, surface molecules and the extracellular matrix.

PARTICIPANTS

Amador, Roberto, M.D., Rockefeller University, New York, New York
Audigier, Yves, Ph.D., CNRS-INSERM de Pharmacologie/Endocrinologie, Montpellier, France
Baldwin, Timothy, B.Sc., University College London, England
Breer, Heinza, Ph.D., University Osnabruck, Federal Republic of Germany
Calof, Anne, M.S., University of California School of Medicine, San Francisco
Cohen-Cory, Susana, B.S., Centro de Investigation sobre Ingenieria Genetica y Biotecnologia, UNAM, Mexico City, Mexico
Earnest, Thomas, M.A., Boston University, Massachusetts
Estacion, Mark, B.S., University of California, Irvine
Hescheler, Jurgen, Universität des Saarlandes, Hamburg, Federal Republic of Germany
Hsiao, Karen Ke-jui, Ph.D., University of California, San Francisco
Jennings, Charles, B.A., University College of London, England
Kadan, Michael, B.S., Johns Hopkins University, Baltimore, Maryland
La Gamma, Edmund, M.D.. Cornell University, Ithaca, New York
Lai, Yvonne, M.S., Rockefeller University, New York, New York
Maelicke, Alfred, Ph.D., Max-Planck Institut, Dortmund, Federal Republic of Germany
McCabe, Joseph, Ph.D., Rockefeller University, New York, New York
Michelsohn, Arie, B.A., California Institute of Technology, Pasadena
Miller, Stephen, B.S., California Institute of Technology, Pasadena
Sarlieve, Louis, Ph.D., INSERM et Centre de Neurochimie du CNRS, Strasbourg, France
Silverstein, Faye, M.D., University of Michigan, Ann Arbor
Tipnis, Ulka, Ph.D., University of Texas Health Science Center, Dallas

SEMINARS

Claudio, T., Columbia University, R. Scheller, Stanford University. Characterization of DNA. Cloning.
———. Northerns. Southerns. Restriction maps.
———. Mapping. Synthetic DNA. cDNA and genomic libraries.
———. Sequencing DNA. Electron microscopy.
———. Hybridization section.
Angerer, R., University of Rochester. In situ hybridization.
Claudio, T., Columbia University. Introducing foreign genes into cells.
Costantini, F., E. Lacy, Columbia University. Introducing foreign genes into the germ line.
Sharp, P., Massachusetts Institute of Technology. Introduction to eukaryotic gene expression: processing on the mRNA level.
Maniatis, T., Harvard University. Role of DNA sequences in regulating gene expression.
Evans, R., Salk Institute. Genomic expression and regulation in bacteria.
Scheller, R., Stanford University, R. Evans, Salk Institute. Current molecular research on the nervous system.
Kennedy, M., California Institute of Technology. Protein phosphorylation.
Solomon, F., Massachusetts Institute

of Technology. Cytoskeleton modification.
Siegelbau, S., Columbia University. Phosphorylation and learning.
Gefter, M., Massachusetts Institute of Technology. Nature of the immune response.
———. Cell interactions in the immune response.
Schinnick, T., Scripps, Clinic and Research Center. Synthetic peptides as antigens.
Alt, F., Columbia University. Molecular basis of antibody diversity.
Lindstrom, J., Salk Institute. Functional domains of the AChR.
Aldrich, R., Yale University. Introduction to patch-clamping.
Miller, C., Brandeis University. Reconstitution of channels in membranes.
White, M., California Institute of Technology. Synthesizing and injecting mRNA.
Khorana, G., Massachusetts Institute of Technology. Genetic modification of a proton pump.
Bender, W., Harvard University. Genetic approaches to development using *Drosophila*.
Jan, L., University of California. Identifying the K^+ channel in *Drosophila*.
Gusella, J., Massachusetts General Hospital. Mapping human genetic defects.
Horvitz, R., Massachusetts Institute of Technology. Genetics of nematode development.

Molecular Embryology of the Mouse

July 8–July 21

INSTRUCTORS

Hogan, Brigid, Ph.D., ICRF, Mill Hill, England
Costantini, Frank, Ph.D., Columbia University College of Physicians & Surgeons, New York, New York
Lacy, Liz, Ph.D., Memorial Sloan-Kettering Cancer Center, New York, New York

ASSISTANTS

Robertson, Liz, University of Cambridge, England
Chada, Kiran, 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 in early embryos was stressed. Through laboratory exercises and lectures, the participants were introduced to the following procedures and their possible applications: the isolation and in vitro culture of germ cells and preimplantation and early postimplantation embryos, the microinjection of DNA into fertilized eggs, nuclear transplantation, the formation of chimeras, dissection of 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.

PARTICIPANTS

Brulet, Philippe, Ph.D., Institut Pasteur, Paris, France
Emerson, Beverly, Ph.D., National Institutes of Health, Bethesda, Maryland
Gimlich, Robert, B.A., University of California, Berkeley
Gross, Kenneth, Ph.D., Roswell Park Memorial Institute, Buffalo, New York
Linney, Elwood, Ph.D., La Jolla Cancer Research Foundation, California
Mendel, Jane, B.A., Massachusetts Institute of Technology, Cambridge
Murray, Ben, Ph.D., Rockefeller University, New York, New York
Nepveu, Alain, Ph.D., Centre Hospitalier Universitaire, Sherbrooke, Canada
Selsing, Erik, Ph.D., Brandeis University, Waltham, Massachusetts
Sorge, Joe, M.D., Scripps Research Institute, La Jolla, California
Vogt, Thomas, B.S., Fox Chase Cancer Institute, Philadelphia, Pennsylvania
Weaver, David, Ph.D., Massachusetts Institute of Technology, Cambridge
Kollias, George, National Institute of Medical Research, London, England

SEMINARS

Wasserman, P.M., Harvard Medical School. Nature and expression of the egg's receptor for sperm.

Damsky, C., Wistar Institute. Cell adhesion molecules in embryonic tissues.

Chapman, V., Roswell Park Memorial Institute. Cell adhesion molecules in embryonic tissues.

Silvery, L., Cold Spring Harbor Laboratory. Mouse genetics.

Stuhlman, H., Whitehead Institute. Microinjection.

Copeland, N., University of Cincinnati. Demonstration of blastocyst injection.

Jenkins, N., University of Cincinnati. Uterine transfer.

Solter, D., Wistar Institute. Post-implantation mouse development.

———. Transnuclear mice.

Silver, L., Cold Spring Harbor Laboratory. The T complex.

McLaren, A., MRC Mammalian Development Unit. Origin and differentiation of germ cells.

———. Sex determination.

Mulligan, R., Whitehead Institute. Retroviral vectors.

Adamson, E., La Jolla Cancer Center. Expression of growth factors, their receptors, and oncogenes in mouse embryos and teratocarcinoma cells.

Rigby, Imperial College. Microinjection.

Posakony, J., Harvard University. Gene insertion in *Drosophila*.

Developmental Neurobiology

July 20–August 2

INSTRUCTORS

Purves, Dale, M.D., Washington University School of Medicine, St. Louis, Missouri
Patterson, Paul H., Ph.D., California Institute of Technology, Pasadena

The aim of this intensive two-week course is to expose a group of approximately 20 students to the history of ideas and classical literature on neural development, with special emphasis on recent advances and controversies in this rapidly growing field. The course is offered every other year, and the session in 1984 represented its fourth cycle. In general, the information covered is not readily available in a coherent form to students (especially those from abroad) because (1) many important results are quite new, and (2) results both old and new rely on a wide variety of technical approaches and are therefore not encompassed in any systematic way in courses arranged along conventional lines (e.g., courses on neurocytology and neurophysiology). The course on developmental neurobiology seeks to fill this gap inviting about 15 guest lecturers, distinguished by their didactic skills as well as by their scientific accomplishments, to present their area of interest to students in a relatively informal setting where student-faculty interchange and discussion are much encouraged. The lecturers are required to teach their material as broadly as possible so the students will take away some idea of the overall direction of research in neural development rather than specific accounts of the endeavors of a few individuals.

This year's class of 23 students (a somewhat larger number of students were accepted in 1984 because of the quantity of outstanding applicants) was composed of 7 women and 16 men from 7 countries. The students were about equally divided between postdoctoral fellows and advanced graduate students already involved in some phase of developmental neurobiology. The research interests of the students were extremely varied, ranging from behavior to molecular biology; the only common denominator was an interest in pursuing research related to neural development.

PARTICIPANTS

Bovolenta, Paola, Ph.D., New York University, New York
Buxbaum, Joseph, M.S., Weizmann Institute, Rehovot, Israel
Camp, Ann, B.A., Vanderbilt University, Nashville, Tennessee
Coopersmith, Robert, B.S., University of California, Irvine
DeGennaro, Louis, Ph.D., Max-Planck-Institut, Martinsried. Federal Republic of Germany
Ferrari, Giovanna, Ph.D. New York University, New York
Fladby, Tormod, University of Oslo, Norway
Geijo, Emilio, M.D., University of Alicante, Spain
Hitchcock, Peter, Ph.D., Salk Institute, San Diego, California
Janowsky, Jeri, B.A., Cornell Medical Center, New York, New York
LaMantia, Anthony-Samuel, B.A., Yale University, New Haven, Connecticut
Lefcort, Francis, B.A., University of California, Berkeley
Levy, Daniel, Ph.D., Harvard University, Cambridge, Massachusetts
Lo, Donald, B.S., Yale University, New Haven, Connecticut
Mackler, Scott, B.A., University of Pennsylvania, Philadelphia
Mikkelsen, Bente, M.D., University of Oslo, Norway
New, Helen, B.S., University College London, England
Ross, Jenny, B.S., University of Otago, New Zealand
Schrank, Ethan, B.S., University of North Carolina, Chapel Hill
Stretavan, David, B.S., Stanford University, California
Tessier-Levigne, Marc, B.S., University College London, England
Voyvodic, James, B.S., Washington University, St. Louis, Missouri
Wolf, Werner, M.D., Max-Planck-Institut, Göttingen, Federal Republic of Germany

SEMINARS

Jacobson, M., University of Utah. Early events in embryogenesis.
Rakic, P., Yale University. Cell proliferation and neuronal migration.
Bate, M., Cambridge University. Gradients, compartments, and positional information in morphogenesis.
Horvitz, R., Massachusetts Institute of Technology. Studies of cell lineage in *C. elegans*.
Goodman, C., Stanford University. Development of the grasshopper nervous system.
Stent, G., University of California, Berkeley. Development of the leech nervous system.
Bray, D., Kings College. The growth cone and mechanisms of axon elongation.
Landmesser, L.T., University of Connecticut. Axon outgrowth in vertebrates.
Goodman, C., Stanford University. Axon outgrowth in invertebrates.
Aguayo, A., McGill University. Axon elongation in the central and peripheral nervous systems of mammals: the role of glial cells and other factors.
Greene, L., New York University Medial Center. Studies of the mechanism of action of nerve growth factor.
Sanes, J.R., Washington University School of Medicine. Formation of synapses in the central and peripheral nervous system.
Easter, S., University of Michigan. Specificity in the retinotectal system.
Edelman, G., Rockefeller University. CAM and its role in development.
Nirenberg, M., National Heart and Lung Institute. A possible molecular basis of positional information.
Shatz, C., Stanford University School of Medicine. Development of the mammalian visual system.
Hildebrand, J., Columbia University. The development of behavior in insects.
Nottebohm, F., Rockefeller University. The development of birdsong and its relevance to learning.

Advanced Electrophysiological Methods

July 24–August 13

INSTRUCTORS

Ascher, Phillippe, D. Sc., Ecole Normale Superieure, Paris, France
Schuetze, Stephen, Ph.D., Columbia University, New York, New York
Siegelbaum, Steven, Ph.D., Columbia University, New York, New York

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.

PARTICIPANTS

Chuman, Mary, Ph.D., Rush Presbyterian St. Luke's Medical Center, Chicago, Illinois
Frederiksen, Kristen, M.S., Cold Spring Harbor Laboratory, New York
Hay, Bruce, B.A., University of California, San Francisco
Madison, Daniel, Ph.D., University of California, San Francisco
Marshall, Cameron, B.S., Brown University, Providence, Rhode Island
Pitchford, Simon, B.S., University of Bristol, England
Sigihara, Izumi, M.D., Tokyo Medical School, Japan
Tobin, Thomas, Ph.D., State University of New York Downstate Medical Center, New York
Worley, Jennings, B.S., University of Maryland, College Park
Zeitoun, Irene, M.S., Tel Aviv University, Israel

Advanced Techniques in Molecular Cloning

July 24–August 13

INSTRUCTORS

Atkinson, Thomas, B.S., University of British Columbia, Vancouver, Canada
Fiddes, John, Ph.D., California Biotechnology, Inc., Palo Alto
Shortle, David,, Ph.D., State University of New York, Stony Brook
Smith, Michael, Ph.D., University of British Columbia, Vancouver, Canada
Zoller, Mark, Ph.D., Cold Spring Harbor Laboratory, New York

ASSISTANT

Pielak, Gary, University of British Columbia, Vancouver

This was a laboratory and lecture course on advanced aspects of molecular cloning designed for scientists who are familiar with basic recombinant DNA techniques. It included the chemical synthesis of oligodeoxyribonucleotides of defined sequence and their characterization by the Maxam-Gilbert sequencing

method. These oligonucleotides were used in various ways, including their use as probes for the isolation of specific genes from libraries and as reagents for directing site-specific in vitro mutagenesis. Specific mutants were isolated, and the sequence of the altered genes was determined. Other methods of in vitro mutagenesis such as nucleotide misincorporation into DNA, sodium bisulfate treatment of DNA, and linker insertion were covered in both laboratory exercises and lectures.

Guest lectures and demonstrations covered the application of these techniques to analysis of various cloned genes, as well as the use of alternate methods of mutagenesis, expression, and analysis of cloned genes by immunological screening.

PARTICIPANTS

Cane, Jerome, B.S., Indiana University, Bloomington

Chaudhuri, Bhabatosh, Ph.D., Friedrich Miescher Institut, Basel, Switzerland

Dean, Carolyn, Ph.D., Advanced Genetic Sciences, Oakland, California

Geller, Ruth, Ph.D., Walter Reed Army Institute of Research, Washington, D.C.

Giroux, Craig N., Ph.D., NIEHS, Research Triangle Park, North Carolina

Goff, Stephen, Ph.D., Harvard Medical School, Boston, Massachusetts

Hilt, Dana, M.D., National Institutes of Health, Bethesda, Maryland

Hopper, Peggy, B.S., Tufts University, Medford, Massachusetts

Mostov, Keith, Ph.D., Rockefeller University, New York, New York

Mroczkowski, Barbara, Ph.D., Vanderbilt University, Nashville, Tennessee

Rdest, Ursula, Ph.D., University of Wurzberg, Federal Republic of Germany

Robinson, Mary Ann, Ph.D., National Institutes of Health, Bethesda, Maryland

SEMINARS

Gumport, R., University of Illinois. The role of homology in bacteriophage λ site-specific recombination.

Goff, S., Columbia University. Mutations in the *gag* and *pol* genes of Moloney murine leukemia virus.

Bogenhagen, Daniel, State University of New York, Stony Brook. Transcription of vertebrate mitochondrial DNA.

Russell, D., University of Texas, Dallas. The structure of human low-density lipoprotein receptor.

Davis, M., Stanford University. Isolation and characterization of the T-cell receptor gene.

Essigmann, J., Massachusetts Institute of Technology. Extrachromosomal probes for mutagenesis by chemical carcinogens.

Meyers, R., Harvard University. Saturation mutagenesis of eukaryotic regulatory sequences.

Yeast Genetics

July 24–August 13

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

Chiu, Isabel, 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.

PARTICIPANTS

Bibus, Claudia, B.A., University of Basel, Switzerland
Butt, Tauseef, Ph.D., Smith Kline & French Laboratories, Philadelphia, Pennsylvania
Chin, Jean, Ph.D., Harvard University, Cambridge, Massachusetts
Eisenberg, Shlomo, Ph.D., Weizmann Institute, Rehovot, Israel
Folger, Kim, Ph.D., University of Utah, Salt Lake City
Harmony, Judith, PH.D., University of Cincinnati, Ohio
LaPorte, David, Ph.D., University of Minnesota, Minneapolis
Lautenberger, Jams, Ph.D., National Cancer Institute, Bethesda, Maryland
Martin, Charles, Ph.D., Rutgers University, New Brunswick, New Jersey
Nelson, Daniel, Ph.D., University of Houston, Texas
Nolan, Catherine, Ph.D., National Cancer Institute, Bethesda, Maryland
Pines, Ophrey, Ph.D., State University of New York, Stony Brook
Rosendahl, Mary, Ph.D., Rockefeller University, New York, New York
Sawadogo, Michele, Ph.D., Rockefeller University, New York, New York
Varshavsky, Alexander, Ph.D., Massachusetts Institute of Technology, Cambridge

SEMINARS

Fox, T., Cornell University. Mitochondrial genetics.
Warner, J., Albert Einstein College of Medicine. Ribosomal genes: structure and regulation.
Kolodner, R., Dana Farber Cancer Institute. Genetic recombination catalyzed by cell-free extracts.
Wigler, M , Cold Spring Harbor Laboratory. *RAS* oncogenes in yeast.

Cantor, C., Columbia University. Separation of chromosome-sized DNA molecules.

Fitzgerald-Hayes, M., University of Massachusetts. Base-specific mutations affecting centromere functions.

Carlson, M., Columbia University. Regulation and structure of yeast invertase genes.

Lindquist, S., University of Chicago. Yeast sporulation: heat shock proteins and a whole lot more.

Tye, B.-K., Cornell University. Functional components of yeast chromosomes.

Broach, J., State University of New York, Stony Brook. Control of plasmid replication in yeast.

Zoller, M., Cold Spring Harbor Laboratory. Mutagenesis of the HO cut site.

Botstein, D., Massachusetts Institute of Technology. Genetics of the cytoskeleton and cell cycle.

Szostak, J., Dana Farber Cancer Institute. Yeast telomeres and artificial chromosomes.

Silver, P., Harvard University. Nuclear localization of proteins in yeast.

Prakash, S., University of Rochester. Structure and regulation of DNA repair genes.

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.

1983–1984

September

Tom Curran, Salk Institute, San Diego, California: A comparative analysis of the viral and cellular *fos* oncogenes.

Richard Treisman, Harvard University, Cambridge, Massachusetts: Expression of cloned globin genes in cultured cells.

October

James Alwine, University of Pennsylvania, Philadelphia: Control of Simian virus 40 late gene expression.

Edward Trifonov, Weisman Institute, Rehovot, Israel: Typical secondary structures around splicing junctions.

Pat Hearing, State University of New York, Stony Brook: Analysis of the Ad5 E1A-transcriptional control region and sequences required for packaging of viral DNA.

Rob Jackson, Albert Einstein College of Medicine, Bronx, New York: A genetic analysis of voltage sensitive sodium channels in *Drosophila*.

Pat Levitt, Medical College of Pennsylvania, Philadelphia: Molecular specificity in stem cells in functional systems of the brain.

Sayeeda Zain, University of Rochester School of Medicine and Dentistry, New York:

I. Two different human liver mRNAs encode serum albumin.

II. Development of a simple and efficient in vitro mRNA splicing system.

November

Eric Hunter, University of Alabama, Birmingham: Mutations that alter the biosynthesis of the Rous sarcoma virus envelope glycoprotein.

Saran Narang, National Research Council of Canada, Ottawa: Total synthesis, cloning, and expression of human preproinsulin.

Nils Loneberg, Harvard University, Cambridge, Massachusetts: Pyruvate kinase genes.

Elizabeth Blackburn, University of California, Berkeley: Molecular structures of telomeres in lower eukaryotes.

Paul Ludden, University of Wisconsin, Madison: Regulation of nitrogenase in photosynthetic bacteria.

David Lowe, University of Toronto, Canada: The mouse *hsp 70* gene family: Cloning of cDNAs from inducible and constitutive mRNAs.

Rob Jackson, Albert Einstein College of Medicine, Bronx, New York: A genetic analysis of voltage sensitive sodium channels in *Drosophila*.

Claude Dery, University of Sherbrooke, Quebec, Canada: Structure of adenovirus chromatin in lytic infection.

December

Michael Young, Rockefeller University, New York, New York: Transposable elements and mutations of a complex locus in *Drosophila*.

Dirk Elseviers, New York University Medical College, New York: Readthrough of nonsense codons: A tool for genetics of proteins.

Neil Copeland, University of Cincinnati, Ohio: Endogenous murine retroviruses: Molecular probes for dissecting the mammalian genome.

Nancy Jenkins, University of Cincinnati, Ohio: Retroviruses as insertional mutagens of mice.

Fred Alt, Columbia University, New York: Rearrangement of endogenous and introduced heavy chain variable region gene segments in pre-B cell lines.

David Grumwald, University of Oregon, Eugene: Genetics of zebra fish: A new approach to the study of vertebrate development.

January

Hans Lehrach, European Molecular Biology Laboratory, Heidelburg, Federal Republic of Germany: Molecular studies of mouse chromosome 17.

Joe Moscal, National Institutes of Health, Bethesda, Maryland: Characterization and purification of a topographically distributed molecule in chick retina.

Richard Novick, Public Health Research Institute of the City of New York, New York: Counter transcription regulation of plasmid replication.

Bertrand Jordan, Center of Immunology, Marseille, France: Structure and expression of human histocompatibility genes.

James McCubrey, Basel Institute for Immunology, Sweden: Gene transfer experiments with the functionally rearranged immunoglobulin genes.

February

Joanne Williams, University of California, San Diego: Reaction center genes from Rhodopseudomonas sphaeroides.

Walter Keller, German Cancer Research Center, Heidelberg, Federal Republic of Germany: In vitro splicing of mRNA precursors.

Malcolm Casadaban, University of Chicago, Illinois: In vivo genetics manipulations with bacteria phage Mu.

March

Marcello Siniscalco, Memorial Sloan-Kettering Cancer Center, New York, New York: Fragile sites of human chromosomes and meiotic recombination.

Michael A. Lischwe, Baylor College of Medicine, Houston, Texas: Nucleolar proteins: Phosphorylation, arginine methylation, immunofluorescence, and scleroderma antigen.

Charles Craik, University of California, San Francisco: Expression and site-specific mutagenesis of rat pancreatic trypsin.

April

Kirsten Fischer Lindahl, Basel Institute for Immunology, Sweden: Mitochondria and the mouse MHC.

Adrian Hayday, Massachusetts Institute of Technology Cancer Center, Cambridge: Activation of c-*myc* in human B-cell lymphoma.

Alan Senior, University of Rochester School of Medicine, New York: The use of mutants in studies of *E. coli* proton-ATPase.

Masayori Inouye, State University of New York, Stony Brook: A novel regulatory mechanism using an RNA transcript complementary to a specific mRNA (micRNA).

Rick Myers, Harvard University, Cambridge, Massachusetts: Saturation mutagenesis of eukaryotic regulatory sequences.

David Housman, Massachusetts Institute of Technology Cancer Center, Cambridge: Gene mapping in mouse and man.

Paul Bingham, State University of New York, Stony Brook: Molecular Genetics of *Drosophila* transposons.

Tom Braciole, Washington University, St. Louis, Missouri: Major histocompatability complex restricted cytotoxic T-cells: What do they see?

May

Steve Cheley, University of Western Ontario, London, Canada: Intracellular mouse hepatitis virus polypeptides and RNAs.

Leonello Bossi, University of Utah, Salt Lake City: Unusual conformation of the DNA in a region upstream from a bacterial promoter is associated with maximal levels of expression.

June

Dave Pauza, MRC University Medical School, Cambridge, England: Early events in human lymphocyte activation.

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, 260 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 the J.M. Foundation, Burroughs Wellcome Fund, and Bayer Aspirin (Glen Brook Labs). Students are listed below with their Laboratory sponsors and topics of research.

Mark Alfenito, Cornell University
Research Advisor: **S. Dellaporta**
Genetic and molecular study of maize controlling elements.

Catherine Chen, Massachusetts Institute of Technology
Research Advisor: **B. Stillman**
Analysis of the *deg* phenotype associated with mutations in the gene encoding the adenovirus E1B 19K tumor antigen.

Shinta Cheng, Yale University
Research Advisor: **F. Daldal**
Molecular genetics of cytochrome C_2 of *Rhodopseudomonas capsulata*.

Susan Euling, Columbia University
Research Advisor: **D. Kurtz**
Determination of tissue-specific alpha 2μ globulin.

William Howell, University of Wisconsin
Research Advisor: **P. Scolnik**
Characterization of the end-sites of the DNA of the gene transfer agent of *Rhodopseudomonas capsulata*.

Phyllis Kristal, Massachusetts Institute of Technology
Research Advisor: **L. Silver**
Developmental analysis of lethal mutations on mouse chromosome 17.

Mark Montgomery, Princeton University
Research Advisor: **J. Lewis**
Transcription studies with the cell-cycle-regulated Chinese hamster thymidine kinase gene.

Ramona Morfeld, Wheaton College
Research Advisor: **J. Hicks**
Investigation of a transformation system in maize.

Jon Rubin, Harvard University
Research Advisor: **D. Helfman**
Isolation and characterization of cDNA clones encoding non-muscle tropomyosin.

David Southern, University of Glasgow
Research Advisor: **R. Sadaie**
PCNA: The need for research.

Olney Fellow
Nasrollah Samiy, Harvard University
Research Advisor: **F. Tamanoi**
Expression in two oncogenes.

Cold Spring Harbor Curriculum Study

The lag time between new discoveries in the laboratory—the basic stuff of science—and their effective integration into secondary science teaching can be as much as 10–15 years. Unfortunately, during this interval there has occurred a revolution in biology whose vast social and economic implications promise to influence the lives of the current generation of youth profoundly.

The explosive advent of powerful techniques to probe the molecular structure of DNA and proteins has fundamentally changed the focus of modern biology. Unfortunately, present school curricula do not reflect this significant revolution or its product—biotechnology. We are faced with an untenable situation: The biology now being taught in high schools is of a different era and of a separate mindset than that which is currently being practiced at the laboratory bench.

In an effort to help close this gap, Cold Spring Harbor Laboratory has joined with eight Long Island school districts in forming the Cold Spring Harbor Curriculum Study—a cooperative project to improve teacher competence and to develop model curricula on molecular biology, recombinant DNA, and biotechnology. The program is funded by a $10,000 grant from Citibank, N.A., and appropriations from the participating school districts: Cold Spring Harbor, East Williston, Great Neck, Herricks, Jericho, Northport-East Northport, Oyster Bay, and Syosset. Included in the $90,000 budget for the Curriculum Study's initial year is over $40,000 in equipment needed to perform state-of-the-art experiments that culminate in the production of recombinant DNA molecules.

For many years, the Laboratory was considered an ivory-tower institution, aloof in an esoteric cloud from the average person. Now, through the Curriculum Study, the institution is reaching out to share its knowledge and expertise with the local community. By acting as a resource for public schools, we hope to help establish Long Island as a nationwide leader in advanced science education. The largest coordinated effort in the country to teach the "new biology," the Curriculum Study will serve as a model for similar efforts, and the materials developed will find use in schools throughout the United States. The program is designed to take a broad approach to the problems of biology education by improving teacher competence and by developing new classroom materials and methods. It is hoped that by effecting change at the top of the education pyramid—at the level of administrators, science chairpersons, and teachers—benefits will filter down to the largest number of students.

Teachers from participating districts take part in seminars presented by Cold Spring Harbor Laboratory staff members and meet regularly to develop laboratories and instructional materials. Enrichment activities for students include "honors colloquia" at the Laboratory, a site visit to each school district by a Cold Spring Harbor staff scientist, and an intensive 6-day workshop to be held during the summer.

Science and Society

Few would argue the extent to which scientific advancements and new technologies affect our socioeconomic environment. On a personal level, technology reorders our life-styles at a bewildering rate. On a public level, science- and technology-related issues play an increasingly important role in public policy. Al-

though our involvement in technology is increasing at an exponential rate, public understanding of the scientific factors that shape our lives lags far behind. Thus, another goal of the Curriculum Study is to prepare students to evaluate more accurately the social and ethical issues generated by the "new biology."

Recent survey data indicate that Americans attribute our global prestige and influence to our technological know-how. However, there is mounting evidence that the United States is beginning to lose its advantage in international technological competition. Clearly, maintaining our technological edge demands a strong commitment to developing new scientific talent. Yet, it appears that we are failing to stimulate expansion of our scientific base at a grass-roots level in our elementary and secondary schools. We seem to have forgotten that increased emphasis on science education is the only way to keep up with the technological realities of our rapidly changing world. Our technological competitors have long realized this fact and have taken pains to expand their science curricula. Yet, we have been too smug or too blind to see that we are in the same race with technology; science curricula in our secondary schools are steadily shrinking.

American students are required to take far fewer science and math courses than are their peers in other countries. In Japan, the Soviet Union, and East Germany, the vast majority of high school graduates have taken chemistry, physics, and biology; the majority of American students take only 1 year of biology. Specialized science study in these countries begins in the 6th grade, with separate courses in biology, chemistry, and physics.

Science Education Coming Full Circle

The scenario is not new. The launch of the Soviet satellite Sputnik in 1957 triggered similar fears that the United States was losing its position as world leader in technology. A public outcry demanded increased support of science education. During the 1960s, an infusion of federal funds and leadership from the National Science Foundation (NSF) stimulated a renaissance in secondary science education–new curricular materials were developed and science teaching was brought up-to-date.

By the mid-1970s, concern was replaced by complacency. The spectacular results of the space program and great advances in computer technology seemed to indicate that we were, once again, ahead in the technology race. In an era of social unrest, NSF-developed curricula were increasingly attacked as a source of breakdown in traditional values. The NSF precollege program declined and was essentially dismantled between 1980-82; curriculum development ground to a halt.

Now we have come full circle. We stand at another watershed in science education in this country. Over the past several years, numerous major assessments of precollege science education have pointed to the urgent need for new efforts to update science teaching. The government appears to have heeded the call; the NSF budget for precollege education rose from $15 million in 1983 to over $54 million in 1984. The Cold Spring Harbor Curriculum Study will stand at the headwaters of this new surge in American science education.

Why Johnny Doesn't Learn Science

Children start out as "natural scientists." They routinely carry out investigations to understand the world around them. Although science teaching should build on

this natural inquisitiveness, recent surveys indicate that children acquire a dislike for formal science at an early age and that these negative attitudes increase with schooling. For the majority of students, there appears to be little growth in interest or knowledge of science during the high school years.

The lack of student interest in science may stem, in large part, from inadequacies in the way it is taught. The vast majority of science teachers rely heavily on standard texts that tend to stress terminology and definitions. Thus, rote learning of terms, which emphasizes only the academic aspects of science, is the major component of precollege biology education. Little attention is paid to the real-world applications of biology or its underlying unity with the chemical and physical sciences.

Although learning by doing has long been a goal of effective science teaching, the sad fact is that our schools place too little emphasis on discovery learning. Too often, teachers faced with overambitious syllabi and large classes opt to skip the laboratory work that could make science more relevant to students. Shortages in supplies and equipment have drastically reduced laboratory time in the secondary schools. Even when laboratories are used, they tend to be demonstrations of information already presented, rather than true discovery learning. It seems clear that although they may impart some formal knowledge of science, current secondary biology curricula are failing miserably to prepare individuals to understand better or participate in an increasingly technological world.

Why Teach Molecular Biology?

Despite the fact that DNA is the starting point of all life, its seminal importance is not emphasized in current science curricula. Intuitively, it makes great sense to focus biology teaching more closely on DNA. It provides a molecular basis upon which can be built the major concepts of biology: the difference between living and nonliving, development and the differentiation of tissues, reproduction, the relationship between structure and function, and the diversity of life.

One criticism of science education is that it has become too aligned with discrete academic disciplines. Survey data indicate that high school students are frightfully ignorant of chemistry as it relates to life. This is a shame, because, in practice, science is becoming less and less compartmentalized. A synthesis of several disciplines, including genetics, microbiology, biochemistry, physical chemistry, and physics, molecular biology highlights the unity of science. Since many high school students' only introduction to science is in a general biology course, it makes sense to stress chemistry and physics as it relates to life. Thus, emphasizing a molecular biological approach would be a step toward teaching "integrated" science. By providing an entrée to biotechnology, such a molecular approach would feed students' interest in technology and demand for practical relevance.

In addition to increasing knowledge of the pivotal role of DNA in life processes, a major aim of the Curriculum Study is to stimulate awareness of career opportunities in biotechnology. Molecular biology is a science that will figure largely in the future economic development of this nation. Like the explosive growth of computer technology, biotechnology will provide unprecedented professional opportunities in research and development. Students who participate in this project will have a clear advantage in the competition for acceptance in science programs at prestigious universities. These students will be well prepared to pursue

other enrichment activities, such as individualized research, projects for science competitions, and part-time work at research institutions. This experience will be especially crucial for students planning careers in biological research.

Nature Study Program

The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, students can engage in such introductory programs as Nature Detectives, Seashore Life, and Pebble Pups, as well as more advanced programs such as Marine Biology, Nature Photography, and the Geology of Long Island.

During the summer of 1984 a new high of 525 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 100 foot Sloop Clearwater chartered from Hudson River Sloop Clearwater, Inc. of Poughkeepsie, New York. Students were able to study the Sound chemically, physically, and biologically using the ship's instrumentation. The three-day Adventure Education class took 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 Sloop Clearwater.

PROGRAM DIRECTOR

Edward Tronolone, M.S., P.D., Science Curriculum Associate,
East Williston Public Schools

INSTRUCTORS

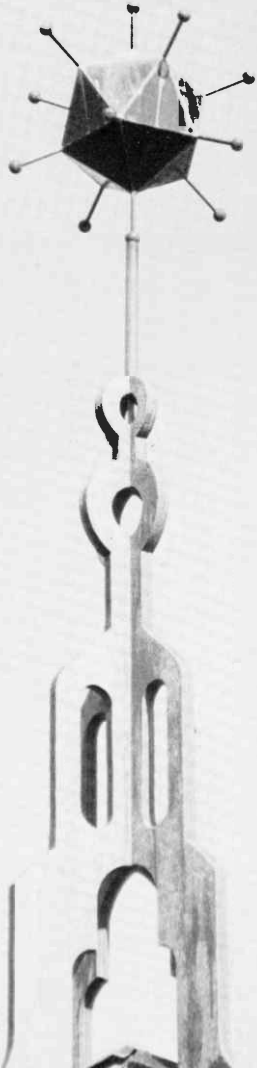
Kathryn Bott, M.S., science teacher, Friends Academy
Ruth Burgess, B.A., naturalist, Nassau County BOCES
Eric Knuffke, M.S., science teacher, Mineola High School
Fred Maasch, M.S., science teacher, Islip High School
Paul Paino, M.S., science teacher, The Wheatley High School
Bill Payoski, M.S., science instructor, Nassau Community College
Linda Payoski, B.A., naturalist, Nassau County BOCES

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

Facing page: A replica of an adenovirus particle decorates the gazebo built on top of the Laboratory's waste water treatment plant.

FINANCIAL STATEMENT



FINANCIAL STATEMENT

BALANCE SHEET
year ended December 31, 1984
with comparative figures for year ended December 31, 1983

COLD SPRING HARBOR LABORATORY

ASSETS

	1984	1983
CURRENT FUNDS		
Unrestricted		
Cash and Short-term investments	$10,963,401	$ 4,119,413
Accounts Receivable	317,217	375,687
Prepaid expenses and other assets	679,516	289,940
Inventory of books	209,329	194,111
Due from restricted fund	351,938	–
Due from Banbury Center	–	115,810
Total unrestricted	12,521,401	5,094,961
Restricted		
Marketable securities (quoted market 1984 – 287,500)	212,500	–
Grants and contracts receivable	4,433,529	3,779,375
Due from unrestricted fund	–	660,906
Total restricted	4,646,029	4,440,281
Total current funds	$17,167,430	$ 9,535,242
ENDOWMENT FUNDS		
Robertson Research Fund		
Cash	3,308,119	3,536,782
Marketable securities (quoted market 1984 – $14,435,055; 1983 – 13,466,772)	12,772,766	11,446,963
Total Robertson Research Fund	16,080,885	14,983,745
Olney Memorial Fund		
Cash	1,693	4,623
Marketable Securities (quoted market 1984 – $33,247; 1983 – $23,252)	29,418	27,538
Total Olney Memorial Fund	31,111	32,161
Total endowment funds	$16,111,996	$15,015,906
PLANT FUNDS		
Investments	759,318	454,212
Due from unrestricted fund	6,608,932	983,773
Land and improvements	1,129,875	1,129,875
Buildings	11,318,113	9,621,165
Furniture, fixtures and equipment	3,490,933	2,485,774
Books and periodicals	365,630	365,630
Construction in progress	1,461,489	960,709
	25,134,290	16,001,138
Less allowance for depreciation and amortization	4,861,975	4,013,770
Total plant funds	$20,272,315	$11,987,368

LIABILITIES AND FUND BALANCES

	1984	1983
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 747,261	$ 835,166
Deferred income	120,000	140,000
Due to Banbury Center	74,580	–
Due to plant fund	6,608,932	983,773
Due to restricted fund	–	660,906
Fund balance	4,970,628	2,475,116
Total unrestricted	12,521,401	5,094,961
Restricted		
Accounts payable	65,792	–
Due to Banbury Center	8,952	–
Due to unrestricted fund	351,938	–
Fund balance	4,250,458	4,440,281
Total restricted	4,646,029	4,440,281
Total current funds	$17,167,430	$ 9,535,242
ENDOWMENT FUNDS		
Fund balance	$16,111,996	$15,015,906
PLANT FUNDS		
Accounts payable	121,055	–
Loan payable	8,000,000	–
Fund balance	12,151,260	11,987,368
Total Plant Funds	$20,272,315	$11,987,368

ASSETS

BANBURY CENTER

	1984	1983
CURRENT FUNDS		
Unrestricted		
Cash	$ 700	$ 700
Prepaid and deferred expenses	19,184	15,025
Inventory of books	46,763	36,857
Due from Banbury restricted fund	–	95,505
Due from CSHL unrestricted fund	16,497	–
Total unrestricted	83,144	148,087
Restricted		
Grants and contracts receivable	111,450	142,988
Due from CSHL unrestricted fund	58,083	–
Due from CSHL restricted fund	8,952	–
Total restricted	178,485	142,988
Total current funds	261,629	291,075
ENDOWMENT FUNDS		
Robertson Maintenance Fund		
Cash	597,661	494,054
Marketable securities (quoted market 1984 – $2,347,644; 1983 – $2,314,259)	2,077,778	1,978,853
Total endowment funds	2,675,439	2,472,907
PLANT FUNDS		
Land	772,500	772,500
Buildings	846,028	845,967
Furniture, fixtures and equipment	180,383	176,318
Construction in progress	1,385	1,386
	1,800,296	1,796,171
Less allowance for depreciation	357,019	320,450
Total plant funds	1,443,277	1,475,721
Total Banbury Center	$ 4,380,345	$ 4,239,703
Total – All funds	$57,932,086	$40,778,219

LIABILITIES AND FUND BALANCES

	1984	1983
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 18,466	$ 32,277
Due to CSHL unrestricted fund	–	115,810
Fund balance	64,678	–
Total unrestricted	83,144	148,087
Restricted		
Accounts payable	1,394	–
Due to Banbury unrestricted	–	95,505
Fund balance	177,091	47,483
Total restricted	178,485	142,988
Total current funds	261,629	291,075
ENDOWMENT FUNDS		
Fund balance	2,675,439	2,472,907
PLANT FUNDS		
Fund balance	1,443,277	1,475,721
Total Banbury Center	$ 4,380,345	$ 4,239,703
Total – All funds	$57,932,086	$40,778,219

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1984
with comparative figures for year ended December 31, 1983

COLD SPRING HARBOR LABORATORY

	1984	1983
REVENUES		
Grants and contracts	$12,418,740	$11,650,901
Indirect cost allowances on grants and contracts	4,141,453	3,626,022
Contributions		
Unrestricted	154,046	27,800
Restricted and capital	421,918	868,528
Long Island Biological Association	515,252	33,300
Robertson Research Fund Distribution	475,000	453,000
Summer programs	487,966	491,049
Laboratory rental	20,732	20,732
Marina rental	58,528	56,170
Investment income	775,231	481,992
Publications sales	1,512,667	1,655,691
Dining Hall	556,931	490,678
Rooms and apartments	309,924	216,179
Other sources	56,998	26,371
Total revenues	21,905,386	20,098,413
EXPENSES		
Research*	10,628,482	10,098,170
Summer and training programs*	1,388,942	993,338
Publications sales*	1,276,015	1,306,539
Dining hall*	735,044	671,602
Research support	289,493	256,676
Library	236,255	223,117
Operation and maintenance of plant	2,179,543	1,921,985
General and Administrative	1,805,567	1,632,045
Depreciation	848,205	643,097
Total expenses	19,387,546	17,746,569
TRANSFERS		
Capital building projects	2,003,456	1,738,298
Banbury Center to (from)	80,420	(80,420)
Total transfers—net	2,083,876	1,657,878
Total expenses and transfers	21,471,422	19,404,447
Excess of revenues over expenses and transfers	$ 433,964	$ 693,966

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

BANBURY CENTER

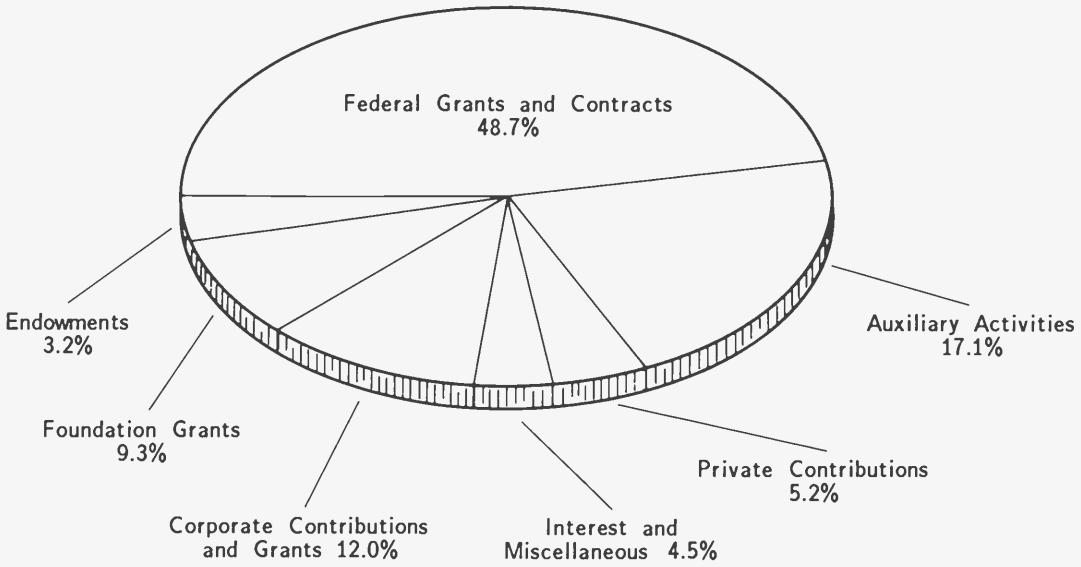
	1984	1983
REVENUES		
Endowment income	$106,000	$100,000
Grants & contributions	407,806	261,436
Indirect cost allowances on grants and contracts	2,060	13,576
Rooms and apartments	65,757	76,522
Publications	102,410	191,993
Conference fees	14,697	54,096
Dining Hall	34,884	4,708
Transfer from (to) Cold Spring Harbor Laboratory	80,420	(80,420)
Total revenues	814,034	621,911
EXPENSES		
Conferences	171,095	206,659
Publications	128,181	126,618
Operation and maintenance of plant	120,103	93,548
Program administration	196,244	202,651
Depreciation	36,569	45,107
Capital plant	4,125	–
Total expenses	656,317	674,583
Excess (deficit) of revenues over expenses	$157,717	$(52,672)

NOTE: Copies of our complete, audited financial statements, certified by our independent auditors, Peat, Marwick, Mitchell & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

COLD SPRING HARBOR LABORATORY

SOURCES OF REVENUE

YEAR ENDED DECEMBER 31, 1984



GRANTS AND CONTRIBUTIONS

Recto: Detail of Carnegie Library, built in 1905 by the Carnegie Institution of Washington. Originally designed as a laboratory, the building was refitted as a library in 1953 and partially renovated in 1980 to house the Marketing offices.

GRANTS

January 1, 1984–December 31, 1984

COLD SPRING HARBOR LABORATORY

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Cancer Research Center, Dr. Roberts	11/77–12/86	18,604,426
	Gene Organization, Dr. Sambrook	4/81– 3/86	5,626,997
Research Support	Dr. Beach	12/84–11/87	361,511*
	Dr. Blose	12/78–11/87	1,094,594
	Dr. Feramisco	7/80– 7/86	686,968
	Dr. Gething	2/83– 1/87	976,587
	Dr. Hicks	7/81– 6/86	1,710,283
	Dr. Hockfield	2/82– 1/85	282,400
	Dr. Klar	7/81– 6/86	1,572,978
	Dr. Klar	12/83–11/84	149,678
	Dr. Kurtz	4/80– 3/86	606,389
	Dr. Lin	9/82– 2/84	134,049
	Dr. Mathews	4/80– 3/86	729,283
	Dr. Matsumura	7/83–12/86	469,737
	Dr. McKay	7/81– 6/84	288,546
	Dr. Silver	2/82– 9/84	587,862
	Dr. Stillman	7/83– 6/86	677,384
	Dr. Topp	3/82– 2/84	279,134
	Dr. Watson	4/84– 3/85	103,479*
	Dr. Watson	4/83– 3/84	111,124
	Dr. Watts	9/84– 8/87	254,731*
	Dr. Welch	4/84– 3/86	279,316*
	Dr. Zipser	12/81–11/84	299,219
	Dr. Zipser	12/83–11/86	358,081
Fellowships	Dr. Bautch	9/83– 8/86	57,244
	Dr. Flanagan	9/83– 9/85	36,204
	Dr. Gerard	5/84– 4/86	38,204*
	Dr. Ivy	1/82– 1/84	38,776
	Dr. Krangel	10/82– 9/85	54,584
	Dr. Livi	10/83–10/86	57,244
	Dr. Quinlan	6/84– 5/87	57,244*
	Dr. Roth	9/83– 8/85	36,776
	Dr. Taparowsky	10/82–10/84	34,116
	Dr. Welch	9/82– 3/84	29,332
Training	Institutional, Dr. Grodzicker	7/78– 8/89	1,291,646
Course Support	Advanced Bacterial Genetics, Dr. Hicks	5/80– 4/88	302,523
	Cancer Center, Dr. Grodzicker	6/82–12/86	657,174
	Neurobiology, Dr. Hockfield	6/79– 3/86	646,143
	Neurobiology, Dr. Hockfield	5/82– 4/85	131,277

*New grants awarded in 1984.

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Meeting Support	Ciliate Molecular Genetics, Dr. Watson	4/84– 3/85	7,000*
	Modern Approaches to Vaccines, Dr. Watson	9/84– 8/85	10,000*
	RNA Processing, Dr. Mathews	4/82– 4/84	12,500
	Symposium: Recombination at the DNA Level, Dr. Watson	4/84– 3/85	27,000*
	Tubulin and Cytoskeletal Proteins, Dr. Watson	4/84– 3/85	10,500*
NATIONAL SCIENCE FOUNDATION			
Research Support	Dr. Malmberg, Dr. Hicks	3/84– 2/87	121,676*
	Dr. Lewis	11/83–12/85	140,000
	Dr. Roberts	1/83– 8/86	28,442
	Dr. Roberts	1/83–12/86	200,000
	Dr. Roberts	7/83–12/86	252,000
	Dr. Tamanoi	7/82–12/84	88,000
Course Support	Plant Molecular Biology, Dr. Hicks	5/81– 4/84	99,600
	Plant Molecular Biology, Dr. Malmberg	5/84– 4/87	121,676*
Meeting Support	Photosynthetic Apparatus, Dr. Watson	4/84– 3/85	4,000*
	RNA Processing, Dr. Mathews	5/84– 4/85	5,000*
	Symposium: Recombination at the DNA Level, Dr. Watson	5/84– 4/85	6,480*
	Tubulin and Cytoskeletal Proteins, Dr. Watson	4/84– 3/85	3,000*
Construction	Plant Genetics Facility, Dr. Watson	12/83– 5/85	406,350
DEPARTMENT OF AGRICULTURE			
Research Support	Dr. Dellaporta	9/84– 8/86	100,000*
Meeting Support	Photosynthetic Apparatus	1984	9,280*
DEPARTMENT OF ENERGY			
Meeting Support	Symposium: Recombination at the DNA Level	1984	9,000*
NORTH ATLANTIC TREATY ORGANIZATION			
Meeting Support	Photosynthetic Apparatus	1984	4,852*
NONFEDERAL GRANTS			
Research Support			
A.B.C. Foundation	Dr. Wigler	5/82– 4/87	600,000
Rita Allen Foundation	Dr. Stillman	1/83–12/87	150,000
American Cancer Society	Dr. Chow	1/82– 8/84	85,255
	Dr. Rossini	1/82–12/84	110,000
	Dr. Stillman – Institutional	7/82– 6/85	80,000
	Dr. Wigler	7/84– 6/86	200,000*
Cancer Research Institute	Dr. Garrels	7/83– 6/85	72,000
Russell and Janet Doubleday Fund	General Support	1/84–12/84	20,000*

Grantor	Program/Principal Investigator	Duration of	Total Award
Exxon Research and Engineering Company	Cooperative Research	1/82–12/86	7,500,000
Robert G. Hatfield Fund	General Support	1/84–12/84	2,000*
International Foundation for Cancer Research	Dr. Hanahan	10/84– 9/85	50,000*
McKnight Foundation	Dr. Malmberg	3/83– 2/86	105,000
Monsanto Company	Cooperative Research	10/84– 9/89	2,060,252*
Muscular Dystrophy Association	Dr. Garrels	1/80–12/84	100,000
	Dr. Lin	1/81– 2/84	105,311
	Dr. Mathews	7/83– 6/85	50,000
	Dr. Matsumura	1/83–12/84	32,228
New England Biolabs	Dr. Roberts	1/82–12/84	30,065
New York State Science and Technology Foundation	Dr. Garrels	10/84– 9/85	25,000*
William and Maude Pritchard Charitable Trust	Plant Molecular Biology	12/84–11/85	60,000*
Marie Robertson Memorial Fund	Neurobiology	1/84–12/84	125,000*
SUNY Stony Brook (Subcontract)	Dr. Topp	8/82– 1/84	9,597
Surdna Foundation	Plant Molecular Biology	11/84–10/85	50,000*
Fellowships			
American Cancer Society	Dr. Young	8/84– 7/85	16,500*
Jane Coffin Childs Memorial Fund for Medical Research	Dr. Sundin	7/82– 6/84	32,000
Leukemia Society of America	Dr. Hanahan	7/84– 6/86	44,280*
	Dr. Powers	12/83–12/85	34,000
	Dr. Sadaie	7/84– 8/85	34,000*
Muscular Dystrophy Association	Dr. Helfman	7/83– 6/85	37,000
Robert P. Olney Memorial Cancer Fund	Graduate Research	1984	2,800*
Rockefeller Foundation	Dr. Hicks	9/84– 8/85	25,900*
Damon Runyon–Walter Winchell Cancer Fund	Dr. Bhagwat	7/82– 6/84	35,000
	Dr. Broek	6/84– 5/86	38,000*
	Dr. Gallagher	7/84– 6/86	38,000*
	Dr. Gerard	5/83– 4/84	17,000
	Dr. Hearing	9/84– 8/86	38,000*
	Dr. Hiatt	8/84– 7/86	38,000*
	Dr. Mains	4/83–11/84	29,030
	Dr. Patterson	4/84–12/84	12,743*
	Dr. Sass	9/84– 8/86	38,000*
	Dr. White	11/83–10/85	35,000
Helen Hay Whitney Foundation	Dr. Herr	1/83– 6/84	39,250
Training			
Metropolitan Life Foundation	Undergraduate Research Program	1984	5,000*
Grass Foundation	Neurobiology Scholarships	1980–1984	63,190
Alfred P. Sloan Foundation	Undergraduate Research Program	1984	20,000*
Visiting Nurse Service	Undergraduate Research Program	1984	150*

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Course Support			
Bayer Company (Glenbrook Labs)	Developmental Neurobiology	1984	3,500*
Charles E. Culpeper Foundation	Developmental Neurobiology	1984	10,000*
Epply Foundation for Research	Neurobiology of Human Diseases	1984	21,000*
The Esther A. and Joseph Klingenstein Fund	Neurobiology	1982–1985	150,000
Metropolitan Life Foundation	Developmental Neurobiology	1984	10,000*
Meeting Support			
Advanced Genetic Sciences, Inc.	Photosynthetic Apparatus	1984	1,500*
Amicon Corporation	Ciliate Molecular Genetics	1984	100*
Arco Plant Cell Research Institute	Photosynthetic Apparatus	1984	1,500*
J.T. Baker Chemical Company	Ciliate Molecular Genetics	1984	100*
Boehringer-Manheim Biochemicals	Ciliate Molecular Genetics	1984	100*
Ciba-Geigy Corporation	Photosynthetic Apparatus	1984	1,500*
Dow Chemical Company	Photosynthetic Apparatus	1984	1,500*
Exxon Research and Engineering Company	Photosynthetic Apparatus	1984	1,500*
Fotodyne, Inc.	Ciliate Molecular Genetics	1984	100*
H.P. Genenchem	Ciliate Molecular Genetics	1984	320*
Hana Biologics, Inc.	Ciliate Molecular Genetics	1984	250*
Johnson & Johnson Biotechnology Center Inc.	Modern Approaches to Vaccines	1984	5,000*
Kontes Glass Company	Ciliate Molecular Genetics	1984	100*
Nikon, Inc.	Ciliate Molecular Genetics	1984	200*
Promega Biotec	Ciliate Molecular Genetics	1984	50*
Rhone-Poulenc, Inc.	Photosynthetic Apparatus	1984	750*
Schleicher & Schuell, Inc.	Ciliate Molecular Genetics	1984	100*
Shell Development Company	Photosynthetic Apparatus	1984	1,000*
Society for Developmental Biology, Inc.	Ciliate Molecular Genetics	1984	1,500*
Standard Oil Company (Amoco)	Photosynthetic Apparatus	1984	500*
Stauffer Chemical Company	Photosynthetic Apparatus	1984	1,500*
Wellcome Biotechnical Ltd.	Modern Approaches to Vaccines	1984	500*
			5,000*
Construction			
Pew Memorial Trust	Monoclonal Antibody Facility	1981–1984	400,000
Equipment			
Samuel Freeman Charitable Trust	Monoclonal Antibody Facility	1984	15,000*
William Randolf Hearst Foundation	Monoclonal Antibody Facility	1984	30,000*
McKnight Foundation	Neurobiology Course	1984–1985	110,000*
Fannie E. Rippel Foundation	Monoclonal Antibody Facility	1983–1984	100,000

BANBURY CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*Total Award*
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Meeting Support	Genetic Manipulation of Mammalian Ovum and Early Embryo, M. Shodell	9/84– 8/85	9,550*
DEPARTMENT OF ENERGY			
Meeting Support	Risk Quantitation	4/84– 3/85	8,000*
OFFICE OF NAVAL RESEARCH			
Meeting Support	Gap Junctions	1984	4,700*
NONFEDERAL GRANTS			
Meeting Support			
Council for Research Planning in Biological Sciences, Inc.	EPA Workshop	8/84–12/84	4,000*
Diamond Shamrock	Biological Mechanisms of Dioxin Action	4/84– 3/85	2,000*
Dow Chemical, U.S.A.	Biological Mechanisms of Dioxin Action	4/84– 3/85	2,000*
Hoffmann-La Roche, Inc.	Biological Mechanisms of Dioxin Action	4/84– 3/85	5,000*
March of Dimes	Genetic Manipulation of Mammalian Ovum and Early Embryo	10/84– 9/85	7,500*
Monsanto Company	Biological Mechanisms of Dioxin Action	4/84– 3/85	5,000*
	Risk Quantitation	4/84– 3/85	5,000*
National Distillers & Chemical Corporation	Risk Quantitation	4/84– 3/85	1,000*
Alfred P. Sloan Foundation	Journalists' Workshop	1984–1986	162,000*
	Computational Neuroscience Workshop	7/84– 6/85	21,000*

Corporate Sponsor Program

A heritage of pioneering research and prominence as a gathering place for scientists from many disciplines make Cold Spring Harbor Laboratory an ideal window on the world of molecular biology and its applications to medicine and industry. The Corporate Sponsor Program was established in 1984 to offer a select group of companies an opportunity to share that vantage point.

Considering that high-caliber research goes on within industry, as well as within academia, the future demands that we foster closer ties within the entire molecular biological community. Thus, a major goal of the Corporate Sponsor Program is to encourage the flow of biotechnical information between academic and industrial laboratories. Cold Spring Harbor and Banbury conferences provide an ideal context for this interaction: 260 scientists from 60 companies participated in the 1984 meetings program.

The yearly commitment of $15,000 by each Sponsor ensures a stable base of support for our meetings program, which had long been at the mercy of fluctuations in government and foundation support. This core support has done much to abate the insecurity that once shadowed our summer meetings program. Now we can devote more time to planning timely conferences that meet the information needs of the research community and less time scurrying about for funding.

Proceeds from the Corporate Sponsor Program entirely support a series of Special Banbury conferences that deal with technical aspects of genetic engineering especially relevant to industry. Combining high-level science with a restful country setting conducive to informal exchange, these meetings are unique in science. The 1984 meetings were "Site Directed Mutagenic Approaches to Protein Structure and Function," "Yeast Cloning Vectors," and "Protein Transport and Secretion."

Benefits to Sponsor companies include gratis attendance for six representatives at Cold Spring Harbor meetings and Special Banbury conferences, gratis Cold Spring Harbor and Banbury publications, and recognition in meeting abstracts and publications.

1984 Members

Agrigenetics Corporation
Biogen S.A.
CPC International, Inc.
E.I. du Pont de Nemours & Company
Genentech, Inc.
Genetics Institute
Hoffmann-La Roche Inc.
Johnson & Johnson
Eli Lilly and Company
Molecular Genetics, Inc.
Monsanto Company
Pall Corporation
Pfizer Inc.
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Long Island Business Cancer Campaign

For many years, the international scientific community has known what a great asset Cold Spring Harbor Laboratory is to science. It is a sad irony that the laboratory is not well known to many local people and, thus, remains one of Long Island's best-kept "secrets." However, just as the world belatedly recognized the achievements of CSH researcher Dr. Barbara McClintock with the 1983 Nobel Prize, so, too, are Long Islanders now becoming aware of the great asset that has quietly been at work in their own backyard since 1890.

The Long Island Association, the official chamber of commerce for Nassau and Suffolk Counties, voted Cold Spring Harbor Laboratory as recipient of its 1984 Medal of Honor. This award was given "in recognition of the Laboratory's vital work for the good of Long Islanders and all mankind."

In an era of shrinking government funding the private sector must assume a larger responsibility for supporting biomedical research. The Long Island Business Cancer Campaign offers businesses an opportunity to participate in the Laboratory's research on the molecular basis of cancer. We hope that this local cancer initiative will help build a greater sense of community on Long Island.

The 1984 campaign netted $50,000 in cash and donated equipment, which was used in the development of the new monoclonal antibody laboratory. We thank the following companies who joined in saying "Long Island is doing something about cancer."

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FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of the State of New York. Contributions are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory has been designated a "public charity" and, therefore, may receive funds resulting from the termination of "private" foundations.

Because its endowment is limited and the uses of research grants are formally restricted, the Laboratory depends on generous contributions from private foundations, sponsors, and friends for central institutional needs and capital improvements.

Over the years, the Laboratory has won a reputation for innovative research and high-level science education. By training young scientists in the latest experimental techniques, it has helped spur the development of many research fields, including tumor virology, cancer genes, gene regulation, moveable genetic elements, yeast genetics, and molecular neurobiology. However, the continued development of new research programs and training courses requires substantial support from private sources.

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities You can generally deduct the full amount of the gift on your income tax return, and, of course, you need pay no capital gains tax on the stock's appreciation. We recommend any of the following methods:

(1) Have your broker sell the stock and remit the proceeds to Cold Spring Harbor Laboratory.

(2) Deliver the stock certificates to your broker with instructions to him to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.

(3) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724. In a separate envelope send an *executed* stock power.

Bequests Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

Appreciated real estate or personal property Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Conversion of privation 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.

LABORATORY STAFF

Recto: Demerec laboratory extension, erected in 1983.

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Joseph Sambrook, Assistant Director for Research
William R. Udry, Administrative Director
John Maroney, Assistant Administrative Director
William Keen, Comptroller
Jack Richards, Director Buildings & Grounds
Michael Shodell, Banbury Center Director

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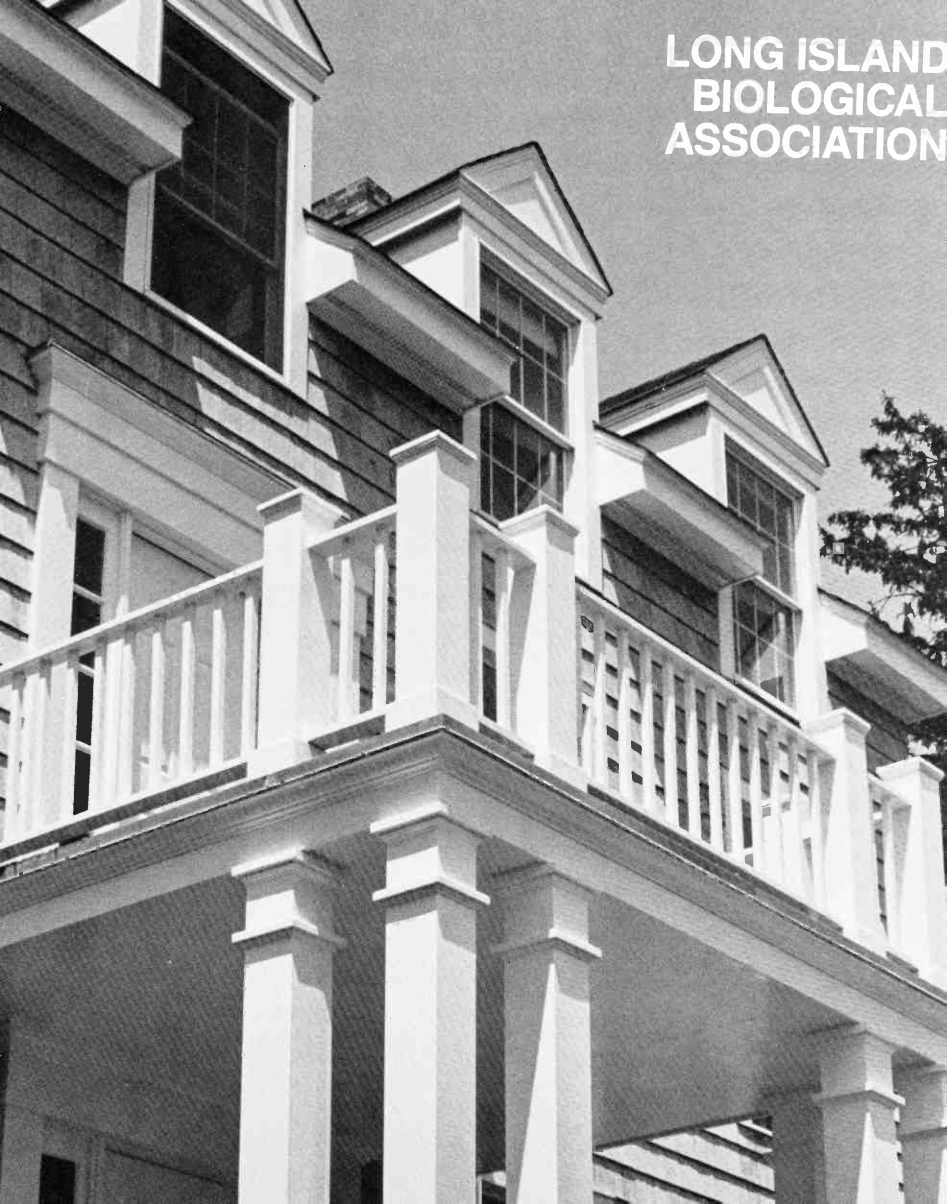
LONG ISLAND
BIOLOGICAL
ASSOCIATION

Recto: Williams House (ca. 1850), rebuilt in 1977 with funds raised by LIBA, provides five apartments for staff scientists. It is named for Col. Timothy S. Williams, first president of LIBA.

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The LONG ISLAND BIOLOGICAL ASSOCIATION (LIBA) and Its Relation to the Cold Spring Harbor Laboratory

Biological research at Cold Spring Harbor began in 1890 when the Brooklyn Institute of Arts and Sciences was looking for a place to set up a summer laboratory as the headquarters of its Department of Zoology. Representatives of the Institute were invited by Eugene S. Blackford, at that time the Fish Commissioner of the State of New York, to inspect a site on Bungtown Road across Northern Boulevard (25A) from the Fish Hatchery. The site was found to be ideal, and so the original Laboratory was organized with Mr. Blackford as president of the Board of Trustees. The land was leased from Mr. John D. Jones, whose family since 1819 had operated various industries including shipbuilding, coopering, and textile manufacture (later whaling, also) at the head of Cold Spring Harbor. Bungtown Road, which runs through the Lab property, got its name from the factory that specialized in making bungs—or stoppers—for barrels.

In 1892 the Laboratory's land was leased for a dollar a year from the Wawepex Society, which Mr. Jones had organized as a corporation for holding real estate and for investing funds for the propagation of fishes and for scientific research. In 1904 the Wawepex Society leased additional land to the Carnegie Institution of Washington, which wanted to locate a Department of Experimental Evolution in the Cold Spring Harbor area. Charles B. Davenport, who had been directing the Laboratory since 1896, assumed the additional duties of director of the Carnegie Institution's experimental station. It is interesting to note that Mr. Davenport lived in what came to be known later as the Carnegie Dormitory, the Victorian house on 25A built by John D. Jones before the turn of the century, and recently repainted in its original colors.

The Long Island Biological Association was established in 1924 when the Brooklyn Institute decided to discontinue its research at Cold Spring Harbor and offered its laboratory to two universities. Fortunately, a local group of interested neighbors decided to assume responsibility for the Lab, and thus LIBA came into being. For 38 years LIBA actually operated the Laboratory in conjunction with the Carnegie Institution, but in 1962 it seemed advisable for the Laboratory to be reorganized as an independent unit. Therefore, the property on which it now stands was conveyed to it by LIBA, which, however, still retains reversionary rights. Today LIBA is one of twelve institutions participating in the support of the Laboratory, each institution being represented on the Laboratory's Board of Trustees.

What has happened, in effect, is that LIBA has become an expanding group of local "Friends of the Laboratory" who help support it through annual contributions. Also, from time to time, the Association undertakes campaigns to finance special important projects for which the Lab cannot obtain funds from the Federal Government or from other sources. For instance, in recent years, building the James Laboratory Annex and the renovation of Blackford Hall, the rebuilding of Williams House, the acquisition of the land formerly belonging to the Carnegie Institution, and (in part) the construction of the new Grace Auditorium.

The affairs of LIBA are handled by a board of 28 directors who are elected to office by the membership at an annual meeting. At least twice a year LIBA members are invited to bring their friends to a lecture or an open house at the Lab.

Membership in LIBA requires a minimum annual contribution (tax deductible) of $25 for a husband and wife, $15 for a single adult, $5 for a junior member (under 21). Further information can be obtained from the Long Island Biological Association, Box 100, Cold Spring Harbor, N.Y. 11724, or by telephoning the Laboratory at (516) 367-8300.

CHAIRMAN'S REPORT

At the Autumn meeting of the Board of Directors I was pleased to announce a substantial growth in the number of LIBA members during the year, also that the generous response to our campaign to raise at least $500,000 towards the cost of the new auditorium indicated that we would be able to increase our contribution to $600,000. At the annual meeting of LIBA members in December it was voted to do this. Approval was given also to the directors' recommendation that our next project should be the financing of a much needed documentary movie (or tape) depicting the history and current research of the Laboratory.

As usual, LIBA members and their guests were invited to two lectures during the year. At the first, the Dorcas Cummings Memorial Lecture, in May, our speaker was Professor Ian Sussex of the Department of Biology at Yale University. Because of the recently established Department of Plant Genetics at the Lab, Dr. Sussex appropriately spoke on "The new plant genetics and its implications for biotechnology". He lucidly explained how independent lines of research had come together in the past few years to allow us to get a whole new approach to understanding how plants develop and are regulated.

At the annual meeting in December we were most fortunate to secure as our speaker Captain Eugene A. Cernan, U.S.N., who made three space flights for NASA and, as commander of Apollo 17, was the last man to leave his footprints on the surface of the moon. Captain Cernan described his fascinating experiences as an astronaut and then went on to explain the advantages of outer space for scientific research. As the overflow audience was leaving the auditorium we could hear on all sides the comment that Captain Cernan's lecture was exceptionally interesting and also inspiring.

The custom of giving dinner parties for visiting scientists during the June Symposium was successfully continued in 1984. It is a custom enjoyed equally by the scientists and by their hosts and hostesses. This year's parties were given by

Mrs. Donald Arthur
Mrs. Gilbert A. Ball
Mr. & Mrs. John P. Campbell
Mr. & Mrs. Miner D. Crary
Mr. & Mrs. James A. Eisenman
Mr. & Mrs. Henry U. Harris, Jr.
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Mrs. H. Irving Pratt
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Mr. & Mrs. Franz Schneider
Mr. & Mrs. Richard S. Storrs
Mr. & Mrs. Martin B. Travis

At the Annual Meeting regret was expressed that the terms of office as directors for Messrs. Charles S. Gay and William F. Parsons, Jr. had expired. Their places were filled by the election of Mrs. R. Duncan Elder and Mr. Roderick H. Cushman.

At a subsequent meeting of the Board of Directors the previous officers and members of the Executive Committee were re-elected.

Edward Pulling, Chairman
Long Island Biological Association

LIBA audience listens to . . .

Capt. Eugene A. Cernan, U.S.N., describing life in outer space.

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