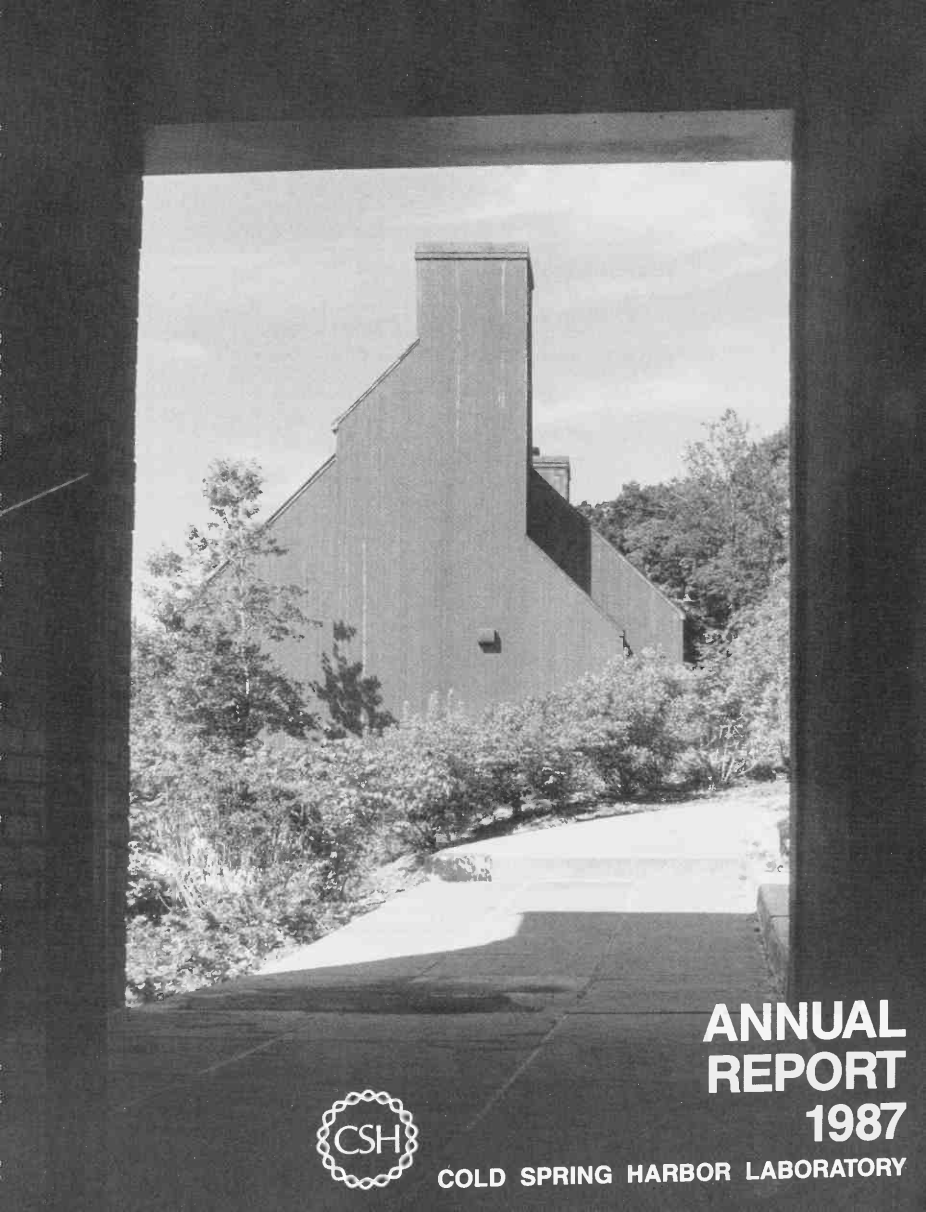
ANNUAL REPORT 1987

COLD SPRING HARBOR LABORATORY



ANNUAL REPORT 1987

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

Book design Emily Harste
Editors Elizabeth Ritcey, Dorothy Brown, Annette Kirk
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Front cover: The Arthur W. and Walter H. Page Laboratory
(Photo by Susan Zehl)
Back cover: Partial view of Laboratory from Cold Spring Harbor
(Photo by David Micklos)

Contents

(*Back row*) T. Whipple, T. Maniatis, B.D. Clarkson, J.D. Watson, J. Darnell, Jr., D.B. Pall, O.R. Grace, J. Warner, S. Strickland (*Middle row*) T.J. Knight, F.M. Richards, D.D. Sabatini, D.L. Luke III, Mrs. C. Dolan, W.S. Robertson, G.W. Cutting, Jr. (*Front row*) Mrs. H.U. Harris, Jr., J. Klingenstein, T.J. Silhavy, Mrs. G.N. Lindsay, D. Botstein
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Representation on the Board of Trustees itself is divided between community representatives and scientists from major research institutions. Ten such institutions are presently represented on the Board of Trustees: Albert Einstein College of Medicine, Columbia University, Harvard University, Massachusetts Institute of Technology, Memorial Sloan-Kettering Cancer Center, New York University, Princeton University, The Rockefeller University, The State University of New York at Stony Brook, and Yale University.

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

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

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Director's Report

The recombinant DNA age is now 15 years old. During this brief period the doing of biological research has changed beyond recognition, and the ability to manipulate DNA has industrial and agricultural consequences that modern nations can ignore only at the expense of their long-term futures. Even more important, DNA research is beginning to have medical and ethical consequences that will affect how we lead our daily lives and plan for the future of our children and they for their children's future.

We thus have to assume that the number of scientists who work with DNA will steadily increase, soon to exceed 100,000. In contrast, when, in 1953, Francis Crick and I found the structure of DNA to be the double helix, those of us who focused exclusively on DNA numbered at most only several hundred. This massive increase in our numbers naturally has had many consequences both scientific and human, some of which are obviously good and others not so wanted. To understand where we now stand, we must realize that we are living in the midst of a virtual biological gold rush, searching in areas where no one has ever before tread and where the rules for decent behavior in more civilized territories are not easily transferable to the new frontiers.

That DNA is today's gold first became apparent when the double helix began to have real consequences on experimental science and the possibility of working out the genetic code gripped our imagination. Francis Crick epitomized this calculated bravado and proclaimed as "The Central Dogma" the transfer of genetic information from DNA to RNA to protein. At the same time, he called his newly expanded Cambridge row house "The Golden Helix" and hung on its exterior wall a helically shaped strip of copper to inform his guests that they were at the right door. What seemed to Francis like common sense, however, could only annoy his more conservative peers, who shuddered at the possibility that they might have to follow his lead along the double helix or disappear into oblivion.

Initially, prospecting for DNA was a civilized affair, and the pace of our daily lives did not measurably change through the remainder of the 1950s and even into the mid-1960s. Important discoveries occurred frequently enough to keep our minds alert, but not so often that long summer vacations in the mountains or on the beaches would fall victim to fears that new discoveries would blow our labs out of the water. For the most part, each major lab worked on its own unique problems, and when, by chance, direct competition existed, the winning or losing of a particular race never was a life or death matter. In this way, the genetic code became established, and the culminating event, the 1966 CSH Symposium on "The Genetic Code," displayed a DNA world that knew and liked each other

enough to take pleasure in each other's successes. Helping make possible this camaraderie was the then high level of financial support. Capable scientists virtually never had a grant turned down, and since the DNA world was then still relatively small, supporting it was not a significant drain on any federal treasury. Scientific success, particularly on the part of younger scientists, could always be rewarded through obtaining the larger grants needed to exploit real breakthrough discoveries.

By the late 1960s, however, this semi-idyllic world almost began to come apart. The Vietnam War was now on, and federal monies for health-related research were no longer ever-increasing. Moreover, the constant influx of new scientific blood, coupled with the fact that few DNA scientists were old enough to retire, meant that the number of individuals applying for the same pot of money had to increase. For the first time since the end of World War II, we could not assume that our next grant application would be routinely successful. So our morale would have badly deteriorated had it not been for the "War on Cancer" that was decalred by Congress in 1972. Though initiated over the opposition of most pure molecular biologists, who thought this war promised too much, it was only through the availability of copious cancer funds that the DNA world of the 1970s remained one that outsiders could envy for its ability to do what it wanted.

Until the 1973 advent of recombinant DNA, only the general facts of how DNA functions could be established, and there had never been enough obviously solvable, important objectives for a gold rush to develop. But with the recombinant DNA procedures for gene cloning and the equally spectacular new methods for sequencing DNA, the exact details of virtually any genetic message were up for grabs. At long last, the genetic nature of cancer was a solvable problem and so might be the mysteries of embryological development and the nature of the immune response. Moreover, methods soon developed to introduce functional DNA into a variety of key biological species, opening up the possibilities of using such genetic manipulations for industrial and agricultural ends.

The rush for all this potential gold, however, did not start off with a bang due to worries that a genetically modified organism might upset the current balance of nature, conceivably even annihilating the human race through new forms of cancer-causing human pathogens. So an effective worldwide moratorium on all recombinant DNA work came into effect in mid-1974. The inherent implausibility of these doomsday scenarios, however, led eventually to general disbelief, and in 1977 an NIH-sponsored committee gave permission for the first experiments with genetically manipulated *E. coli* cells to begin. By 1979, further permissions were on hand to do experiments on the genetic determinants that give rise to cancer, and within two years the first human oncogenes were cloned.

To begin to train the vast numbers of scientists who now wanted to work with recombinant DNA, a formal course on gene cloning was initiated here in the summer of 1980, and today we hold seven advanced techniques courses that deal with recombinant DNA procedures. Equally important has been our publishing of a massive gene cloning laboratory manual, written by Tom Maniatis, Ed Fritsch, and Joe Sambrook. *Molecular Cloning* has already sold over 60,000 copies, becoming in effect the bible of the recombinant DNA world. Most of its sales appear to be to individual scientists, not to students taking prescribed university courses, and the number sold may be a good measure of the number of scientists who now work with DNA.

The DNA world thus is no longer a collection of friends who have grown up with each other and know each other's strengths and weaknesses. Instead, none of us can personally know more than a small fraction of our peers. All we can now

do is to try to maintain real contacts with that subset of the recombinant DNA world that focuses on a common research objective (e.g., nuclear oncogenes). At the same time, it is becoming impossible to read thoroughly all the new journal articles that common sense tells us we should be conversant with. So, increasingly, we have to rely on the writings of others, say, in *Nature*, *Science*, or *TIBS*, to tell us what is happening in areas outside our immediate research focus. And because of the much faster rate at which the new discoveries occur, it is more important than ever to attend specialized meetings where you can meet your true peers. So, we are steadily increasing the number of such meetings held here. During 1988, we will host 25 high-level gatherings, in contrast to the two gatherings (the Symposium and the phage meeting) held the year I became the director. Within five years, I can easily see this number rising to at least 35, and it takes little imagination to predict that by the year 2000 we will have to host some 50 meetings if we are to continue as the premier meeting place in the world for biology.

In so expanding, the DNA world is not getting duller. The new facts being reported are every bit as important as those found, say, 25 years ago and very often reflect deeper insights into the nature and functioning of living organisms. Many fields that totally bored me 25 years ago (embryology and the functioning of the human brain) now begin to excite me. So it is hard to imagine that our rate of growth will soon level off. Who will pay for this seemingly never-ending expansion, however, is far from clear, particularly in the United States where our borrowing from abroad cannot go on forever in Ponzi-like fashion. With the NIH budget now effectively capped at $6 billion, we have reached the point where more and more highly meritorious grant applications are arbitrarily being turned down. The dangerous situation has arrived where real scientific success is no longer necessarily being rewarded.

The running of even our best research institutions in a first-class manner thus cannot be taken for granted. No matter how hard we work, one or more of our staff may be intellectually strangled by a peer-review group that has too many hungry mouths to feed. For now, a complete lack of faith in the peer-review system is only held back by the recent arrival on the scene of the vastly expanded Howard Hughes Medical Research Institute. It is yearly adding $300 million to the funding of basic biological research. Those several hundred key scientists whom they now support will be able to do science to the best of their ability, as will many of those individuals who work under the umbrella of program project support from the National Cancer Institute. But for the vast majority who are not so fortunate, the future must look increasingly troubled.

Increasingly we hear the assertion that American science is a zero sum game, with every winner happy only at the expense of a loser's gloom. This must not be accepted! To go this route is to accept the inevitability of the decline of the United States as the major intellectual force in the world. If we do not rise to the occasion and do the science that can be done today, then other nations, particularly those in the Orient, will fill the vacuum. The head start that the NIH has given American scientists to find the gold of DNA could easily be dissipated within a decade. The aspirations of the youth of Singapore, for example, will not be limited to the assembly of Apple computers, and serious DNA research is bound to commence there soon. So, to keep our lead, we must be able to follow up our latest successes with the resources needed for their exploitation. To do this, the NIH budget must be approximately doubled in real terms by the year 2000.

This new money cannot realistically come from the already poor, the aged, or any of our middle-class entitlement programs. Nor are our nation's better-off

farmers going to lose their political clout. Instead, the monies should and can come from our bloated military-industrial complex, which increasingly is producing weapons that either do not work as promised or have costs totally incomparable with any addition they may give to true national security. There is no way we can continue to make albatrosses like the B1 bomber without further hastening our relative industrial decline. Admittedly, it will take courage for our national leaders to admit that our overpreoccupation with national security is destroying our fabric for future greatness. Unless, however, we act soon, we shall be economically dominated by an Orient that has the good sense not to spend money for exotic weapons incapable of rational use. Planning for military engagements in which computers must of necessity replace the human brain is inherently a losing game in which we should not gamble our future.

Fortunately, this may be the time in history when we can reverse our aberrant course. The realization by the current Soviet leadership that their economy is in a shambles and that their fancy weapons lead them even deeper into bankruptcy creates the possibility that our two nations can work together to reduce our military-industrial complexes to levels commensurate with those of our true industrial rivals in Asia and Western Europe. Were this to happen, more than sufficient monies would be released here in the United States to support the increases in our science budgets needed to retain our scientific leadership. At the same time, there would be the money needed by our industries to constantly modernize themselves. Our highest political priority as scientists must be to work toward arms reduction efforts that lower the true costs of our military establishment. So arguing may help our science much more than directly lobbying for more grant funds. Last year's disaster for NSF funding showed how hard it is to get monies that must be stolen from other good causes.

In so arguing, we must take care that we are not outflanked by a growing belief that just as the military-industrial complex is loaded with sleaze, today's DNA world in its search for its own form of gold has lost not only its civility, but its soul. Are we in our bidding for federal monies all too often deceiving our public benefactors through both overstating our achievements and covering up all too frequent cases of hasty, sloppy, if not fraudulent, research?

Admittedly, there have been some real crooks among us over the last few years, and conceivably their relative numbers are increasing. A dishonest undergraduate worked in the lab next to mine while I was at Harvard, and here at Cold Spring Harbor, we had a postdoctoral fellow whom we should not have trusted. The oncogene field, moreover, was temporarily excited by a blatantly crooked graduate student who temporarily rose to great heights at Cornell. But the consequences of these charlatans' deceptions have seldom spread seriously beyond the labs in which they worked, and as a profession, we have not been effectively harmed. More often, we are faced with situations where we cannot distinguish between deliberate deceit and wishful thinking, but here again the scientific truth usually soon sorts itself out, with the individuals concerned having a lingering reputation for often claiming more than they should. So at least in the DNA world that I personally know, there have been no major misuses of federal money that in any sense are comparable with the major Pentagon scandals of the recent past. In those latter cases, it has been virtually impossible to abort quickly the ineffective weapon systems on whose continued production depend the jobs of thousands of innocent workers.

In stating that fraud is not a serious factor in our profession, we should not, however, argue that science must remain essentially a private club that can correct itself without outside help. The thought that a shoplifter might go to jail while a

dishonest scientist is just locked out of our laboratories is bound to bother many decent outsiders. Why should only blue-collar crooks go to jail, with the so-called victimless crimes being treated so much more leniently? Moreover, there are often real victims of scientific fraud who, because of the dishonesty of their associates, have months of lost experiments and may lose not only their research grants, but even the opportunity of staying in science.

I therefore find it hard to defend the notion that the perpetuators of scientific fraud should not be penalized beyond expulsion from the scientific fraternity. To be sure, there will often be difficulties in establishing definitive guilt, and the offenses in many cases will be too trivial to warrant formal legal action. But this is also the case with many other forms of criminal activities, and yet firm penalties are enforced when the circumstances warrant. Stealing a case of soda may only deserve a warning, but a truckload is another matter.

There is, of course, the response that the perpetrators of scientific fraud must invariably be mentally unstable and should be helped back into society rather than incarcerated with common criminals. With no real gold to bank away, they cannot skip off to Brazil and live the good life when their scientific claims fall apart. But if they are to be judged mentally incompetent, this should be left for the courts to decide, not their embarrassed colleagues who don't want it known that they might have been asleep at the helm.

I realize that many of my friends strongly disagree with me, believing that the prospect of jailing our deliberate deceivers will cause more harm than it will ever prevent. Legalizing the right to demand evidence for scientific assertions could easily provide open field days for the malcontents and nonachievers who have nothing better to do. But if we really have the goods to prove our assertions, it costs little to make Xerox copies, and then the shoe is on the other foot. Of course, it is annoying to be attacked by flies, but we virtually never hear of anyone seriously harmed by their bites.

The question must also be faced as to what we should do when we discover that a scientific claim that we honestly believed correct and so published later turns out to be invalid. The obvious response of a decent stranger to science would be to withdraw publicly the claim as soon as possible. Such clear expulsions of mistruths, however, do not always occur, and, in fact, straightforward retractions of misleading claims now are the exception rather than the rule. Yet, as scientists, we always like it to be known that we are offended by other than straightforward pursuit of the truth.

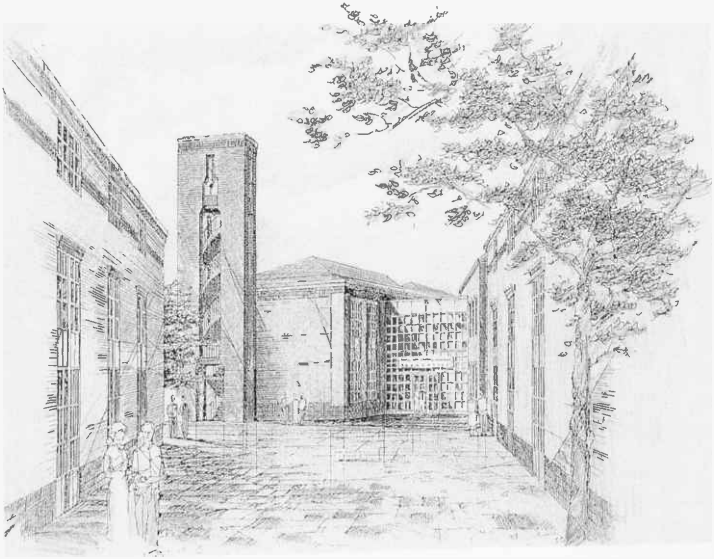
There are many reasons for our apparent hypocrisy in seeming indifferent to setting straight the record of our discoveries. In the simplest cases, the facts that have disappeared were not crucial to the main claims and calling attention to minor errors will serve no valid end and only make our journals even heavier to carry about. In other cases, we will argue that scientific claims that are not followed up by further details tend to be forgotten. If no one cares about an already forgotten and by now irrelevant observation, no harm will come by failure to make a public retraction. But then there are the cases where we don't retract for reasons of embarrassment or semihumiliation. Who wants one's peers to know that we may have been too hasty in doing the right controls, possibly having been driven by the greed to be the first to strike a claim for a particularly pure form of gold? Publicly being perceived as hasty is bound to upset members of the peer-review groups who will judge your next grant, your promotion to tenure, or even your right for a trip to Stockholm.

Being nimble in covering up one's mistakes often may seem as important for one's future as finally getting the right answer. The basest perpetrators of such

false infallibility generally are well known, and there is much talk behind their backs. Occasionally, it bursts into the open when an outraged peer who has gotten the answer without false claims is made to share credit with a fellow scientist whom he thinks he has reason to despise.

We would be making a mistake, however, to neatly divide ourselves into the good and bad guys, for we as scientists are also human beings. True gods exist only in our imagination, and we will seldom find a peer who when faced with even temporary loss of respect does not feel threatened and behaves inappropriately. That we are all imperfect, however, does not mean that we should not try to better ourselves and the society in which we live. Not retracting a false claim is not always a victimless act. Many individuals can be hurt badly by following up supposedly firm observations which by then are known to be false by the original claimant. Making the excuse that all interested parties already know the truth by the grapevine, say, from New York to Cambridge to San Francisco, then on to Berkeley, does not gel well with those working in less-watered areas.

So although it is easy to sneer at the Wall Street Ivan Boeskys and the Pentagon Melvin Paisleys, we must take care that we as scientists do not become the newest victims of the corruption that comes from gold. There are already too many fallen idols in our country, and if we do not fight to keep our standards for honesty and decency above reproach, we will not command the respect we so need.



Neuroscience Center

HIGHLIGHTS OF THE YEAR

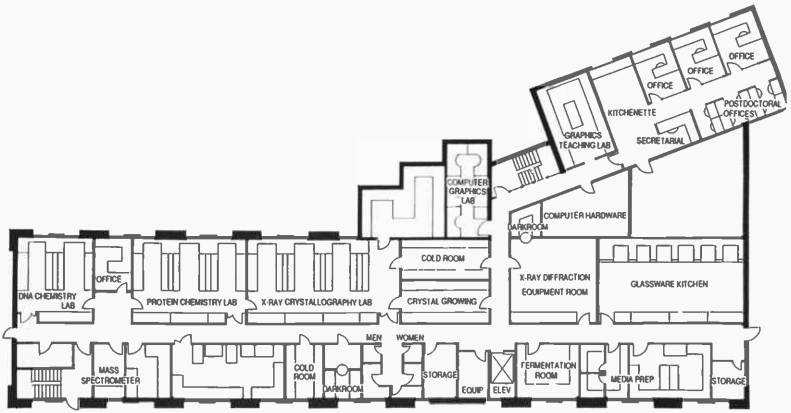
Major Grant from Howard Hughes Medical Institute to Increase Our Teaching Activities

In the fall of 1987, we received the largest foundation grant in our 98-year history. Seven million dollars was pledged by the Howard Hughes Medical Institute toward development of an expanded Neuroscience Education Program. The three-year grant will allow us to establish a year-round training program in advanced areas of neurobiology and to maintain a permanent research program emphasizing molecular approaches to neural development. The grant provides $5 million toward the construction of and equipment for the Neuroscience Education and Research Facility. An additional $2 million is slated for faculty development and ongoing support of the teaching program, to be named the Howard Hughes Education Program.

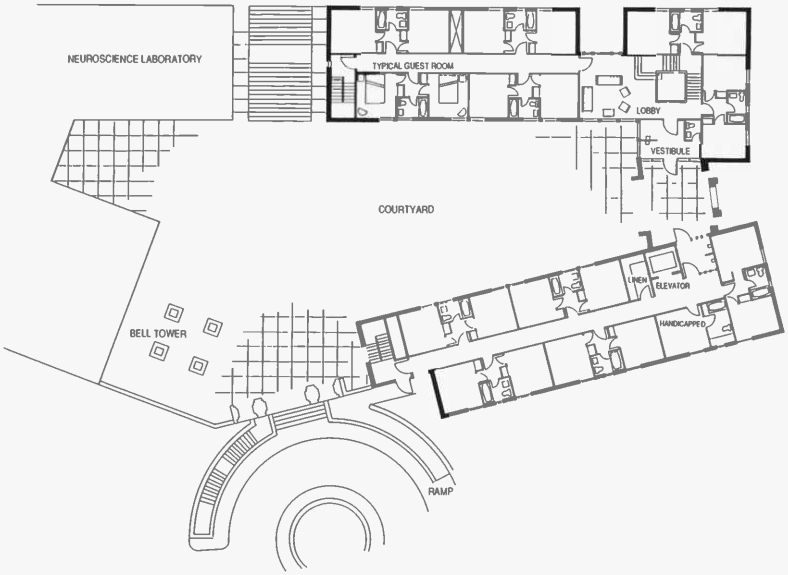
We are proud to have received the largest award among the first group of recipients of grants under the Hughes Institute's new research resource grant program. Officials at the Institute stated that the grant was in recognition of the Laboratory's role as "the schoolhouse for modern biology."

Greatly Expanded Plans for the Upper Campus

The plan for development of the upper campus presented in last year's Annual Report included three separate components: a 24,000-square-foot neuroscience research facility, a 60-bed lodge, and a complex of 6 visitor cabins. However, receipt of the Hughes grant prompted a major rethinking by ourselves and our architects (Centerbrook, Essex, Connecticut) of both the scope and style of the upper campus project.



Neuroscience Facility, Bio-technology Center



Neuroscience Facility, Visitor Lodge

First, the research-teaching facility and 60-room visitor lodge were reconceptualized as a coordinated unit—the Neuroscience Center—linked by a common courtyard. Second, the square footage of the research-teaching facility was nearly doubled to 45,000 square feet. This was cleverly accomplished by resiting large mechanical and storage rooms, formerly on the lower level of the research facility, underneath the central courtyard. This freed up an entire floor of usable research space in which will be situated all of the campus' facilities for structural analysis of biologically important molecules.

As redesigned, the facility will contain ten research laboratories housing some 60 scientists and three specialized teaching laboratories for up to 45 course participants. In keeping with Cold Spring Harbor Laboratory tradition, the teaching components of the facility will be located within a strong research environment that provides course participants with a constant flow of state-of-the-art methods and ideas.

Both the size and the style of the upper campus were reconceived. The original plan called for rustic buildings styled in the manner of the great Adirondack camps of the early 1900s. This fit well with our traditional role as a summer camp for science. However, as it became increasingly clear that the Neuroscience Center would be the hub of the Laboratory's future year-round development, it was felt that its architecture should reflect a greater sense of permanence, as already exemplified here by the highly successful eclectic features of our Grace Auditorium.

In October, we presented plans for the Neuroscience Center, and other potential construction projects, to representatives from the Village of Laurel Hollow. Bill Grover, the principal Centerbrook architect, explained how the structure had been styled to give the impression (when viewed from the other side of the harbor) of a large estate rather than of an institutional building. He conveyed well our concern for maintaining the beauty of our Long Island community. We were especially pleased when the Zoning Board of Appeals formally approved our construction plans in June 1988.

The Beckman Name to Grace Our Neurobiology Building

The new plans were enthusiastically endorsed at the annual meeting of the Board of Trustees in November, to which we invited Dr. Arnold Beckman, the celebrated founder of the Beckman Instrument Company and noted philanthropist. Happily, he liked our proposal to him for major help in the construction of the Neuroscience Center. In the spring, we were awarded a $4 million grant from the Arnold and Mabel Beckman Foundation toward construction of the neuroscience education and research facility, which will be named for the Beckmans. We thought the grant especially appropriate in light of the fact that Mrs. Beckman was raised in the Bayside region of Queens.

Further major support for this project has come through $1 million grants from the Esther A. and Joseph Klingenstein Fund and from the James S. McDonnell Foundation. We also have received a $200,000 grant from the Samuel S. Freeman Charitable Trust to expedite the preparation of the architectural drawings.

Dr. Arnold O. Beckman
(photo by Ingbert Gruttner)

Neurobiology Course Program Expanded

A new course offered in summer of 1987, "Molecular Cloning of Neural Genes," was an indication of the future expansion of our programs in neuroscience education. The organizers, Jim Eberwine of Stanford University and Marian Evinger of Cornell University, saw a need among neuroscience researchers for the recombinant DNA tools that have so revolutionized cancer research.

Reverse genetics—where one first finds the gene, then the protein, and finally a biochemical function within the cell—should provide keys to unraveling the function of the human brain. The new course was made possible through a generous core grant from the Esther A. and Joseph Klingenstein Fund, which has been largely responsible for the expansion of the Neurobiology Teaching Program to its present size of eight courses.

Alumni Fund Helps Build First Cabin Complex

In the fall, we broke ground on construction of six heated visitor cabins to replace dilapidated ones erected here in the 1950s. Each of the new log structures contains four double bedrooms, two baths, and a common living area; multiple gables and planed, clapboard-like siding give them an architectural flair. Completion of the cabins in fall 1988 will make the first phase of development of the upper campus site. Over the next several years, we hope to get village approval to construct an additional four cabins.

Major gifts from Herb Boyer and Mark Ptashne provided the impetus to begin

Cabin Complex under construction

Commemorative plaque in Page Laboratory

Dr. Barbara McClintock

construction of the cabin complex. But just as heartening were gifts from 80 scientists worldwide, to date totaling $60,000, toward an "Alumni Cabin." This cabin will honor all the remarkable biologists who have enriched the legacy of Cold Spring Harbor Laboratory—as staff members and as meeting and course participants.

Advanced Teaching Program to Become a Year-round Effort

Since most of our on-site accommodations lack heating, our advanced-training workshops have always been confined to the summer months. With completion of the first six cabins in fall 1988, we will be able to institute our first spring and fall teaching sessions. Supported by our Hughes grant will be four courses: Cloning and Analysis of Large DNA Molecules, Macromolecular Crystallography, Protein Chemistry, and Molecular Biology of the Yeast, *Schizosaccharomyces pombe*.

Page Laboratory Is Dedicated

The dedication of the Arthur W. and Walter H. Page Laboratory this October marked the culmination of our three-year capital program in plant genetics. More than 350 people gathered in Grace Auditorium to honor the remarkable contributions of a father-son team whose commitment to the Laboratory has stretched unbroken for more than 60 years. Among the day's speakers was Barbara McClintock, whose seminal work on transposable elements in maize (corn) forms the intellectual basis of research carried out in the Page Laboratory. The dedication was followed by a special dinner in Bush Lecture Hall for friends of the Page family.

Arthur Page was a key player in founding the Long Island Biological Association (LIBA), which assumed administrative responsibility for the Laboratory when the Brooklyn Institute of Arts and Sciences withdrew its support in 1924. Including

Renovated Delbrück teaching lab

13 years as president, Arthur Page served continuously on the LIBA Board until 1958, when his son Walter Page became president, serving until 1973. Walter Page, whose tribute appeared in last year's Annual Report, was instrumental in effecting the merger of the Biological Laboratory with the Carnegie operation to establish, in 1962, what we now know as Cold Spring Harbor Laboratory. Walter was a founding member of our Board of Trustees, serving as our Chairman from 1980 to 1986, at which time he became an Honorary Trustee.

The 6500-square-foot Page facility was designed by Centerbrook of Essex, Connecticut. On the ground level are three laboratories occupied by the corn genetics group led by Steven Briggs, Vankatesan Sundaresan, and Tom Peterson. The main level includes scientists' offices and the laboratory of yeast molecular biologist Kim Arndt.

In addition to the new construction, the adjoining original section of Delbrück Laboratory (1929) was completely remodeled. A new glassware kitchen and darkroom were installed on the lower level, and the teaching laboratory on the upper floor was updated. The focal point on the main floor, the Robert L. Cummings Seminar Room, honors Bob's near decade of service on the Laboratory's Board of Trustees, including seven years as treasurer.

Mr. and Mrs.
Robert L. Cummings

Cancer Center Grant Gets Fourth Renewal

Our five-year DNA Tumor Virus Program Project grant from the National Cancer Institute was renewed for the fourth time since its inception here in 1972. This time, Mike Mathews oversaw preparation of the massive grant proposal and now serves

as the program director. This grant was the initial basis of our long-term investigation of the molecular basis of cancer and has enabled us to nurture the careers of numerous young scientists. Equally important was the awarding to us by the National Cancer Institute of a large core grant for our activities as a specialized cancer center. Here, Rich Roberts played the vital role in coordinating the successful grant proposal. Combined, these two grants now provide some $6 million of support to our cancer research efforts.

Uniting Oncogenes and Anti-oncogenes

Since the early 1970s, Cold Spring Harbor has been a leader in understanding the action of oncogenes that play critical roles in oncogenesis. Important work on viral oncogenes, such as E1A and T antigen, was done here, and Mike Wigler's group helped launch the study of human oncogenes with the discovery of *ras* in 1981.

Initially, all oncogenes isolated from both viral and mammalian cells were found to be dominant-acting; i.e., the presence of the gene is a prerequisite for cancer formation. Oncogene proteins play crucial roles in relaying signals that tell a cell when to divide. The alteration or deregulation of the oncoprotein causes cancer cells to replicate inappropriately.

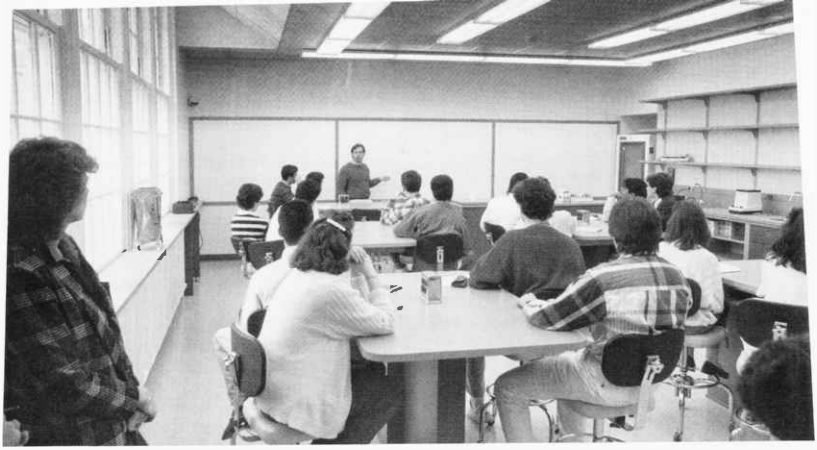
More recently, a fundamentally different type of oncogene was found involved in the rare childhood cancer, retinoblastoma. This malignancy results from the absence of a functional copy of the Rb (retinoblastoma) gene. Rb is considered an anti-oncogene, because its presence inhibits cancer formation. Presumably, the Rb protein in some way tells cells when not to divide. Thus, there was great excitement when, early this year, Senior Staff Investigator Ed Harlow and his group discovered the first physical connection between an oncogene and an anti-oncogene. Working in collaboration with scientists from the Whitehead Institute for Biomedical Research in Cambridge, they found that a protein produced by the viral oncogene E1A binds to the Rb protein. This result suggests that the proteins of dominant-acting oncogenes may bring about malignancy by deactivating anti-oncogene proteins that are needed to suppress cell proliferation.

This most important conceptual breakthrough relied on monoclonal antibodies generated in the specialized facilities that became available with the opening of Sambrook Laboratory in 1984. Long before we even started its construction, we argued that monoclonal antibodies would be powerful tools for understanding cancer, but we never dared to believe that they soon would prove to be so decisive.

DNA Learning Center Opens

Our effort to bring biotechnological literacy to the nation has culminated in the development of a new facility, the DNA Learning Center. In the fall, we assumed a long-term lease (with option to buy) on the former grade school building at the foot of Goose Hill Road in Cold Spring Harbor Village. By spring 1988, we had completed Phase I renovations, which included preparation of office space for a full-time staff of four and the installation of a teaching laboratory where high-school students have the opportunity to perform experiments with DNA.

We also reached agreement with the National Museum of American History, a branch of the renowned Smithsonian Institution of Washington, to display its



DNA Learning Center

exhibit—The Search for Life: Genetic Technology in the Twentieth Century—at the Learning Center from September 1988 to September 1989. Acceptance of the exhibit required us to accelerate our renovation schedule and to entirely revamp the building's antiquated heating and air-conditioning systems. The opening of the exhibit will firmly establish the DNA Learning Center as a leader in its field and also serve as the kickoff for our centennial celebration.

The DNA Literacy Program was founded in 1985 by David Micklos as a community relations project. For several years, Dave administered the program in conjunction with his duties as Director of Public Affairs and Development. However, with receipt of major grant support from the National Science Foundation and the Josiah Macy, Jr., Foundation in 1987, and the subsequent decision to open the Learning Center, it became clear that DNA education had become for Dave more than a full-time job. So, early in 1988, he was named Director of the DNA Learning Center, and Susan Gensel Cooper assumed the title of Director of Public Affairs (while continuing also as Director of Libraries). At the same time, Konrad Matthaei was recruited as the new Director of Development.

Fifty-second Symposium Explores the Origin of Life

Coming on the heels of last year's Symposium on "The Molecular Biology of *Homo sapiens*," the 52nd Symposium tackled an equally fundamental and intriguing topic, "The Evolution of Catalytic Function." It is fitting that a science that has come so close to fully understanding the biochemical basis of life should also attempt to find the origin of the molecules that carry the genetic description of life. Topics ranged from how self-replicating molecules arose in the "primordial soup" of the primitive earth to how protein structure relates to chemical activity. This made

for an extremely intense schedule that included Dr. Ernst Mayr's Dorcas Cummings Lecture, "What Is Evolution All About?" It was a great personal pleasure to welcome back Ernst and his wife Gretal, whom I first met when they spent their summers here living in Hooper House, in the years following World War II. Ernst was then a member of the staff of the American Museum of Natural History, moving in 1953 to the Museum of Comparative Zoology of Harvard University. Scientists and LIBA members alike packed Grace Auditorium for the talk, which was followed by some 15 dinner parties held at the homes of LIBA members.

Undergraduate Research

Our Undergraduate Research Program has, since 1959, been a stepping stone in the careers of many excellent scientists. Under the commendable direction of Winship Herr, the program flourished in 1987, growing to 19 students compared to 15 in 1986. This was accomplished with major support from the National Science Foundation, four-year core support from the Alfred P. Sloan Foundation, and grants from the Burroughs Wellcome Fund, American Cyanamid Company, Pfizer, Inc., and the Samuel Freeman Charitable Trust. One student was supported by the Olney Memorial Fund of the Long Island Biological Association.

A Remarkable Presentation on Brain Research at Banbury

We were honored to collaborate with Shearson Lehman Brothers in October to present an informational weekend for senior corporate executives on "The Human Brain." The purpose was to offer leaders of the nation's pharmaceutical and health-

Max Cowan leads seminar on "The Human Brain"

care industry a view of the frontiers of research on the human nervous system. The meeting opened with Max Cowan's entertaining "field guide" to the human brain, followed by dissection of a real one during a lab session on the second day. One highlight was Terry Sejnowski's description of his success in building a computer that had learned to speak (but not understand) English. The meeting closed with Patricia Smith Churchland's talk on the relationship between philosophy and neuroscience.

Molecular Cloning, Second Edition

When the first edition of the laboratory manual *Molecular Cloning* was published in 1982, the size of its success came as a welcome surprise; sales reached an unprecedented 60,000 volumes. So in 1986, authors Joe Sambrook, Ed Fritsch, and Tom Maniatis began the large task of producing an expanded and updated second edition. The editorial task proved greater than anticipated—the seven existing chapters were completely revised and ten new chapters are being written. As a result, the second edition will be issued in two volumes and is due out in spring of 1989. Despite its large size, this edition will remain moderately priced so that it can continue to be the standard reference on the lab bench of every recombinant DNA scientist.

New Director For Banbury Center

We were very fortunate in October to attract Jan Witkowski as the new director of our Banbury Center. Jan brings to Banbury an intimate view of the sociological aspects of molecular biology, as well as hands-on experience in laboratory work and administration. Before coming to the Laboratory, Jan was the director of the Kleberg DNA Diagnostic Laboratory at Baylor College of Medicine, one of the first labs in the world to use DNA techniques to diagnose Duchenne muscular dystrophy and other genetic diseases. His talents have been made evident by the recent successful meetings held at Banbury, including one on gene expression in

Jan Witkowski, John Inglis

the AIDS virus. Jan's organizational flair is matched by his ability and eagerness to speak to nonscientific audiences on the social impact of DNA science.

New Executive Director of Publications

We were also pleased to welcome, in the fall, John Inglis as the new Executive Director of Cold Spring Harbor Laboratory Publications. John is no stranger either to science or to publishing. After receiving his Ph.D. in immunology from the University of Edinburgh, he chose scientific publishing as a career and soon moved to Elsevier Publications, Cambridge, England. While there, he was the managing editor of several successful journals, including *Immunology Today* and *Trends in Biotechnology*. He has also published numerous scientific articles and edited two books. Presently, he is working on a proposal for a scientific journal dealing with current cancer research.

Staff Investigator
N. Hernandez

Robertson Research Fund Provides Key Support

Although young scientists are the core of our research program, they often find it difficult to obtain the initial grant needed to establish themselves as independent scientists. For several years, income from the Robertson Research Fund, the Laboratory's largest endowment, has helped many scientists take this first step, by supplementing salaries and helping with travel and relocation expenses.

The Robertson Research Fund supports entirely two Cold Spring Harbor Laboratory Fellows. Selected during their final year of graduate work, the CSHL Fellows, in effect, skip the postdoctoral period during which most young scientists work under the direction of a senior scientist. Cold Spring Harbor Fellows are instead given the status of independent junior investigators and are free to work entirely on projects of their own choosing. Each receives a full stipend, as well as funds for supplies and a full-time technician. Adrian Krainer, who joined the Laboratory in 1986 from Harvard, works on factors necessary for nuclear pre-mRNA splicing in mammalian cells. Arriving in January 1988, from Berkeley, Carol Greider studies the RNA-containing enzyme telomerase, which synthesizes telomere sequences found on the ends of all chromosomes.

The Robertson Research Fund also provided full stipend suport for seven postdoctoral fellows and several graduate students. Start-up funds were given to Bruce Futcher, David Frendewey, and Nouria Hernandez to help them establish their labs and hire personnel needed prior to receipt of federal support. Equipment and support funds supplemented the plant genetics program, which receives primary support through a joint agreement with Pioneer Hi-Bred International, Inc. Honoraria and travel expenses were provided for visiting scientists presenting talks at the Laboratory's in-house seminars and winter symposium.

LIBA Initiates New Start-up Funds

This past year, under the adept chairmanship of George W. Cutting, Jr., LIBA continued to provide the key community relations and support that are so vital to the Laboratory's well-being. At the LIBA annual meeting in January, George reported on the creation of a new fund to help answer the needs of young scientists for laboratory equipment. The New Investigator Start-up Fund this year

provided much needed supplies and equipment for Nouria Hernandez and David Spector. LIBA also continued to support four $25,000 LIBA Fellowships to help the Laboratory attract the finest new Ph.D. scientists. Both of these programs, and partial support of our meetings program, were funded through the annual contributions of the almost 600 LIBA members. I was pleased to hear George also report that for the first four months of fiscal 1988, contributions had already exceeded the total for the entire previous year.

Changes in Our Scientific Staff

Senior Staff Investigator Fevzi Daldal, who came to the Laboratory under the joint research agreement with Exxon Research and Engineering Company, accepted a position as Associate Professor in the Biology Department of the University of Pennsylvania, Philadelphia. Fevzi joined the Laboratory in 1983 after receiving his Ph.D. from the Louis Pasteur Institute in Strasbourg, France and doing his postdoctoral work at Harvard Medical School, where he also taught microbiology. A prokaryotic geneticist, Fevzi was interested in the molecular genetics of cytochrome bc_1 complex of photosynthetic bacteria.

Staff Investigators Andrew Hiatt and Takashi Toda moved on to new positions. Andy accepted a position as an assistant member at the Scripps Research Institute in La Jolla. He came to the Laboratory in 1983 as a postdoctoral fellow in Delbrück Laboratory and worked on the molecular genetics of polyamine regulation. Takashi, who worked here with Michael Wigler since 1984, assumed an assistant professorship in the Department of Biophysics at Kyoto University, to resume his studies on genetic control of mitosis in the fission yeast *Schizosaccharomyces pombe*.

New Staff Members

Joining the Laboratory this year was Senior Staff Investigator Tom Peterson, who is doing research on the role of transposable elements in maize gene expression. A native of California, he received his Ph.D. in yeast genetics from the University of California, Santa Barbara in 1984. During his postdoctoral period with the Commonwealth Scientific and Industrial Research Organization (CSIRO) Division of Plant Industry in Canberra, Australia, he switched to the field of maize genetics.

Bruce Futcher also joined the laboratory as Senior Staff Investigator. Bruce is a citizen of Canada and held the position of Assistant Professor of Biochemistry at McMaster University in Hamilton, Ontario, Canada prior to coming to Cold Spring Harbor Laboratory.

Visiting scientists arriving in 1987 were Barbara Knowles, Davor Solter, Stephen Munroe, Janos Posfai, and Paul Young. Both members of the Wistar Institute in Philadelphia, Barbara Knowles and Davor Solter are collaborating with Doug Hanahan. Janos Posfai, from the Institute of Biophysics at the Hungarian Academy of Science, is working with Rich Roberts on sequence analysis and structure prediction. Stephen Munroe, on sabbatical leave from the Biology Department of Marquette University, is working with Rich Roberts on mRNA splicing and RNA-protein interactions. Paul Young, associate professor in the Biology Department at Queen's University, Kingston, Ontario, is working with David Beach on cell-cycle control in *Schizosaccharomyces pombe*.

Several visiting scientists completed their studies at Cold Spring Harbor

Laboratory and went on to new positions: Arie Admon to the Department of Biochemistry at the University of California, Berkeley; Esa Kuismanen to the Robert Wood Johnson Laboratory, Department of Medicine, University of New Jersey, Piscataway, New Jersey; and Junichi Nikawa returned to Gunma University School of Medicine, Japan.

Winship Herr Promoted to Senior Scientist

In recognition of his important research on the fine-structure analysis of the SV40 enhancer region, Winship Herr has been promoted to the position of Senior Staff Scientist. Winship, who works in James Laboratory, received his Ph.D. from Harvard in the spring of 1982 and did his postdoctoral work here under Joe Sambrook.

The Senior Scientist rank carries with it a "Rolling-5" year appointment, which specifies funding for a minimum of five years, continuously rolling forward with time.

Staff Promotions

Robert Franza and Andrew Rice both accepted positions as Senior Staff Investigators. Bob works in McClintock Laboratory, where he is interested in the analysis of oncogenic proteins. He completed his M.D. at Georgetown University, Washington, D.C., did his graduate work at the National Institutes of Health, and completed his medical residency program at Dartmouth Medical School. Bob came to the Laboratory as a postdoctoral fellow with Jim Garrels in July 1982 and shortly after became Staff Investigator.

Andrew completed his Ph.D. at Harvard in 1981 and did his postdoctoral work at the University of Cambridge, England. He came to the Laboratory from the Imperial Cancer Research Fund and since has focused on the regulation of human immunodeficiency virus (HIV) gene expression. He works in Demerec Laboratory.

Jeffrey Field and Dallan Young, formerly postdoctoral fellows, accepted positions as staff associates in the laboratory of Michael Wigler. This newly created position is intended to provide a transition between the postdoctoral period and an independent staff position. The appointment to staff associate is made only in cases where a postdoctoral fellow has demonstrated potential for technical proficiency, scientific creativity, interaction with others, and independent thought. Jeffrey, who came to the Laboratory in 1985, studies *ras* oncogene function in yeast. Dallan, who arrived in 1984, is working on the effector pathway of the *mas* oncogene.

Visiting scientists Steven Briggs and Loren Field were appointed to Senior Staff Investigator and Staff Investigator, respectively. Steven, who shares a joint appointment with Pioneer Hi-Bred International, Inc., works in the area of molecular plant pathology. Loren, previously with Roswell Park Memorial Institute in Buffalo, is developing transgenic mouse models of hyper- and hypotension.

Postdoctoral Fellows

Leaving the Laboratory after completion of postdoctoral terms were Edgar Davidson to the Institute of Structural and Functional Studies (Philadelphia, PA);

Anne Fernandez-Solt to the Centre Nationale de la Recherche Scientifique (Nice, France); Wendy Heiger to the Department of Applied Biological Sciences at Massachusetts Institute of Technology (Cambridge, MA); Mathew Kostura to Merck Sharpe & Dohme Laboratories (Rahway, NJ); and Eugenia Lamas to the Institut National de la Sante et de la Recherche Medicale (INSERM) (Paris, France).

Also leaving were Jeremy (Ned) Lamb to the C.R.B.M. (Centre de Recherche en Biologie Marine), Centre Nationale de la Recherche Scientifique (Montpellier, France); Michael Lambert to the Research Foundation of Scripps Clinic (La Jolla, CA); George Livi to Smith Kline & French Laboratories, Department of Molecular Genetics (Philadelphia, PA); Beat Mollet to Nestle Research Centre (Switzerland); Philip Sass to Lederle Laboratories (Pearl River, NY); Neil Sullivan to the Imperial Cancer Research Fund (London, England); and Frances Purves to the University of Chicago.

Margaret Kelly and Robert O'Malley, both graduate students who received their Ph.Ds. in 1987, left to pursue careers elsewhere. Margaret moved to Charleston, South Carolina, where she accepted a position in the Department of Pharmacology at the Medical University of South Carolina. Robert moved to the Department of Molecular Cardiology at the Children's Hospital in Boston. Another graduate student, Mary Chapman, returned to the University of California, Berkeley, after receiving her M.S. at Stony Brook.

Changes in Our Board of Trustees

At the November Board meeting, we welcomed as new trustees David B. Pall of Roslyn Estates and Mrs. George (Elinor) Montgomery of New York City. David is the founder and chairman of Pall Corporation of Glen Cove, New York. He is generally acknowledged to be the world authority on filtration and has been granted 100 patents.

Elinor joined us following 15 years of active service on the Board of Trustees of Memorial Sloan-Kettering Cancer Center and experience as a teacher at the Brearly School in New York City. Her presence on our board brings back memories of her wonderful mother Mrs. Alex (Posie) White, who served for many years both as a Laboratory Trustee and as a LIBA Director.

Preparing for Our Second Century

I was most pleased when David Luke accepted the chairmanship of a high-level trustee committee with primary responsibility for coordinating the fund-raising effort that will prepare the Laboratory for its second century of scientific excellence. One of his first tasks was to lead the search for a full-time development director.

We were lucky to find Konrad Matthaei, who joined the Laboratory in April 1988. Before then, Konrad was associated with the fund-raising efforts of the New York State Republican Committee. Earlier, he served as director of the Shakespeare Festival in Stratford, Connecticut. He and his staff took up new quarters renovated in the lower level of Wawepex.

The public affairs group of David Micklos, Susan Zehl, and Ellen Skaggs (aided by science writer Dan Schechter and Librarian/Archivist Susan Cooper) designed and produced a most attractive package of development materials. "The First

Hundred Years" gives a fascinating history of the Laboratory, while "The Second Hundred Years" describes development objectives that will launch a new era of productivity. A third major piece describes the Neuroscience Center and begins with a thought-provoking analogy that compares the challenge of neurobiological research to the puzzle of the mythical Gordian knot.

Our Board Is among the Best

At the time of this report, $13.2 million has been pledged toward the total $20 million cost to construct and equip the Neuroscience Center. Major proposals for the remaining sums are currently being reviewed by several leading foundations, and we have reason to be hopeful that the funding for the Center will be in place by the beginning of 1989.

Our extraordinary good fortune in finding funding for this and other centennial projects would not have been possible without the strong leadership and participation of our board. Personal contributions totaling more than $6 million in the last several years have gone a long way in convincing others that our goals are realistic and our management sound. In terms of both willingness to give and personal expertise, we have a board that compares favorably with that of any major university.

We Desperately Need Better Accommodations for Our Visitors

Attendance at Cold Spring Harbor and Banbury meetings registered a 14% rise, from 3600 scientists in 1986 to 4100 in 1987. Two factors contribute to the continued growth in our meetings program. First, in Grace Auditorium, we have perhaps the nation's most beautiful meeting facility. Second, the annual support of our Corporate Sponsors, which now number 30, has given us the flexibility to schedule meetings whose critical importance may not be apparent to federal funding agencies.

Unfortunately, our arrangements for the housing and feeding of visitors suffer greatly in comparison to the quality of our meetings and meeting facilities. Far too many meeting participants, housed in the plastic surroundings of the Jericho strip motels, must commute to and from meeting sessions. They miss the opportunity for a late-night walk down Bungtown Road and informal camaraderie of those who are housed on grounds. Yet, even those housed on the Laboratory campus must endure accommodations that really go several steps beyond rustic. Off-grounds housing has another detrimental impact: at $60.00 per night, motel accommodations cost the Laboratory nearly twice as much as on-site rooms.

A more subtle problem is the great inadequacy of our food service facilities in Blackford Hall, which is mitigated only by the good food and good-natured staff orchestrated by our chef Jim Hope. During large meetings, his staff serves some 1500 meals per day out of a kitchen and service area designed for a third that volume. Enduring long lines and cramped seating (in a non-air-conditioned environment) can make cranky even those newly inspired by the report of important research results.

Our centennial fund-raising agenda includes three projects that will help alleviate these problems: construction of 10 eight-bed cabins, construction of a 60-bed visitor lodge, and complete renovation of Blackford Hall. However, most foundations and private individuals view these projects as secondary to hard research. Therefore, one of my major goals for the upcoming year is to find

benefactors who realize, as trustee Mary Jean Harris wisely put it: "I don't see how anyone can find a cure for cancer if he hasn't had a good night's sleep."

My Twentieth Anniversary as the Director

This summer marks my twentieth year as the Director, having formally taken over the reins in the early winter of 1968. Soon afterward, I married Elizabeth Lewis, then a student at Radcliffe. When the Harvard term ended, we came down for the summer, living briefly in Cole Cottage and then in a rented much larger home on Shore Road, where we could look after my then very ill father. Our permanent home in Cold Spring Harbor was to be a much repaired and expanded Osterhout Cottage, dating from the late 18th century and in which Alfred Hershey earlier had lived. When construction was to start, the contractor told us that Osterhout had decayed beyond redemption. We were soon relieved when we learned that a brand new house could be built for the same price and with ceilings more appropriate for my height and with air-conditioning thrown in for nothing. This was a very good turn of fate, and we found the new Osterhout a perfect home for our then growing family. In 1974, upon the closing down of our Cambridge house, we moved into a totally renovated Airslie, the product of the extraordinary imagination of Charles Moore, then on the faculty of the Yale School of Architecture. At first, the grand size and spaces of Airslie overwhelmed us, making us wonder whether we deserved it. By now, however, it seems very much the perfect family country house.

In coming here, I never asked myself whether we could continue to do inspired science in the past manner of Barbara McClintock and Alfred Hershey. Then, my thoughts focused primarily on the Lab's survival, as opposed to whether we would do science competitive with that I had experienced personally before at Cambridge, Caltech, and Harvard. So, the fact that we have operated at a world-class level for virtually all of the last 20 years gives me pleasure that I never anticipated and so never initially worried about. Now it is natural to ask whether our current period of real glory is coming to an end. Need it be the inevitable consequence of so many institutions of much greater resources also deciding to take DNA seriously? This moment of time, however, is one of our most scientifically productive periods. We continue to reveal ourselves to be an extraordinarily fertile site for young investigators to do their first world-class science. As a consequence, we are all too often subject to raids by prestigous universities who need new young stars on their faculties. Virtually every year I must accept the loss of one to several colleagues that I have depended on for friendship as well as for intellectual stimulation.

At all times since our arriving here, I have strived to strengthen the Lab physically while somehow keeping it very much a village for science. Ever since the summer of 1948, when I first came here as a graduate student of Salvador Luria, I have loved this institution both for its intrinsic beauty and for the unpretentious way it does innovative science, and encourages others to take the same quiet path. No eager mind is too young to be thought important and, correspondingly, no senior scientist is too old to be a student in one of our advanced courses, as long as he or she lives for science and does not demand respect on the basis of age alone. So, I worry each time we undertake a new building project whether we shall lose our past sense of intimacy and the feeling that grand ideas can be at home in simple surroundings. In particular, I was concerned whether the Grace Auditorium might make us an alien place to our real old-timers. Already,

however, the verdict is in. Absolutely no one bemoans the disappearance of the hard cramped seats of Bush or the TV screens for those unable to push themselves in. We cannot imagine functioning without the listening ease made possible by the extraordinary design of Grace Auditorium.

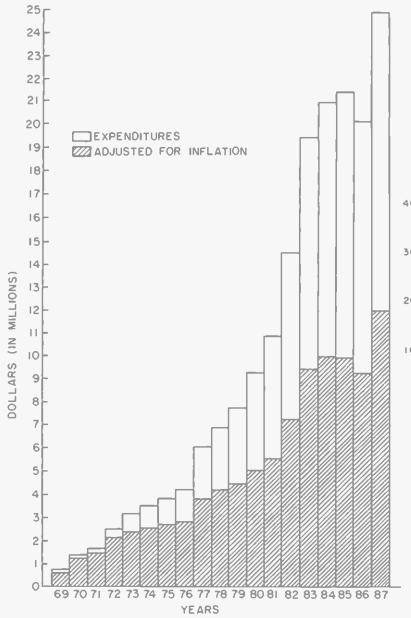
Now, I am not unduly worried by what we will be like upon the completion of the Neuroscience Center, which is bound for the first time to give us the feeling of an academic institution. That is what we already are and if we are to continue to attract the cream of the world's advanced students, we must have the facilities equal to our role. These new buildings must be compatible with our architectural heritage or we could quickly look overblown among our neighbors' wooden homes. But both the new Residence Hall and the Neuroscience Center have been designed with care, and I'm sure they will further enhance the beauty of our inner harbor scene. We must also take great care in the finding of the right scientists to go into these new labs. As long, however, as we continue to have the assistance of our many friends whose careers we have helped shape, we are bound to make the right choices.

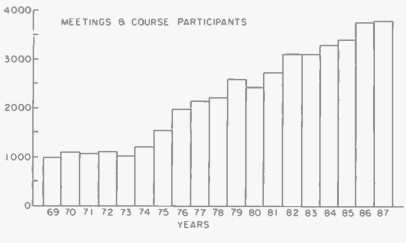
We thus have every reason to believe that we will continue to be an Institution that not only our nation, but also the world can both admire and cherish.

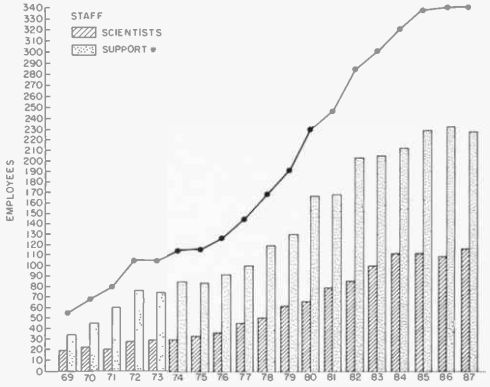
July 27, 1988

James D. Watson

DEPARTMENTAL REPORTS







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

CSHL operations during 1987 resulted in an excess of revenues over expenses, but not in sufficient amount to fully fund depreciation, which has come to exceed $1 million per year.

These financial results were better than expected considering that a major five-year research support agreement with Exxon Corporation ended on December 31, 1986. In addition, there was an unexpected delay in the publication of the second edition of the *Molecular Cloning Manual*, the Laboratory's heretofore best-selling publication, which is now scheduled for early 1989. The last few months of the year were positively affected by the August start of a three-year NIH Cancer Center Core Facility Grant, which will now be a major help in funding the increasing cost of scientific support services.

It is clear, however, that we must continue to seek additional sources of funding, both from government and from among our many friends in the private sector, while emphasizing programs to control the cost of operating the Laboratory. Increasingly, we find that it is more difficult to obtain money for construction of facilities than for program support. Limited laboratory space for research and teaching activities is a serious concern and lends urgency to the need to fully fund depreciation. This is a real expense and absolutely vital to the proper maintenance and replacement of the extraordinary facilities here.

It is also important that overall operations result in a surplus each year. This strengthens the confidence of those we will be asking to fund the expensive new facilities needed for our soon to begin "Second Century" and makes it possible to maintain and build endowment.

Although Cold Spring Harbor seems far removed from Wall Street, October 19th did not pass entirely unnoticed here. The Laboratory's endowment, consisting of the Robertson Research and Maintenance Funds and the Cold Spring Harbor Fund, had risen nicely with the market early in the year; but following October 19th, it ended the year essentially unchanged from the close of 1986, with a total value of $31,608,778. Over the past five years, the endowment has appreciated substantially and has outperformed the popular indices, even though it has been invested conservatively. The conservative investment posture helped to avoid any serious damage from the October collapse of the stock and bond market.

During 1987, it was decided to establish a full-time development office. In past years, Dave Micklos did an outstanding job of obtaining foundation grants and creating the very successful Corporate Sponsor Program, all while attending to his many other responsibilities at CSHL. Now Dave will be heading full time the Lab's new DNA Learning Center. In April of 1988, we were most fortunate to have Konrad Matthaei join us as Director of Development. Konrad has been Executive Finance Director for the New York State Republican Committee and was previously President and Chief Executive Officer of the American Shakespeare Theater in Stratford, Connecticut. He is a graduate of Yale University and received his MBA from the University of Michigan. Konrad is experienced in all aspects of development and is a most welcome addition to the Laboratory. In June, the Development Department moved into new offices in the recently renovated ground floor of Wawepex, from where they and their many community volunteers will have one of the Laboratory's very best views of the harbor.

Our Director of the Library, Susan Cooper, has taken on the additional responsibilities and title of Director of Public Affairs. Susan will also be orchestrat-

ing the many programs and events now in the planning stage, leading up to the Laboratory's 100th Centennial birthday in 1990. She will continue to be responsible for special Laboratory events, such as the Page Laboratory Dedication and the "Nothing But Steel" Exhibit, which she has handled so well.

Since the close of 1987, several good friends and valued associates have left the Laboratory for personal reasons or for other pursuits. We shall miss particularly Bill Putnam and Jacquie Maidel. Bill supervised the Personnel Department and a myriad of other activities, including Meetings, the telephone system, the parking problem, and the many social events that make the Laboratory a civilized and enjoyable place for all. Jacquie was Bill's right hand in Personnel and brightened the Lab with her good nature and grace. Together they made the Lab a caring institution.

In February, Peter Rice departed abruptly, but he left as his legacy a first-class Purchasing Department. His staff, temporarily led by John Maroney and Sande Chmelev, managed with great competence and professionalism in the difficult days following Peter's leaving, and we owe them a debt of gratitude. We are now fortunate indeed to have as our new Director of Purchasing, Chuck Haibel, who comes to CSHL from Rochester, New York, with extensive experience in procurement management, buying of scientific equipment, and contract management in the construction industry. Chuck exhibits a remarkable combination of leadership, overall competence, and good humor and already it is difficult to think of him as a relative newcomer.

Recently, we received the good news that Tony Napoli will be joining us shortly as director of Personnel. Tony lives in East Norwich, New York, with his wife and four children. He is coming to CSHL from Gulf & Western Industries with extensive experience in the personnel field.

Much time was spent last year in planning the Laboratory's expansion, particularly the new Neuroscience Center, on which it is hoped construction can begin this coming Fall and be completed by the end of the 1990 centennial year. A five-year pro-forma operating projection was prepared, including the new guest house complex. The overall project is at least three times larger than any previously undertaken here and will increase the Lab's overall operating budget by more than one-third once it is fully in operation. CSHL's administrative departments are led by highly motivated and professional managers; but now each will be challenged to plan the additional personnel, space, and methods required to properly support this major expansion.

G. Morgan Browne

BUILDINGS AND GROUNDS

In 1987, the Buildings and Grounds Department continued in the fast lane with an ever-growing workload as well as an ever-growing staff to do all the jobs that need to be done. Our Maintenance Department has improved immeasurably under the guidance of Peter Stahl. Thanks to a very competent mechanical, electrical, and plumbing crew and their preventive maintenance programs, air-conditioning and heating problems have been reduced considerably. Stubborn problems, such as

poor temperature control, inadequate wiring, and boiler shutdowns, were solved with both patience and persistence.

Both the custodial and housekeeping crews have also increased their workloads. With the addition of Uplands Farm and the new Page Laboratory to their list, the custodians have taken on an ever-increasing burden of maintenance as well as the collection and disposal of all of the laboratories' waste. The housekeepers silently go about their tasks of linen changing and cleaning as hundreds of meeting participants come and go. Maintaining all of the residences, both permanent and temporary, takes all of their time and effort.

While all this is going on inside the buildings, the grounds crew continually grooms the grounds. However, the job is made even more difficult as they also must do all the new landscaping and are constantly on call to move heavy lab equipment from location to location. Keeping the Laboratory operating efficiently is an enormous job that ALL of our crews have a hand in. This year, again, they have done a fine job.

The Firehouse

In 1986, the Firehouse was moved to its new location and set on its foundation. This year, we continued to work on the interior of the building. The existing apartments were renovated and a new basement apartment was completed. We also furnished the apartments and helped the tenants, who were very patient, to move in. On the exterior, brick and cement staircases were installed, and a historically acceptable entrance porch was constructed. We regraded the north side, putting in a drainage system for a future pond and constructed massive retaining walls. Landscaping was completed after all the concrete work was finished. The Firehouse was then painted red as it was years ago, when it housed the Cold Spring Harbor Fire Department.

Page Laboratory

With remarkable energy, the Buildings and Grounds crew worked hard and long to complete the Page Laboratory in time for the dedication held on Sunday, October 25, 1987. With the dedication only hours away, painters, carpenters, floormen, housekeepers, and custodians were literally backing out of just-completed rooms in order to exit the building while people were coming in the front door for the dedication.

On the lower level of Page, we completed the remaining labs, and on the upper level, we installed two new offices and completed the post-doc offices. Planters were also installed in the hallway. In Delbrück, the first and second floors were also totally renovated, including installation of a new kitchen on the lower level and a brand new darkroom. On the second floor, we ripped out everything and put in an Early American oak Seminar Room, with projection screens, room-sized chalkboards, and a conference table for 20 people. We also installed new tiled bathrooms. The whole area around Page/Delbrück was graded and landscaped and sod was put down.

The building of Page also included the construction of the Delbrück icehouse and a generator shed. The icehouse, which is 300 feet from the lab, houses the air-conditioning chillers for Delbrück/Page buildings. The generator shed is an

addition to the Treatment Plant and houses the emergency generator for Delbrück/Page Labs.

Other Alterations and Renovations

Hershey: Many changes were made at the Hershey X-ray Crystallography Lab. The second floor was completely transformed from an apartment into office space. The first floor saw changes as well—the space that had been Equipment Service was reworked and made into a computer office for the crystallographers. All of the new offices also had air conditioning installed in them.

Doubleday House: During the summer, the Lab assumed ownership of the Doubleday House. A total cleanup was needed, including interior painting throughout and furnishing all the rooms. This was a trick to accomplish because our hassled custodial and housekeeping departments are extremely busy during the summer meetings.

Yellow House: The renovation of the Yellow House was finished during the past year. We replaced the old boiler with a more efficient one and updated two bathrooms, including new fixtures in one of them. The windows were repaired, the interior and exterior were painted, and all the floors were refinished. It all turned out beautifully—just in time for our new Director of Publications and his family to move in.

Blackford Pathway: We completed the pathway that connects Blackford/Bush patio to the Grace patio. A stairway, with seating areas between the two buildings, leads up to Bungtown Road, and a new masonry stair in the rear of Blackford leads to the Blackford lawn.

DNA Learning Center: Although starting late in the year, we managed to remove the asbestos from the boiler and piping and to install new electrical services in the west room Teaching Lab and the three offices. Carpentry was also begun in the same area as well as interior painting and window replacements. This work will continue into the new year.

Uplands Field Station: The second floor was converted into offices and the new Farm Manager's apartment was completed, plus four air-conditioning units were installed. Much landscaping was also done, including the construction of a retaining wall.

Barbara McClintock, Owen Stewart, Louise Chow

We All Miss Owen

We all regret the loss of Owen Stewart. Owen was our electrician for many years and we respected him for his skill and for what he could accomplish on his own. We will miss him sorely. We will also miss all his ghastly jokes, which he so often repeated, and his stories about old Huntington.

Jack Richards

PUBLIC AFFAIRS AND DEVELOPMENT

Since the first Symposium on Quantitative Biology in 1933, scientists have come to Cold Spring Harbor to exchange information on the latest advances in research. However, not until 50 years later did the Laboratory establish a Public Affairs Department to explain science to the nonscientific public. Today, the major goal of the Public Affairs staff is to share the unique resources of the Laboratory with many audiences requiring accurate, up-to-the-minute information about DNA science.

The DNA Literacy Program, founded in 1985 (and explained in greater detail in the education section of this report), is the embodiment of the Laboratory's commitment to public education. The Literacy Program staff, traveling in two Vector Mobile DNA Laboratories, have presented week-long workshops on recombinant DNA techniques to more than 700 high school teachers from New York to California and from Wisconsin to Alabama. Bolstered by receipt of long-term core grants from the National Science Foundation and from the Josiah Macy, Jr. Foundation, the Laboratory began development of a DNA Learning Center in nearby Cold Spring Harbor Village. The nation's first "museum" devoted to explaining biotechnology, the Learning Center is a unique educational resource for Long Island, the New York metropolitan area, and the nation. Dedication of the facility in fall 1988, coordinate with the opening of a major exhibit, "The Search for Life," on loan from the Smithsonian Institution National Museum of American History, will provide tangible evidence of the Laboratory's leadership in stimulating public understanding of genetic biology.

During 1987, two conferences were arranged to bring together scientists and business leaders. In September, at a conference held in cooperation with Pioneer Hi-Bred International, Inc., Wall Street financial analysts heard research briefings on plant genetics from Cold Spring Harbor scientists; they also toured the main Laboratory campus and the Uplands Farm Agricultural Experiment Station. A weekend workshop entitled The Human Brain, cosponsored with Shearson Lehman Brothers, Inc., included scientists from leading research institutions throughout the United States and corporate executives from health care and investment companies.

Local high school students and teachers received an in-depth view of molecular biology from practicing researchers in the continuing *Great Moments in Science* lecture series. The highlight of the series was a talk by Dr. Charles Cantor of Columbia University College of Physicians & Surgeons on Determining the Structure of the Human Genome. Other topics were Techniques of Gene Splicing, Restriction Enzymes and RNA Splicing, Molecular Regulation of Glucose Transport, Microscopic Localization of Oncoproteins, Molecular Diagnosis of Genetic Diseases, Tracking Down the Huntington's Gene, and Genetic Screening for Duchenne Muscular Dystrophy.

A three-part public forum on Gene Manipulation: Personal and Social Implications was cosponsored in April with Hutton House Lectures of Long Island University. Following an introductory session, *Newsday* science writers Robert Cooke and B.D. Colen moderated panel discussions on Human Gene Therapy and Release of Genetically Altered Plants.

Cold Spring Harbor Laboratory Associates participated in two hands-on workshops designed to help them to better understand recombinant DNA techniques that are central to biomedical research. The basis of gene splicing was

shown in the Cutting DNA with Enzymes workshop, where Associates manipulated DNA and separated the resulting fragments using electrophoresis. The Inserting DNA into Cells workshop showed participants how new DNA molecules are introduced into living bacterial cells. Associates also were given an historical walking tour of the Laboratory campus and a research briefing, in which Senior Staff Scientist Dr. James Feramisco demonstrated microinjection techniques used to inject cancer-causing proteins directly into living cells.

To increase awareness of the Laboratory's centennial anniversary in 1990, the Cold Spring Harbor logo was redesigned, and centennial colors of maroon and grey were chosen. The maroon color matches the covers of volumes of proceedings of Cold Spring Harbor Symposia and was originally selected by Reginald Harris, who organized the first Symposium. The logo has been used on a variety of products and publications, including stationery, full-color notecards depicting scenes of the Laboratory, the *Harbor Transcript* newsletter, and announcements of LIBA's new Associates Program. Posters announcing Cold Spring Harbor courses on neurobiology and on molecular genetics were redesigned in centennial colors, and, for the first time, posters were printed to publicize Cold Spring Harbor meetings and to announce the 53rd Cold Spring Harbor Symposium on Quantitative Biology.

Of special importance was the design and printing of an articulated set of literature to explain the significance of the Laboratory's centennial. *The First Hundred Years* uses events that occurred at Cold Spring Harbor as an allegory of the development of modern American science. It explains how the quest to understand the nature of heredity, beginning with the work of Charles Darwin, has led biologists to the brink of understanding the molecular basis of life. *The Second Hundred Years* sets forth development objectives necessary to ensure a second century of scientifc excellence at Cold Spring Harbor. A third major piece describes plans for development of the Neuroscience Center, which will be a focal point of research and education in the Laboratory's second century.

The dedication of the Arthur and Walter Page Laboratory in October marked the successful completion of the Plant Genetics Capital Program initiated in 1983. The Department coordinated efforts that resulted in a total of $3 million in capital funds for the development of the Uplands Farm Agricultural Experiment Station, as well as construction of the Page Laboratory. It also helped to negotiate a joint research agreement with Pioneer Hi-Bred International, Inc. and other grants that resulted in an additional $1.75 million in program support.

The Corporate Sponsor Program, initiated by the Department in 1984, continues to provide core support for the Laboratory's professional meetings and has helped to boost industrial attendance by 73%. Membership grew from 20 companies in 1986 to 29 companies in 1987. Donations from sponsoring companies totaling more than $1.2 million since 1984 have stimulated significant expansion in the number of scientific meetings and overall attendance.

David Micklos

LIBRARY SERVICES

Library Director Celebrates 15 Years at Laboratory

Celebrating 15 years in one organization is certainly a personal milestone; celebrating it at Cold Spring Harbor Laboratory is to have witnessed an extraordinary contribution to scientific research and education, and growth in all manner of things.

	1972	1987	Increase
Laboratory staff	94	339	260%
Library staff	3	6	100%
Annual Report pages	52	366	604%
CSHL titles in print	7	87	1,143%
Buildings*	21	31	48%
Sites	1	3	200%
Nobel Prize winners	2	3	50%
Courses	9	13	44%
Meetings	6	13	117%

*Does not include renovations

To say that the Laboratory has flourished is an understatement. Typewriters without correcting ribbons have been replaced with microcomputers, word processers, and laser printers. Scientific searches, then gleaned from 4-inch encyclopedic volumes, are now done by accessing massive computerized databases. Parking lots have gone from dirt to blue stone. Wild foliage has given way to a plush landscape. The adaptive reuse of our victorian era architecture, along with the addition of several new buildings, has retained the eclectic atmosphere while completely modernizing the facility.

Growth in Permanent Collection

In 1972, the book collection numbered 14,200 and the bound journal volumes equalled 10,800. In contrast, in 1987, the book collection numbers only 8,546 and bound journals have more than doubled to 23,201. This pattern is indicative of the need for rapid exchange of scientific information, as contrasted with the more sedentary nature of the book.

Reference Services Expanded

Our interlibrary loan service has been automated to achieve a rapid response to requests for articles and books for our scientists. Steps have been taken to improve access to clinical and research journals not held in our collection. The library is now a reciprocal member of the Basic Health Sciences Library network, consisting of over 250 medical and health science libraries in the Northeast. This network is also part of Docline, the automated interlibrary loan system of the National Library of Medicine. We have received grants to test several electronic

loan request systems. New document delivery systems are being used, including telefacsimile.

The use of our computerized scientific databases has increased by 42% over the last year, and photocopying by the library staff for patrons has increased by 46%.

In December 1986, the library began an experiment to distribute journal content pages to our senior scientific staff; this is now a regularly established library service. Photocopies of the contents of 53 journals are sent on a daily basis to 28 senior scientists. The program has been very well received.

Continued Use of Storage Facility

Our retrieval of older materials from our storage facility continues to work very well. However, the number of volumes requested has decreased from 287 volumes in 1986 to 158 in 1987, a decline of 45%. This indicates that as time goes on, fewer of these materials will be required, and eventually we will be able to withdraw the unused titles from our collection.

Banbury Center Library

The new director of the Banbury Center, Jan Witkowski, arrived in October and participated in the reorganization of the Banbury Library. Several journal titles were cancelled and others were ordered, changing the emphasis from strictly risk assessment to include more in molecular biology. The book collection has also been weeded, and new titles were ordered.

Special Projects

The Library Director and the staff have been involved in organizing several special events this year, among them the ongoing sculpture exhibit "Nothing But Steel" and the dedication of the Arthur W. and Walter H. Page building.

New Plans for Library Building

To accommodate increased staffing in the Publications Department housed in the Library, plans were completed for the renovation of the second floor and attic of the library building in 1988. The results of this renovation will locate the journal offices, the office of the Director, and the Marketing Department on the second floor. By moving the book and reference collections to the basement, more quiet study space will be available.

Susan Cooper

PUBLICATIONS

A Year of Major Upheavals

1987 was a year of major upheavals within the Publications Department. By far the most disturbing of these was the loss in February of Steve Prentis, who had been the Executive Director of Publications and Director of the Banbury Center. Steve's death in an auto accident, besides being a personal loss for his colleagues here, greatly disrupted the publishing projects he was in the process of initiating. Although we were able to devote the utmost attention to the more developed projects, such as the journal *Genes & Development*, others were put on hold as we began the search for Steve's successor.

Meanwhile, it had been decided to reorganize in such a way that would require the hiring of two new people, one for the Publications position and another to head the Banbury Center, the Laboratory's facility in nearby Lloyd Harbor. In October, the role of Executive Director of Publications was filled by Dr. John Inglis, who came to us from Cambridge, U.K., where he had been Managing Editor of several Elsevier journals, including *Immunology Today, Parasitology Today, Trends in Pharmacological Sciences*, and *Trends in Biotechnology*. Also in October, Dr. Jan Witkowski came to direct the Banbury Center's meetings and publishing programs. Selected meetings that are held at the Banbury Center are published either as *Banbury Reports* or in the series *Current Communications in Molecular Biology*.

Banbury Center Publishing Program Moves to Urey Cottage

Soon after the arrival of Dr. Witkowski, it was decided to incorporate the editorial and production tasks for the *Banbury Reports* into the program run out of Urey Cottage, on the grounds of the Laboratory in Cold Spring Harbor. Unfortunately, it was at this same time that Judith Blum, who had handled the editing and production of the *Banbury Reports* for more than three years, left to take a supervisory position elsewhere. The editorial assistant from Banbury, Inez Sialiano, was moved into the Urey offices, where she quickly adapted to the needs of the general publishing program, working in crowded office conditions that demand super powers of concentration from all the staff. Editing of the *Banbury Reports* was given out to freelancers until March, when Ralph Battey was hired as Editor.

Banbury Reports published in 1987 included *Antibiotic Resistance Genes: Ecology, Transfer, and Expression*; *Nongenotoxic Mechanisms in Carcinogenesis*; and *Developmental Toxicology: Mechanisms and Risk*.

Still More Staff Changes

The position of Technical Editor left vacant by Doug Owen's departure in December for Massachusetts was filled in March with the hiring of Patricia Barker. In April, Dorothy Brown was promoted to Senior Technical Editor, book division, and Judith Cuddihy was promoted to the new position of Journals Editor and later to Managing Editor of *Genes & Development*.

Genes & Development, Our First Journal

Cold Spring Harbor Laboratory stepped into the journal publishing arena in March with the premiere issue of *Genes & Development*. Published monthly in cooperation with the Genetical Society of Great Britain, the Journal's aims are to explore the relationship between gene expression and phenotype. Almost immediately, *Genes & Development* attracted high-quality research papers, and it quickly became required reading for molecular and developmental biologists. The enthusiastic response of the scientific community, both as authors and as reviewers, enabled us to publish 10 issues in 1987 comprising 1351 pages (including a 312-page issue in December). *Genes & Development* was successful on the subscription and advertising fronts as well, reaching its forecast goals well before year's end.

That this could be accomplished in the aftermath of Steve's death was due to the tireless efforts of the journal staff and the unselfish outpouring of assistance we received from the Editorial Board and the scientific community at large. Our sincere gratitude goes to Mike Mathews, who without a moment's hesitation, so effectively took up the task of Executive Editor of the Journal.

Mention must also be made of the marketing efforts of Susan Cooper and her staff and the efforts of the Fulfillment Department, headed by Charlaine Apsel. With the advent of the Journal came a whole host of new enterprises for these departments, not the least of which were the selling of space advertising and the handling of subscription lists. That it all came off without a hitch is due to the dedication, at times ingenuity, of both of these departments.

Changes in Our Computer System

A Lab-wide decision to eliminate the PDP44 main frame and convert to IBM PCs called for more major adjustments in our procedures. In April, the new computers arrived and both staff and freelancers began training while continuing to use the old equipment to complete work in progress. This dual operation and conversion of existing files to the new software continued through December. Staff worked extra hours for months to minimize any negative impact that an unavoidable learning curve would have upon production.

The new system also required the reworking of all of our typesetting codes, new tests being conducted with our typesetting suppliers, and new suppliers solicited now that we would be transmitting manuscripts on floppy disks instead of by modem. These changes required that we get revised estimates on all of our series, now including Banbury books.

Desktop Publishing Initiated

With the arrival in September of our Postscript laser printer, we began to generate camera copy in-house. Programming was done both for the marketing newsletter and for all titles in the *Current Communications in Molecular Biology* series. For setting tables and for more complex work, a page-makeup software package will be essential, and we began the process of testing several packages.

Inquiries were begun with manufacturers of OCR scanners. This technology will greatly relieve the amount of keying that needs to be done and allow us to

increase the number of pages produced without the need to recruit, train, and supply computers and software to additional freelance typists.

Two Major Laboratory Manuals in Production

The manuscript for the second edition of *Molecular Cloning*, by Sambrook, Fritsch, and Maniatis, grew in size and complexity to a point where it became clear that the book would have to be published in a two-volume set, totaling about 1200 pages. Publication is expected early in 1989.

Antibodies: A Laboratory Manual, by Ed Harlow and David Lane, began production and will appear in Fall 1988. This will be our first two-color text.

Symposium and Other Book Series

The 1987 Symposium brought together a diverse collection of pure chemists, biochemists, molecular biologists, and evolutionary biologists. Their enlightening discussions of the evolutionary events that may have given rise to living organisms that now exist on earth are contained in Volume 52 of our Symposium series.

Other publications in our regular series included the fifth volume of *Cancer Cells: Papillomaviruses*; *Vaccines 88*; four titles in *Current Communications in Molecular Biology: Nuclear Oncogenes*, *Inositol Lipids in Cellular Signaling*, *Angiogenesis*, and *Gene Transfer Vectors for Mammalian Cells*; and the *Methods in Yeast Genetics Course Manual*.

The Newest Volume of a Standard Reference Work

Composed of more than 750 pages, *Genetic Maps 1987*, the newest volume of this now standard reference work, contains over 100 maps and covers more than 80 organisms. With the rapidly increasing amount of mapping data available, we recognize that an innovative approach to the presentation of this material will be required to preserve the usefulness of this series a few years hence when faced with the next volume, which is sure to pass the 1000-page mark. Alternatives are already being discussed.

Books Dedicated to Max Delbrück and Ahmad Bukhari

Dedicated to Max Delbrück, *Phycomyces* is the only comprehensive review of this field since that written by Max in 1969. Although current work is emphasized, the lasting values of older research are preserved in this 1987 publication.

Also published in 1987 was *Phage Mu*, which details how work using this bacteriophage has contributed to our understanding of transposition, site-specific recombination, and DNA modification and its use as a genetic tool in gene manipulation experiments. This book is fittingly dedicated to Ahmad Bukhari, whose contribution to the study of genetic recombination using bacteriophage Mu as the model system helped inaugurate the field of DNA transposition.

When the Dust Settled . . .

It is gratifying to look back and review that, despite the trials and challenges of 1987, we managed to publish 14 new titles, 7 reprints, 13 abstracts, the Annual Report, and a new journal. As the year ended, we had some 22 new titles in contract or in production, and looked to 1988 to be a happier, albeit even more hectic, year.

Nancy Ford

TUMOR VIRUSES

In this section are the reports of the eight laboratories comprising the Tumor Virus group. These laboratories are studying a wide spectrum of biological processes, ranging from cellular transformation and DNA replication to protein modification and RNA structure. The common thread that joins together these diverse endeavors is the use of the DNA tumor viruses, adenovirus and SV40, as the chief objects of study. Their imitation of and interplay with cellular mechanisms provide an unexcelled window onto the ways in which the processes of growth and replication occur in the mammalian cell. The oncogenes carried by these viruses are capable of inducing the profound changes in cellular metabolism that characterize malignant transformation, and one of the recurrent themes in this year's report is the means by which these genes exert their proliferative effects. A particularly exciting development in this area is the discovery by E. Harlow and his group of the interaction between proteins encoded by an adenovirus oncogene and by a cellular oncogene, the retinoblastoma gene. Variations on this theme of gene regulation and growth control will be evident in the pages that follow.

ADENOVIRUS GENETICS

T. Grodzicker L. Arrigoni R. Chisum R. Cone M. Goodwin P. Hinton M. Jaramillo E. Lamas M. Quinlan

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

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

M. Quinlan, E. Lamas, P. Hinton, T. Grodzicker

The 12S genes of the E1A region of adenovirus induces primary baby rat kidney (BRK) epithelial cells to synthesize DNA and proliferate in the presence or absence of serum and immortalizes them. Increased cellular DNA synthesis is first detectable between 8 and 12 hours after infection and is maintained at a level at least tenfold higher than in adenovirus-5 *dl*312 or mock-infected cells. The 12S-infected and 12S-immortalized cell lines retain many of their original epithelial cell characteristics and are

not transformed. This is in contrast to changes induced by the large T antigen of SV40. The early effects of the protein are similar to those of the 12S protein. However, the cells lose many normal characteristics and become fully transformed (Quinlan and Grodzicker, *J. Virol. 61:* 673 [1987]).

We have shown that a growth factor that stimulates primary epithelial cell proliferation is produced by BRK cells infected with the 12S virus. Increased epithelial cell DNA synthesis and proliferation is detectable between 24 and 36 hours after adding conditioned medium from 12S-virus-infected cells to primary cultures. This mitogenic factor is effective in the absence of serum and can override the inhibitory effect(s) of serum on primary epithelial cells. There is a requirement for the continued presence of the growth factor(s) to maintain the epithelial cells in a proliferative mode, which it can do for at least 8 weeks. The stimulatory activity is complexed with high-molecular-weight molecules, from which it can be released by high salt (Quinlan et al., *Proc. Natl. Acad. Sci. 84:* 3283 [1987]). To identify this growth factor(s), we are pursuing several channels. We are testing the ability of other growth factors to stimulate quiescent primary BRK cells to proliferate. We are also trying to purify the growth factor(s) from conditioned medium generated in the absence of serum (in collaboration with D. Marshak, Protein Chemistry).

In our pursuit of the identification of the 12S-induced growth factor, we have been testing the ability of known growth factors to stimulate quiescent primary BRK cells to proliferate. These cells do not respond to TBFβ, PDGF, EGF, bradykinin, or bombesin. TGFβ also does not inhibit the proliferative response of the primary epithelial cells to the 12S-conditioned medium. However, the BRK cells do proliferate in the presence of bFGF or TBFα. Since we know that our growth factor(s) does not bind to the EGF receptor, we have eliminated the possibility that TGFα is present in the conditioned medium. In addition, 12S-conditioned medium induces a morphological alteration of NIH-3T3 cells that resembles that which is produced by the conditioned medium from cells transformed by the Kaposi sarcoma oncogene, a member of the FGF family (provided by C. Basilico, NYU School of Medicine).

In collaboration with E. Lamas, we have found that the adenovirus 12S-induced growth factor enables the maintenance of primary rat hepatocytes in culture for extended time periods, in a proliferative mode. The hepatocytes do not proliferate as rapidly as the epithelial cells. However, the hepatocytes continue to express adult hepatocyte markers, such as albumin and aldolase B, and do not express fetal markers such as α-fetoprotein.

To map the regions of the 12S gene required for the induction of growth factors and to compare these regions with those required for other 12S functions, we undertook a mutational analysis, in collaboration with P. Whyte (Protein Immunochemistry). We have found that the first exon of the 12S gene involves a function(s) that stimulates quiescent primary BRK epithelial cells to synthesize DNA and proliferate. However, it is unable to maintain the cells in a proliferative mode and immortalize them or to induce growth-factor production. The entire first exon is not required for the induction of DNA synthesis and proliferation of BRK cells; rather, about 12 amino acids at both ends of the first exon peptide are dispensable. The carboxy-terminal boundary of first exon sequences required for stimulation of quiescent epithelial cells coincides with the boundary required for E1A cooperation with Ha-*ras* to transform primary cells, as determined by P. Whyte et al. (Protein Immunochemistry) and B. Moran et al. (Protein Synthesis). In contrast, the cooperation with Ha-*ras* and immortalization of primary cells both require a wild-type amino terminus of the E1A 12S protein, whereas induction of DNA synthesis and proliferation does not. Together, these data indicate that an additional function(s) necessary for both immortalization and cotransformation is encoded within the first 13 amino acids of the E1A protein. This demonstrates a new region important for E1A function.

To immortalize primary BRK cells, expression of a 52-amino-acid region encoded near the 3′ end of the second exon is required. Activation of quiescent primary epithelial cells into the cell cycle is a prerequisite in order for second exon sequences to effect extended growth and immortalization.

The induction of the epithelial-cell-growth factor also requires the expression of the 52-amino-acid region near the carboxyl terminus of the 12S protein. This same region also enables primary epithelial cells to be replated, an early function of the 12S gene. Thus, maintenance of primary epithelial cells in a proliferative mode, immortalization, ability to be replated, and growth factor production are cosegregating properties that require expression of the carboxyl terminus of the 12S protein. No function has previously been ascribed to the second exon of E1A. Further analysis of this region is being pursued to

determine whether the sequences for any of these functions are separable.

The subcellular localization of mutant polypeptides has led to the identification of additional regions required for the localization and/or retention of the 12S protein into the nucleus. The five carboxy-terminal amino acids have previously been identified as a nuclear localization signal for E1A (Lyons et al., *Mol. Cell. Biol. 7:* 2451 [1987]). However, E1A polypeptides missing this sequence are not excluded from the nucleus but are localized in both the nucleus and the cytoplasm. Furthermore, the presence of these five amino acids does not ensure complete E1A nuclear localization. The presence of the first 36 amino acids encoded by the second exon may enhance nuclear localization. A sequence resembling the SV40 large T antigen nuclear localization signal is located in this region and may serve as an additional nuclear localization signal. A signal involved in nuclear localization and/or retention seems to be located toward the amino terminus of the protein. 12S or 13S E1A polypeptides missing amino acids encoded between amino acids 29 and 86 are completely excluded from the nucleus. Further analysis of this region is in progress. Thus, multiple signals seem to be operating in the efficient localization and retention of E1A polypeptides in the nucleus.

Establishment of Murine Bone Marrow Stromal Cell Lines That Support CFU-S Cell Proliferation

R. Cone [in collaboration with David Williams, Children's Hospital, Boston]

During this past year, we have continued to examine the use of oncogene-containing retrovirus vectors for the establishment of differentiated cell lines. T.M. Dexter showed some time ago that long-term bone marrow cultures provide an in vitro environment capable of supporting proliferation of hematopoietic stem cells, analogous to the role of bone marrow in vivo. These cultures are very complex, containing a variety of adherent stromal cells and nonadherent hematopoietic cells that are dependent on the stromal cells for continued growth in culture. To simplify the study of stromal cell–hematopoietic cell interactions, we have established a number of murine bone marrow stromal cell lines from long-term Dexter-type cultures by infection of the adherent layer with retroviruses encoding E1A or SV40 T antigen. Three of five lines immortalized with SV40 T antigen were able to support proliferation of the most primitive murine hematopoietic cell known, the CFU-S cell (colony forming unit-spleen), whereas none of the E1A-immortalized lines were able to do so. Assays were performed by cocultivating fresh nonadherent bone marrow cells with feeder layers of immortalized stromal cell lines and quantitating the number of CFU-S cells per culture at 1-week intervals for up to 5 weeks. CFU-S cells are quantitated by virtue of their ability to home to the spleen and form macroscopic hematopoietic colonies there after intravenous injection into irradiated recipient mice.

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

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DNA SYNTHESIS

B. Stillman E. White, J. Diffley, M. Fairman, W. Heiger, N. Heintz, T. Tsurimoto, G. Prelich, S. Smith, C. Bauer, R. Cipriani, A. Denton, S. Longionetti, S. Penzi

The DNA tumor viruses continue to be valuable probes for understanding the molecular biology of mammalian cells. Consequently, the DNA Synthesis Section remains committed to studying these interesting viruses, but we have strayed far from the field of virology. Our purpose continues toward an understanding of DNA replication and its control in eukaryotic cells, as well as solving some functions of the adenovirus E1B-encoded tumor antigens that effect cellular growth properties.

During the past year, we have (1) studied the functions of cellular proteins that replicate SV40 DNA in vitro, (2) continued to characterize factors required for assembly of DNA into chromatin, (3) analyzed cellular proteins that bind to chromosomal origins of DNA replication in the yeast, *Saccharomyces cerevisiae*, and (4) determined the function of the adenovirus E1B-encoded 19,000-dalton tumor antigen in regulation of viral gene expression. Highlights of this research include the continued characterization of the proliferating cell nuclear antigen and its role in coordinating leading- and lagging-strand synthesis at a replication fork. In addition, several cellular proteins have been isolated and characterized that are required for initiation or elongation of SV40 DNA replication or for the assembly of the replicating DNA into chromatin. Two site-specific DNA-binding proteins that interact with yeast origins of DNA replication have been characterized biochemically, and we are now in a position to continue this characterization to include a genetic approach of the function of these two proteins in yeast. Finally, it has now become clear from our cumulative work on the function of the E1B 19K tumor antigen over the last few years that this protein plays an important role in controlling viral gene expression during the productive infection of human cells with adenovirus. The 19K protein negatively regulates the activity or amount of the virus-encoded E1A protein, which itself is a transcriptional activator of gene expression and an oncogene.

In addition to these ongoing projects, we were fortunate to have as a visitor Dr. Nicholas Heintz from the University of Vermont on a sabbatical leave for part of this year. During his stay at Cold Spring Harbor, Nick studied the replication of DNA from an origin of DNA replication located adjacent to the dihydrofolate reductase gene, establishing a potentially valuable cell-free system to investigate the mechanism of elongation of chromosomal DNA replication from specific chromosomal sites.

SV40 DNA Replication

M. Fairman, W. Heiger, G. Prelich, T. Tsurimoto, S. Longionetti, B. Stillman

The development of a cell-free system for the replication of SV40 DNA in vitro in 1984 by Li and Kelly of Johns Hopkins University allowed a detailed analysis of eukaryotic DNA replication to begin. SV40 contains a small double-stranded, circular genome with a single origin of DNA replication. Only one virus-encoded protein, the SV40 large tumor antigen (TAg) is required for SV40 DNA replication, and although this protein plays a critical role in DNA synthesis, the virus relies heavily upon the cellular DNA replication machinery. During the past 3 years, we have focused on the function of TAg and cellular proteins in SV40 DNA replication. More recently, we have concentrated on identifying the cellular proteins that function during SV40 DNA replication, with the ultimate goal of deciphering their regulation and linking this important cell-cycle event to cellular growth regulatory pathways.

IDENTIFICATION OF MULTIPLE CELLULAR REPLICATION COMPONENTS

To identify cellular replication proteins, in the absence of suitable genetic techniques, we have resorted to a direct biochemical fractionation of the cellular replication extract and purification of individual components from these separate fractions. We described in last year's Annual Report the purification of the first replication factor and demonstrated its identity with three previously recognized proteins.

These were the human autoantigen called the proliferating cell nuclear antigen (PCNA), a growth- and cell-cycle-regulated protein called cyclin, and an auxiliary protein for the DNA polymerase δ enzyme, which we demonstrated, in collaboration with K. Downey and A. So (University of Miami Medical School), could greatly increase the processivity of this little known DNA polymerase.

FUNCTION OF PCNA

The role of PCNA in the replication of DNA from the SV40 origin was investigated by comparing the replication products synthesized in the presence or absence of this protein. In the completely reconstituted replication system that contains PCNA, DNA synthesis initiates at the origin and proceeds bidirectionally on both the leading and lagging strands around the template DNA to yield duplex, circular daughter molecules. In contrast, in the absence of PCNA, early replicative intermediates accumulate. Replication forks continue bidirectionally from the origin, but only lagging-strand products are synthesized, and these products remain the size of Okazaki fragments. Thus, two stages of DNA replication have been identified. The first is the initiation of DNA replication at the origin and the formation of the first nascent strands, a PCNA-independent stage. During the second stage, PCNA is required for leading-strand DNA synthesis and for coordinating replication fork progression on both the leading and lagging strands. Since PCNA functions as a processivity factor for the DNA polymerase δ enzyme, we suggested that this polymerase functions as the leading-strand DNA polymerase. Carrying the speculation a little further, we propose that DNA polymerase α, once thought to be the only replicative polymerase, is involved in producing the first nascent strands at the replication origin and subsequently the Okazaki fragments during the elongation stage of DNA replication. A hypothetical scheme showing our current view of a eukaryotic replication fork is shown in Figure 1.

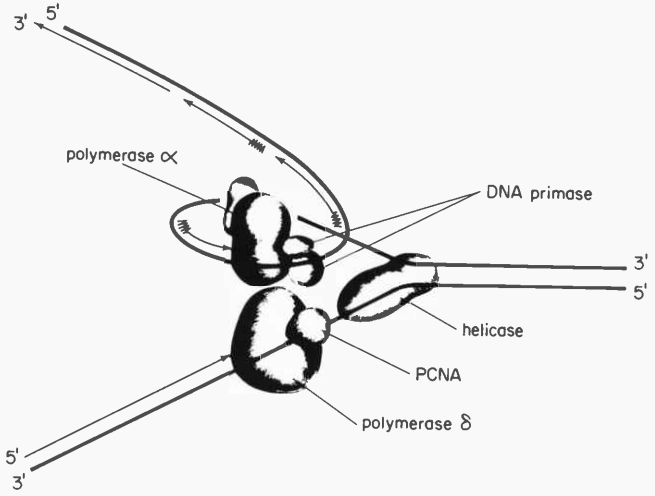

FIGURE 1 A hypothetical view of the eukaryotic replication fork showing two different DNA polymerases that may synthesize leading and lagging strands in a coordinated manner. This diagram highlights the function of PCNA in the synthesis of leading strands at a replication fork.

A MAMMALIAN SINGLE-STRANDED DNA-BINDING PROTEIN

Another cellular protein, purified on the basis that it is absolutely required for SV40 DNA replication in vitro is a multisubunit factor containing polypeptides of 70,000, 34,000, and 11,000 daltons in approximate 1:1:1 stoichiometry. This replication factor, called RF-A, binds to single-stranded DNA cellulose with 30–50-fold higher affinity than to double-stranded DNA and may function as a mammalian cell single-stranded DNA-binding protein (SSB), analogous to the *Escherichia coli* SSB. The three polypeptides are tightly associated in a complex that is resistant to denaturation with urea and thus probably functions in the cell as such. We are currently determining the role that this protein plays in initiation of DNA replication and if it has any regulatory role throughout the cell cycle.

PRESYNTHESIS COMPLEX PROTEIN

A third cellular protein, tentatively labeled SSI, that is essential for the initiation of SV40 DNA replication in vitro has been isolated, although not yet purified. However, this replication factor is of considerable interest because it is the only cellular factor that is required to form a presynthesis complex at the replication origin with SV40 TAg. This factor, together with TAg and the plasmid DNA, when incubated for 15 minutes at 37°C, will allow DNA replication to proceed immediately upon addition of the complete complement of replication factors. In contrast, if this presynthesis factor is not preincubated with TAg and the template DNA, but is added with the other cellular components, then replication is delayed for 15 minutes at 37°C. This presynthesis step, which defines an additional stage in SV40 DNA replication, requires ATP and an incubation temperature of 37°C, since 30°C will not suffice. Since it is possible that this interesting factor is required for a similar stage prior to initiation of chromosomal DNA replication, we are well under way to characterize this protein fully.

OTHER CELLULAR FACTORS

Further fraction of the replication extracts has enabled us to confirm the role of topoisomerases I and II during the elongation stage of SV40 DNA replication. Another cellular protein, which has been extensively purified, is required for the elongation of DNA replication and, as for PCNA, may also be required preferentially for leading-strand, but not lagging-strand, replication.

This analysis, although not complete, has already allowed us to define multiple stages of SV40 DNA replication in vitro (Fig. 2). Some of these steps may be analogous to the multiple stages expected for cell chromosomal DNA replication. It is likely that some of these stages of replication, and the components involved, will be targets for regulatory pathways that control events such as the temporal control of DNA replication throughout the cell cycle, the regulation of initiation and prevention of reinitiation of replication at the origin, and perhaps also elongation from initiated replication complexes at different times in S phase. Finally, we must ultimately understand how these replication proteins are controlled following stimulation of quiescent, nongrowing cells to proliferate.

Replication-dependent Chromatin Assembly

S. Smith, B. Stillman

In addition to our studies on SV40 DNA replication, we have previously described a cell-free system that is capable of assembling the replicating plasmid DNA into a chromatin structure that resembles the structure of mammalian cell chromosomes. The chromatin assembly occurs concomitantly with DNA replication and requires the addition of a nuclear extract obtained from human cells to the DNA replication reactions. A single chromatin assembly factor (CAF) has been fractionated from the nuclear extract, and it appears that this factor can replace the nuclear extract and permit the synthesis of chromatin to occur preferentially on replicating DNA. The focus of current efforts is to identify this interesting cellular protein and determine its function in assembly of chromatin, as well as to characterize fully the structure of the replication products for their histone content and presence of DNase-I-hypersensitive sites.

DNA-Protein Interactions at Cellular Origins of DNA Replication

J. Diffley, B. Stillman

Cell-free systems that faithfully replicate viral DNA have been extremely useful in yielding a deeper un-

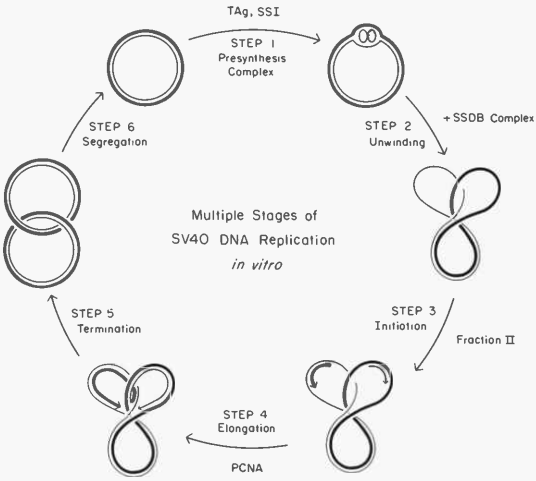

FIGURE 2 A summary of the multiple stages of SV40 DNA replication and the requirement for various cellular replication proteins.

derstanding of the enzymology and mechanism of eukaryotic DNA replication. In comparing viral and chromosomal DNA replication, however, one must ultimately consider the critical role played by virus-encoded proteins, especially in the initiation reactions. The identity of cellular proteins with functions and biochemical activities analogous to the virus-encoded initiator proteins can at present only be inferred.

The identification of *a*utonomously *r*eplicating *s*equences (ARSs) as origins of DNA replication in the yeast *Saccharomyces cerevisiae* allows us to approach the mechanism of initiation of chromosomal DNA replication more directly. That ARSs are, in fact, origins of DNA replication is based on many different lines of evidence. ARSs allow any colinear DNA to be maintained episomally, replicating once per cell cycle under control of the same genes required for chromosomal DNA synthesis. The number of ARSs is approximately equal to the number of origins of replication. Mutations within ARS1 that reduce ARS function increase the frequency of aberrant 1:0 (and not 2:0) segregation events, which is consistent with a defect in replication rather than in segregation of daughters after replication. Finally, replication has been directly shown to initiate at or near ARS sequences in vivo.

Two important generalizations have emerged from analysis of several ARSs concerning the sequences required for efficient ARS function. First, all known ARSs contain and apparently require the ARS core consensus sequence ($^{A}/_{T}$TTTATPuTTT$^{A}/_{T}$). Second, this sequence alone is not capable of efficient ARS function. Sequences flanking the ARS core consensus sequence, which apparently exhibit little homology and can range from as little as 14 bp on only one side of the consensus sequence in the case of the HO ARS to several hundred base pairs, including sequences on both sides of the consensus sequences in the case of ARS1, are important for ARS function.

Not much is known about the molecular mechanisms governing eukaryotic DNA replication, but it is likely that control of cell growth and DNA replication are effected on multiple levels prior to S phase. In yeast, for example, S phase is dependent on the execution of a number of genetically defined events during G_1. Moreover, a number of proteins apparently required for DNA replication are expressed in a cell-cycle-dependent fashion just prior to S phase.

The complexity of eukaryotic DNA replication with respect to the number of origins and the temporal regulation of origin function during S phase makes it unlikely that replication is controlled solely by controlling the level of some initiator protein. Thus, although each eukaryotic origin of replication may have complex and subtle roles during S phase, the sequences required for origin function may be simpler sequences than their prokaryotic counterparts and may not serve as central loci for the control of S phase and, consequently, cell growth. This notion is supported experimentally by the fact that any sequence can be replicated in a controlled fashion when injected into a *Xenopus* oocyte. Additionally, there is some evidence that the yeast ARS core consensus sequence, which is, in fact, a very simple sequence expected to arise randomly once every 10,000 bp in the yeast genome, is capable of functioning alone as an ARS, albeit very weakly. The sequences that normally flank the core and increase mitotic stability may function to make origins more efficient and may not actually be mechanistically required for origin function. It is within this context that the roles of ARS-binding factors I and II (ABFI and ABFII), two proteins which we have purified and characterized, in ARS function must be considered, since both proteins appear to interact specifically with sequences that flank the ARS core consensus sequence, and neither protein appears to interact specifically with the essential core consensus.

We have purified ABFI as a 135-kD sequence-specific DNA-binding protein that binds to a single site at both ARSI and the HMR E region (HRME) but does not bind to all ARSs. Deletion of this binding site at both ARSI and HMRE reduces the mitotic stability of these plasmids but does not affect the ability of these ARSs to transform yeast at high frequency. Deletion of these binding sites therefore does not inactivate either ARS, although it does reduce the apparent efficiency of these sequences to serve as replication origins. HMRE, in addition to being an ARS, also functions in *cis* to repress completely transcription of the mating-type information contained within HMRE in a position- and orientation-independent manner (Brand et al., *Cell 51:* 709 [1987]). HMRE contains three functional elements designated A, E, and B that are required for repression. Element A is the ARS core consensus sequence, element E is a binding site for the cellular protein SBF-E, and element B is a binding site for ABFI. These three elements exhibit some functional redundancy, since any one can be deleted without drastically reducing silencer function. Deletion of any two elements, however, completely inactivates the silencer. Since one of these three elements is an ABFI-binding site, ABFI can apparently function in transcriptional repression. Characterization of the gene encoding the ABFI polypeptide and mutations therein will allow an analysis of these possibilities and yield an understanding of the in vivo role of ABFI. The availability of the purified ABFI protein provides approaches that will allow the identification of this gene.

A role for ABFII in ARS function is more difficult to assign on the basis of biochemistry and sequence analysis due primarily to the fact that this 21-kD protein can bind any DNA with roughly equal affinity, although its binding to ARS-containing DNA is qualitatively different from its binding to nonspecific pBR322 DNA. As shown by DNase I footprinting, ABFII binding to pBR322 DNA appears to occur randomly across the entire fragment, whereas binding to ARSI and the histone H4 ARS occurs at multiple discrete sites within sequence elements that flank the ARS core consensus sequence and are required for full ARS function. A summary of the ABFI- and ABFII-binding sites at ARSI is shown in Figure 3. ABFII, furthermore, can alter the conformation of at least one ARS, ARSI, apparently by DNA bending. It is interesting that both of these biochemical characteristics—the ABFII ARS-binding pattern and the ABFII-induced ARS bending—are reminiscent of initiator protein binding at many origins of DNA replication. *dna*A binds at the *E. coli oriC* both to specific sequences and nonspecifically in a cooperative fashion until 20–40 *dna*A monomers are bound at *oriC*. A number of episome- and bacteriophage-encoded initiator proteins also bind at multiple contiguous sites within their respective origins of replication, and some of these proteins have been shown to bend origin DNA upon binding. Finally, the SV40-encoded large T antigen exhibits both specific and nonspecific DNA-binding activity and binds at multiple sites within the SV40 origin of DNA replication and, upon binding, alters the structure of the origin DNA. The pattern of ABFII binding at both ARSI and the histone H4 ARS is such that the ARS core consensus sequence is at least partially exposed to DNase I digestion, which is interesting since both the *dna*A-binding sites at *oriC* and the TAg-binding sites at the SV40 *ori* lie adjacent to conserved sequences required for DNA replication. *dna*A binds adjacent to the 13-mer sequences that are required to interact with the *dna*B

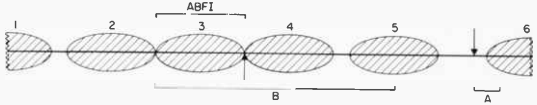

FIGURE 3 Protein binding sites at the ARS1 origin of replication. The positions of ABFII (hatched ovals)- and ABFI (bracket)-binding sites were derived from DNase I footprinting data. Arrows indicate DNase-I-hypersensitive sites induced by the binding of ABFII. Locations of the essential domains (*A,B*) that constitute the ARS1 origin are shown.

helicase, and TAg binds adjacent to an essential 17-bp A+T-rich sequence.

Function of the Adenovirus E1B 19K Tumor Antigen

E. White, A. Denton, R. Cipriani, B. Stillman

Adenovirus early region 1 (E1) is primarily responsible for regulating transcription of adenovirus early genes during productive infection as well as conveying the ability of adenovirus to transform cells in culture. E1 consists of two transcription units, designated E1A and E1B. Although E1A is known to *trans*-activate transcription of viral and cellular genes and cause immortalization of primary cells in culture, proper regulation and manifestation of these processes require expression of the E1B oncogene. The nature of the function of one E1B gene product, the 19K tumor antigen, has been largely unknown, save its requirement for gene regulation and transformation. We have adopted two strategies for delineating the function of this important protein. The first approach was genetic, where we have examined the effect of mutations in the gene encoding the E1B 19K protein on adenovirus gene expression and transformation. The second approach involved the construction of expression vectors to express the 19K protein and specifically define its effects on gene expression and to assess whether the 19K protein possesses any intrinsic biological activity.

Characterization of adenovirus mutants containing point and deletion mutations in the E1B 19K gene yielded a wealth of information regarding the function of the 19K protein in the life cycle of the virus. The mutations induced a number of interesting pleiotropic phenotypes during infection; enhanced and abnormal cytopathic effect (*cyt* phenotype), degradation of the host-cell DNA (*deg* phenotype), formation of large plaques (*lp* phenotype), accelerated growth in quiescent human cells (host range, *hr* phenotype), and defective transformation (*tra* phenotype). The wide scope of these phenotypes suggested that the 19K protein affects fundamental aspects of viral morphogenesis. The two critical findings from this work are (1) that the 19K protein was acting as a negative regulator of adenovirus early gene expression, accounting for the accelerated growth of mutant viruses, and (2) that induction of the mutant phenotypes was dependent on expression of the E1A proteins. Two main points remaining to be elucidated are (1) the mechanism by which the 19K protein repressed gene expression and (2) the involvement of the E1A proteins in inducing the E1B 19K gene mutant phenotypes.

E1A-DEPENDENT REPRESSION OF ADENOVIRUS EARLY GENE EXPRESSION BY THE 19K PROTEIN DURING VIRAL INFECTION

Introducing mutations into the E1B 19K gene-coding region resulted in accelerated growth of the mutant viruses, compared with the wild-type virus in growth-restricted human cells (WI38). Similar results were obtained in HeLa cells, but most dramatically with viruses that expressed an E1A 12S cDNA gene in place of genomic E1A sequences. In the case of the 12S virus, introducing E1B 19K gene mutations caused increased early gene transcription, viral DNA replication, early and late viral protein synthesis, and virus production. Elevated viral gene expression in E1B 19K mutant-infected cells was independent of viral DNA synthesis, since it still occurred under conditions where viral DNA synthesis was inhibited. The basis for the effect of E1B mutations appears to be elevated expression of the E1A proteins, which leads to further increased transcription.

The obvious complicated nature of unraveling the mechanism of gene regulation by the 19K protein has led us to restrict our investigation to a single, well characterized gene promoter (SV40 enhancer and early promoter) driving expression of a marker

gene encoding bacterial chloramphenicol acetyltransferase (CAT), constructed by M. Quinlan (Adenovirus Genetics Lab) and E. Harlow (Protein Immunochemistry Lab). This SV40-CAT indicator gene was placed in an adenovirus chromosome in place of E1 sequences. Effects of the E1A and the E1B 19K proteins on expression of the SV40 promoter/enhancer could be determined simply by coinfection of the SV40-CAT with viruses that expressed the E1A 12S gene product, with or without an intact E1B 19K gene, and measuring CAT activity. The result from this experiment was, as expected, that E1B 19K mutations caused elevated expression of the SV40 enhancer. Demonstrating an effect of E1B on E1A and this specific promoter/enhancer should enable us to ask if interactions of cellular factors with this promoter are effected by the E1B 19K protein.

E1A-INDEPENDENT STIMULATION OF VIRAL REPLICATION BY THE E1B 19K PROTEIN

To examine the effects of E1B 19K gene mutations on adenovirus gene expression and replication in the absence of the E1A proteins, we constructed a virus (9S.R2) that contained an E1B 19K gene point mutation and was capable of expressing only a nonfunctional E1A 9S cDNA gene. Viral gene expression, DNA replication, and virus production were examined and compared to that of a 9S virus capable of synthesizing wild-type E1B proteins. Surprisingly, E1B 19K mutations had the opposite effect in the absence of E1A, as they did when E1A was expressed; i.e., instead of causing increased viral gene expression, an E1B 19K mutation in an E1A 9S cDNA background resulted in diminution of viral gene expression, viral DNA synthesis, and viral replication, albeit at low levels. This effect of 19K gene mutations was manifest primarily at the level of viral DNA synthesis, since inhibiting viral DNA synthesis substantially eliminated any effect of the E1B mutations on gene expression. This indicated that in the absence of E1A, the primary effect of the E1B 19K protein may be to stimulate viral DNA replication, and thereby increase gene expression indirectly.

CONSTRUCTION AND USE OF PLASMID VECTORS AND CELL LINES THAT EXPRESS THE E1B 19K PROTEIN

From the work with adenovirus mutants, it was clear that the E1B 19K protein effected viral gene expression in both a positive (in the absence of E1A) and a negative (in the presence of E1A) fashion. Because examining gene expression during viral infection is complicated by the interaction of many viral and cellular proteins, and indirect effects resulting from replication of viral DNA templates, we decided to examine the role of the E1B 19K protein in regulating gene expression in a more simplified system. Expression vectors were constructed to produce the E1B 19K protein under the control of heterologous promoters for use in transient expression assays. The two promoters that were chosen were the mouse metallothionein promoter, because its expression is inducible, and the cytomegalovirus enhancer and promoter, because it is an extremely strong promoter and is capable of expressing large amounts of the 19K protein, comparable to those found in infected cells. These plasmid expression vectors, MT19K and CMV19K (Fig. 4A), were also used to construct cell lines that express the E1B 19K protein. Once these vectors were constructed and demonstrated to express the E1B 19K protein (Fig. 4B), they were used in transfection experiments with marker genes containing various promoters driving expression of the *cat* gene.

EXPRESSION OF THE E1B 19K PROTEIN RESULTS IN STIMULATED EXPRESSION OF TRANSFECTED GENES

HeLa cells were cotransfected with the plasmid expression vectors MT19K or CMV19K, and the effects of the 19K protein expression on a number of other promoter-CAT constructs were determined (E3CAT, E1ACAT, RSVCAT, SV_2CAT). In all cases, cotransfection with either the MT19K or CMV19K vectors with the CAT plasmids resulted in increased CAT activity over transfection of the CAT plasmids alone. Stimulation of gene expression by cotransfection of the MT19K expression vector was small (2-fold), compared with the CMV19K vector (3–5-fold), which produces considerably more 19K protein. Similar stimulation of gene expression (2–25-fold) was observed when cell lines that express the E1B 19K protein were transfected with promoter-CAT plasmids, compared with cell lines that did not express the 19K protein.

POSSIBLE MECHANISMS BY WHICH THE E1B 19K PROTEIN REGULATES GENE EXPRESSION

From studies with adenovirus mutants that carry mutations in the E1B 19K gene, it is clear that the 19K

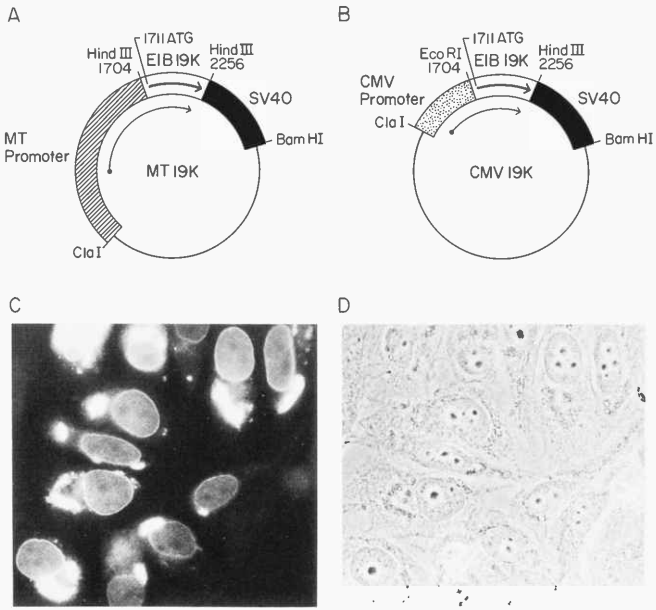

FIGURE 4 E1B 19K expression vectors. (*A,B*) Schematic representation of E1B plasmid expression vectors; (*A*) MT19K; (*B*) CMV19K. (*C,D*) Expression of the E1B 19K protein in HeLa cells. Cells were transfected with the CMV19K vector and examined by indirect immunofluorescence with an E1B 19K-specific monoclonal antibody (*C*) or by phase-contrast microscopy (*D*)

protein negatively regulates E1A-dependent viral gene expression. The absence of this negative regulation leads to an accelerated infection and overproduction of early and late viral gene products and most likely accounts for the enhanced cytopathic effect, large-plaque formation, and the host-range phenotype observed in mutant-infected cells. This negative effect of the 19K protein is an important aspect of viral gene regulation during infection, since without it, premature death of the host cell occurs, resulting in compromised virus yield. Furthermore, since the negative regulation of viral gene expression is E1A-dependent, it follows tnat the E1B 19K protein either directly or indirectly modifies the activity of the E1A proteins during infection.

How the E1B 19K protein stimulates expression of transfected genes may be related to the ability of the 19K protein to prevent degradation of DNA (*deg* phenotype). Since transfected DNAs are inherently unstable and eventually become degraded after transfection, extending the half-life of the transfected DNA template should greatly increase gene expression. Increased DNA stability might similarly account for increased 9S virus replication compared to the 9S virus, which does not express a functional E1B 19K gene product. Evaluation of the role of the 19K protein in stabilizing transfected DNA is being carried out using the CMV19K plasmid expression vector in transient expression assays (in collaboration with C. Gorman, Genentech, Inc.).

These studies have demonstrated that the E1B 19K protein can modify gene expression possibly by two (not necessarily independent) mechanisms: (1) by affecting the activity of the E1A proteins and (2) by

possibly affecting the stability of DNA in the nucleus. Eventually, demonstration of E1B 19K protein function will be carried out with purified proteins in in vitro systems. As a first step toward this goal, we have expressed the 19K protein in *E. coli* using the T7 expression system, which should serve as a source of protein for these experiments.

Role of the E1A Proteins in Inducing the *deg* Phenotype in E1B 19K Mutant-infected Cells

E. White, A. Denton

The E1A proteins are central to the induction of the E1B 19K gene mutant phenotypes. For example, the *deg* phenotype occurs in cells infected with an E1B 19K mutant virus, but the same 19K gene mutation does not cause DNA degradation if the E1A gene is also deleted. How the E1A proteins induce DNA degradation in the absence of E1B is not known, and since the E1A proteins are multifunctional, serving to regulate transcription, stimulate DNA synthesis, and otherwise modify basic cellular functions, the possibilities are numerous. By comparing amino acid homologies between different adenovirus serotypes in conjunction with mutational studies of the E1A gene, the E1A-coding region has been divided into discrete functional domains. We have set out to determine which domains and their associated functions within the E1A-coding region are required for the induction of the *deg* phenotype.

Mapping the functional domains of E1A required for inducing DNA degradation was accomplished by the construction of E1A-E1B 19K double-mutant viruses. Specifically, viruses were constructed that carried deletion or point mutations within each of the three E1A conserved domains, in addition to an E1B 19K gene deletion. These E1A-E1B double-mutant viruses were then assayed for the induction of DNA degradation with the anticipation that eliminating the E1A function required for inducing DNA degradation would result in extragenic suppression of the *deg* phenotype. This was exactly the result that was obtained when E1A domain one was deleted. Domain one has been demonstrated to function in the stimulation of cellular DNA synthesis. Therefore, it appears likely that the means by which the E1A proteins induce DNA degradation in E1B 19K mutant-infected cells may be related to the ability of E1A to stimulate cellular DNA synthesis and alter growth properties of infected cells. This is interesting in light of our previously reported result that the *deg* phenotype does not occur in E1B 19K mutant-infected human WI38 cells, which are strictly growth-controlled. It is conceivable that the E1A proteins alter cell-growth physiology by modulating the expression of cellular genes or by producing changes in chromatin structure that might otherwise require E1B expression to maintain the integrity of the host-cell chromosomal DNA during productive infection.

Differential Localization of the Adenovirus E1A Proteins and Association of E1A with the Cellular Heat-shock Protein 70 in Infected Cells

E. White [in collaboration with D. Spector and W. Welch, Cold Spring Harbor Laboratory]

We originally set out simply to examine the localization of the adenovirus E1A proteins by indirect immunofluorescence and immunoelectron microscopy in adenovirus-infected cells. Much to our surprise, the distribution of E1A was heterogeneous with not one, but five distinct localization patterns being observed in the nucleus of infected HeLa cells. We have designated these E1A localization patterns diffuse, reticular, nucleolar, punctate, and peripheral nuclear staining on the basis of their appearance (Fig. 5). The heterogeneity of E1A localization during infection was quite unexpected, but interestingly, this variable distribution of E1A correlated with both the time postinfection and the cell-cycle stage of the host cell at the time of infection.

To distinguish which localization patterns of E1A were associated with the early versus the late stage of infection, E1A localization was examined in time course experiments. The distribution of E1A was also determined during infection in the presence of hydroxyurea in order to inhibit viral DNA replication and block passage to the late phase of infection. We have found that all E1A distributions, except the peripheral E1A-staining pattern, were associated with the early phase of infection and were therefore not a consequence of the gross changes in nuclear structure accompanying the late phase.

To examine the effect of the host cell cycle on the localization of the E1A proteins, we compared the

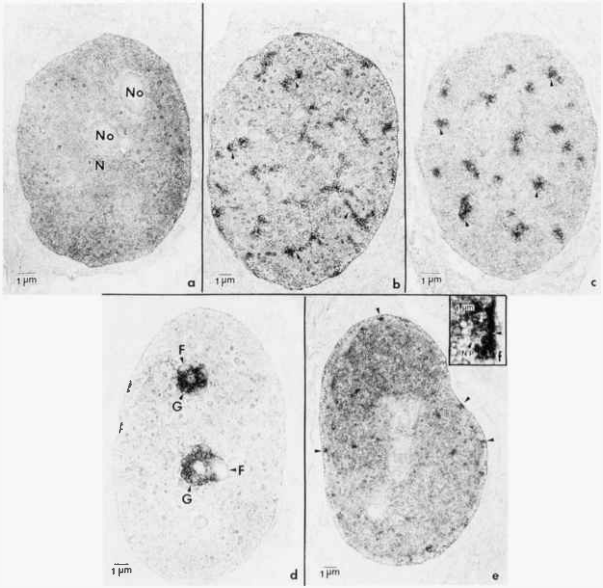

FIGURE 5 Localization of the E1A proteins in infected cells at the electron microscopy level. HeLa cells were infected with Ad2, and 24 hr later, the cells were prepared for immunoelectron microscopy using an E1A monoclonal antibody. Shown are representative examples of the diffuse (*a*), reticular (*b*), speckled (*c*), nucleolar (*d*), and peripheral (*e,f*) staining patterns of E1A. The nucleolar distribution of E1A is limited to the granular region (G) of the nucleolus (*d*). The nucleolar fibrillar regions (F) are devoid of immunoreactivity (*d*). Numerous nuclear pore (NP) profiles can be observed in the regions of peripheral immunoreactivity (*e,f*).

distribution of E1A after infection of normal populations of asynchronized cells to that of cells that had been accumulated in the late G_1 and S cell-cycle stages prior to infection due to inhibition of cellular DNA synthesis. When cells accumulated in G_1 and S were infected, there was a more homogeneous distribution of E1A staining with a predominance of the diffuse nucleus-staining pattern. This is in sharp contrast to the heterogeneous staining observed after infection of asynchronized cells, suggesting that the cell-cycle stage at the time of infection may effect the distribution of E1A within the nucleus.

We have also observed that the localization of the E1A proteins resembles that of the 70-kD cellular heat-shock protein (hsp70) after heat shock of uninfected cells. This is interesting in light of the fact that E1A is known to stimulate transcription of hsp70. Therefore, we investigated whether E1A expression could alter the intracellular localization of hsp70, as well as stimulate hsp70 expression. Examination of the distribution of hsp70 before and after adenovirus infection revealed that hsp70, which is normally predominantly cytoplasmic, was rapidly translocated to the nucleus after infection. hsp70 accumulated in nucleoli, and also showed reticular, diffuse, and punctate nuclear distributions. This translocation of hsp70 to the nucleus upon infection was an E1A-dependent process, since the localization of hsp70 was unaltered by infection with an E1A 9S cDNA virus, which does not synthesize a functional E1A

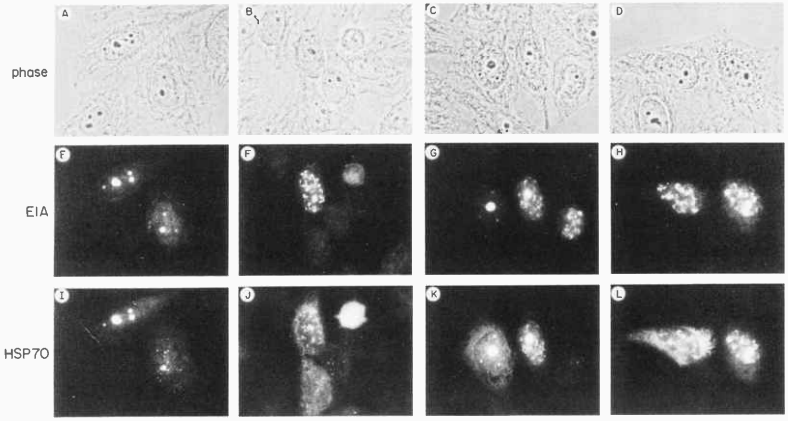

FIGURE 6 E1A and hsp70 colocalize in Ad2-infected HeLa cells. HeLa cells, growing on glass coverslips, were infected with Ad2, and 24 hr later, the cells were fixed and simultaneously analyzed for the distribution of both hsp70 and E1A using double-label indirect immunofluorescence. Detection of the rabbit anti-E1A antibody and of the mouse anti-hsp70 antibody was done by subsequent incubation with rhodamine-conjugated goat anti-rabbit antibodies and fluorescein-conjugated goat anti-mouse antibodies, respectively. Shown are selected fields of cells which displayed colocalization of E1A and hsp70 (roughly 10–25% of the total population in this experiment). Note that the same cells are shown in each vertical column.

gene product. The localization of hsp70 after infection was remarkably similar to the distribution of E1A proteins in infected cells. Double-label indirect immunofluorescence of E1A and hsp70 in infected cells demonstrated colocalization of these proteins within the nucleus (Fig. 6). The discrete nuclear localization patterns of E1A, and the colocalization of E1A with hsp70, were a consequence of adenovirus infection, since only the diffuse nuclear E1A-staining pattern was observed in adenovirus-transformed 293 cells that constitutively express the E1A and E1B proteins.

Demonstration of hsp70 and E1A in the same intracellular locale raised the possibility that the two proteins may be physically associated in vivo. In immunoprecipitation experiments, we have been able to coimmunoprecipitate hsp70 with monoclonal antibody directed against the E1A proteins. These data suggested that the adenovirus E1A proteins may associate with, and possibly form, a physical complex with cellular hsp70 in infected cells. Functional relevance of this association of E1A with hsp70 is not yet known. hsp70 has, however, been found to complex with other oncoproteins and potentially modulate their activity. High levels of cellular hsp70 have been correlated with increased permissivity for adenovirus infection, suggesting that an E1A-hsp70 association may be functionally relevant.

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MOLECULAR BIOLOGY OF SV40

Y. Gluzman D. McVey M. Pizzolato
I. Mohr

Current work in this laboratory is aimed at understanding the role the multifunctional large T antigen plays in the replication of SV40 DNA. In our continuing efforts to correlate various structural regions of large T antigen with a biochemical function, we have assessed the effect of a series of replication-defective point mutations on the DNA helicase activity of large T antigen. This has permitted us to examine the relationship between this newly discovered activity and previously described biochemical activities. Single-amino-acid substitutions that destroyed origin-specific DNA binding had little or no effect on the DNA helicase activity of T antigen. However, a mutation at residue 522, which ablates the ATPase activity of the protein, eliminated the DNA helicase activity of the protein. This mutation also dramatically reduced the ability of large T antigen to bind to single-stranded DNA, whereas it had little or no effect on the ability of the protein to bind specifically to the origin of replication. Other mutants, which failed to bind specifically to origin DNA, displayed wild-type levels of single-stranded DNA-binding activity. Thus, these studies have also permitted us to separate origin-specific DNA binding from single-stranded DNA-binding genetically.

To identify the minimal protein domain that is required for T antigen to bind specifically to the SV40 origin replication, we have constructed a series of T-antigen mutants coding for amino-terminal fragments of different lengths. Mutant DNAs were used to produce truncated T-antigen proteins in *Escherichia coli* using the T7 expression system of Studier and Moffatt (*J. Mol. Biol. 189:* 133 [1986]). The origin-binding properties of truncated T antigens were screened in soluble *Escherichia coli* extracts, and several proteins chosen for further analysis were purified. Whereas full-length T antigen (708 amino acids) produced in *E. coli* binds to fragments containing either the wild-type origin or site I, truncated T-antigen proteins containing the amino-terminal 272 or 266 amino acids bind only to the wild-type fragment with very low efficiency. Shorter proteins, however, displayed a resurgence in specific DNA-binding activity beginning with amino-terminal fragments of 259 amino acids and culminating with a protein containing the amino-terminal 246 amino acids. This 246-amino-acid protein, along with a 249-amino-acid amino-terminal fragment, bound efficiently to DNA fragments containing either the wild-type origin, site I, or site II. When an equimolar mixture of these three DNA fragments was used to evaluate DNA binding, T antigen bound to the wild-type fragment first, followed by the site I fragment, and finally the site II fragment. The 246-amino-acid protein was the

most efficient of all the truncated proteins in the specific DNA-binding assay. Although a 242-amino-acid amino-terminal fragment bound DNA at levels slightly above background, all proteins 240 amino acids in length and shorter failed to demonstrate any specific DNA binding. This allows us to place the carboxy-terminal boundary of the specific DNA-binding domain at amino acid 246. The amino-terminal boundary of the origin-binding domain lies at amino acid 132, and a protein containing only amino acids 132–246 binds efficiently to all sites in the origin of replication. Current work is geared toward purifying this small protein domain for biochemical and structural analysis.

We have continued our interest in crystallizing T antigen for structural analysis (in collaboration with J. Anderson; see Structure Section). Our primary focus has been to purify large amounts of the 259- and 246-amino-acid truncated proteins using the *E. coli*-based expression system. We are currently capable of obtaining 2–5 mg of the truncated T antigens per 500-ml culture. However, degradation products containing an intact amino terminus copurify with the truncated proteins on the immunoaffinity resin employed in the isolation. Most of these contaminants can be removed using various ion-exchange columns. To simplify the isolation of these truncated proteins from their degradation products, we are working with D. Lane (ICRF, London) to identify antibodies that will recognize epitopes present only on the truncated proteins, but not on the degradation products.

In collaboration with R. Lanford (Southwest Foundation for Biomedical Research, San Antonio), full-length T antigen has been expressed using baculovirus vectors. T antigen purified from infected insect cells and T antigen produced in HeLa cells (using adenovirus vectors) both function equivalently in the SV40 in vitro DNA replication system. We are currently modifying the baculovirus vector in order to increase our yield of full-length T antigen. If the production can be substantially increased, this material may be valuable for structural studies on the full-length protein.

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NUCLEIC ACID CHEMISTRY

R.J. Roberts G.C. Conway, J. Harper, D. Kozak, A.R. Krainer, C. Marcincuk, S. Miceli, S. Munroe, M. Wallace

Characterization of Pre-mRNA Splicing Factors

G.C. Conway

My research goal is the purification and characterization of splicing factors. I have used the technique of sucrose gradient sedimentation to separate components of nuclear splicing extracts according to their sedimentation coefficients. Sedimentation analysis of these extracts reveals the presence of slow-sedimenting proteins and small nuclear ribonucleoproteins (snRNPs; $\leq$20S) as well as faster-sedimenting RNP structures ($\geq$20S). When the slow- or fast-sedimenting material is analyzed separately for splicing activity, neither fraction is found to splice; however, when the two fractions are combined, they are found to complement each other. Interestingly, when the nuclear extract is preincubated with ATP before sedimentation, the top fractions (i.e., $\leq$20S) now possess splicing activity. It appears that a splicing factor(s) is released from large endogenous RNP com-

plexes in the presence of ATP and, once freed, is capable of participating in the splicing process. Initial characterization of the released activity suggests that it consists of multiple components. One or more of the factors is micrococcal-nuclease-sensitive, indicating that it is an snRNP or associated with snRNPs.

Another of the factors is SF2, a previously characterized nonmicrococcal-nuclease-sensitive factor. RNA analysis of top and bottom fractions from ATP preincubated extracts and nonpreincubated extracts reveals no difference in the abundance of U1, U2, U4, U5, and U6 snRNAs when comparing tops to tops and bottoms to bottoms. Interestingly, these snRNAs are equally abundant in both top and bottom fractions whether preincubated or not. Protein analysis of top and bottom fractions reveals major differences in composition; for example, heterogeneous nuclear ribonucleoproteins (hnRNPs) are more abundant in the faster-sedimenting fractions. A comparison of the proteins in bottom fractions from extracts preincubated and not preincubated reveals only slight differences. Only seven bands, on one-dimensional gels, are missing in the preincubated bottom fractions when compared to the nonincubated bottoms, and as expected, these bands now appear in the ATP preincubated top fractions. It is possible that the ATP released splicing factors are among these proteins. Further characterization of these released proteins and splicing factors is in progress.

Alternative Splicing of Adenovirus Genes

J. Harper, S. Miceli

The use of alternative splicing to generate multiple mRNAs from a single gene is a common strategy among viral genes and in certain classes of cellular genes. Frequently, splicing is regulated to give differential expression of alternatively spliced mRNAs in a tissue-specific or temporal fashion. We have been trying to identify factors that regulate alternative splicing using the adenovirus lytic cycle as a model system. Most adenovirus transcription units make multiple mRNAs by alternative RNA processing, and many of these show differential expression of mRNAs between early and late phases of infection. We have examined the products of in vitro splicing of two of these.

SPLICING OF E1A PRE-mRNA

Most of our work has focused on alternative splicing of the E1A transcription unit. E1A pre-mRNA has three 5′ splice sites and two 3′ splice sites that are used to generate at least five different mRNAs in adenovirus-infected cells. Of these mRNAs, two (12S and 13S) are expressed throughout infection, and three (9S, 10S, and 11S) are expressed only at late times after infection. The common feature of the three late specific mRNAs is that all of them use the 9S 5′ splice site, suggesting that differential activity of this splice site is responsible for regulation of E1A RNA expression. Our past work has shown that 9S 5′ splice-site activity does not require an adenovirus-encoded factor, because splicing extracts prepared from uninfected cells can produce all of the E1A mRNAs during in vitro reactions. In addition, we found no dramatic differences in the activities of extracts prepared from uninfected cells or adenovirus-infected cells. This year, we have concentrated on examining the effects of variations in extract preparations on the products of the splicing reactions. In general, we find that the splicing pattern is quite sensitive to variations in the method of extract preparation. However, none of the changes we see reflect the changes produced during the course of adenovirus infection. Changing the cell growth conditions also changes the splicing activity of the extracts obtained, although most of these changes are also unrelated to those seen during infection. One potentially relevant result in these experiments is that serum deprivation of adenovirus-infected cells produces extracts that make relatively higher amounts of late specific RNAs than those from cells grown in serum-rich medium. Overall, our results indicate that regulation of E1A splicing is not preserved during in vitro splicing reactions. This may be due to our inability to extract regulatory factors under the conditions used or may indicate that E1A splicing is regulated in vivo by alteration of the substrate pre-mRNA, rather than solely by changes in *trans*-acting factors.

SPLICING OF L1 PRE-mRNA

This work was carried out in collaboration with C. Delsert and D. Klessig (Rutgers University). The

L1 region of the adenovirus major late transcription unit produces three mRNAs (L1a, L1b, and L1c) that share the same 5′ and 3′ ends, but differ in the 3′ splice site used to join the third late leader to the body of the RNA. All three are expressed at late times after infection, whereas only L1b RNA (the 52,55K mRNA) is expressed at early times. We have recently begun experiments to look at L1 splicing in vitro. We have used deleted templates to transcribe pre-mRNA substrates that allow us to compare L1b (52,55K) splicing with L1c (IIIa) splicing. In a survey of more than 40 splicing extracts, most produce only 52,55K RNA; however, we have found 4 extracts (2 nuclear extracts and 2 whole-cell extracts) that contain IIIa splicing activity. These extracts stimulate IIIa splicing in reactions that make only 52,55K RNA, even when the IIIa extract represents only 2% of the total. This indicates that IIIa-splicing activity is due to a positive factor that could be purified relatively simply using a complementation assay. Our major problem is that there is no obvious reason why the four positive extracts should contain this activity while other similar extracts do not. Active extracts were obtained from both uninfected and adenovirus-infected cells and by two different extraction protocols; therefore, we believe that the differences are due to the condition of the cells used. We are currently examining different cell lines and cell-growth conditions to determine optimal conditions for IIIa RNA expression in adenovirus-infected cells so that extracts containing IIIa activity can be obtained routinely.

Purification of Factors Involved in Mammalian Nuclear Pre-mRNA splicing

A.R. Krainer, D. Kozak

We are interested in purifying the components of the general nuclear pre-mRNA splicing apparatus. The long-term goal of these experiments is to identify and characterize all the general splicing factors from mammalian cells. By studying splicing with purified components, we hope to elucidate presently unknown aspects of the pre-mRNA splicing reaction. These include the nature of the catalytic active sites (protein and/or RNA), the molecular basis of the specificity of splice-site selection, the role of ATP, and the evolution of the splicing machinery.

To purify one whole class of factors that are necessary for pre-mRNA splicing, i.e., snRNPs, we have taken advantage of the fact that their RNA constituents possess a unique trimethylated 5′ cap structure. With the help of C. Bautista and E. Harlow (Protein Immunochemistry Section), we have generated mouse monoclonal antibodies against *N2,N2*,7-trimethylguanosine (m_3G)-keyhole limpet hemocyanin nucleoside-protein conjugates. Hybridomas were screened in pools by immunoprecipitation of labeled U1 snRNA in order to obtain high-affinity antibodies. Two monoclonal antibodies were obtained that are capable of immunoprecipitating deproteinized snRNAs or labeled snRNPs. These antibodies are useful for analyzing the polypeptide composition of snRNPs from a wide variety of species, including *Schizosaccharomyces pombe* (A. Krainer and D. Frendewey, unpubl.).

We have employed these monoclonal antibodies for preparative immunoaffinity chromatography of HeLa cell snRNPs from active splicing extracts. The bound snRNPs are recovered by competition with free nucleoside, under gentle ionic conditions. The m_3G-eluted snRNPs do not catalyze any cleavage-ligation reactions when incubated with pre-mRNA under splicing conditions. However, they can fully complement a micrococcal-nuclease-treated nuclear extract for splicing activity. Therefore, all of the nuclease-sensitive RNPs that are necessary for pre-mRNA splicing can be purified by anti-m_3G immunoaffinity chromatography. These snRNPs have a very high degree of purity based on the RNA and polypeptide composition. They lack SF2 and SF3, two activities that were previously shown to be necessary for 5′ splice-site cleavage and lariat formation. The snRNPs do not appear to contain the intron-binding protein that was previously shown by other investigators to be associated with U5 snRNP under some conditions. We are currently characterizing these splicing-competent snRNPs with respect to their physical properties, as well as RNA-binding properties and putative enzymatic activities. Electron microscopy studies of the purified snRNPs, in collaboration with D. Spector (Cell Biology Section), are in progress. We are also attempting to separate the m_3G-snRNPs from each other while preserving their splicing activity. The resulting fractions will be employed to study the biochemical and physical properties of individual snRNPs and for reconstitution studies, as well as to attempt to identify minor snRNPs that may be necessary for splicing.

In addition to the snRNPs, we are attempting to identify and purify the remaining protein factors that

are necessary for the reaction. With this aim, we are developing a rapid quantitative assay for pre-mRNA splicing, which should facilitate the ongoing purification of splicing factors by complementation. Finally, in collaboration with S. Munroe and G. Conway, we are exploring affinity and immunochemical methods to identify and characterize the stable components of the spliceosome.

Antisense RNA as a Probe for Pre-mRNA Splicing

S. Munroe

Although large pre-mRNA transcripts typically contain many sequences that closely resemble functional splice sites, splicing occurs only at a small number of authentic splice sites. Accumulating evidence suggests that sequences some distance from the splice site are important for splice-site selection. Antisense RNAs complementary to adenovirus and globin pre-mRNA sequences were used as probes to assess sequence requirements for pre-mRNA splicing. These antisense RNAs were found to inhibit splicing efficiently in vitro. Several features of this inhibition are of particular interest for understanding the mechanism of splice-site selection and factors that interact with pre-mRNA molecules under conditions of splicing in vitro and in vivo.

First, splicing is strongly inhibited by antisense RNA that anneals to sequences more than 80 nucleotides downstream from 3′ splice sites. This inhibition provides strong evidence that exon sequences affect splice-site function at a distance. The level of inhibition depends on the length, position, and concentration of antisense RNA relative to pre-mRNA. Antisense RNAs that anneal directly across splice sites also inhibit pre-mRNA splicing, although the efficiency of inhibition is no greater than that observed with a number of antisense RNAs hybridizing to downstream exons only.

Second, the effects of antisense RNA appear to be relatively localized to the nearest upstream splice site. Experiments with substrates containing duplicated splice sites suggest that antisense RNAs do not inactivate more distant sites and inhibition at one site does not activate splicing at a more distant site. This result contrasts with the effect of many splice-site mutations that activate splicing at cryptic sites when blocking splicing at a proximal site.

Third, inhibition of splicing is mediated in vitro by proteins that catalyze RNA/RNA annealing. When antisense RNA is added with the substrate directly to the splicing reaction, a factor(s) catalyzes the rapid annealing of complementary RNAs in a manner that closely parallels inhibition of splicing. This activity closely resembles that of several well-characterized single-stranded nucleic-acid-binding proteins, such as T4 gene-32 protein and *Escherichia coli ssb* protein, which both facilitate annealing of complementary nucleic acids. Since many of the proteins that associate with pre-mRNAs in vivo bind specifically to single-stranded molecules, antisense RNAs should provide useful probes for studying the functional role of these protein-RNA interactions in RNA splicing. Finally, there is a specific temporal requirement for inhibition with antisense RNA. When antisense RNA is added to the splicing reaction after the substrate, the efficiency of inhibition is greatly reduced. This suggests that additional factors block RNA duplex formation shortly after splicing is initiated. Thus, antisense RNA appears to block splicing at an early step in the assembly of an active splicing complex.

The inhibition of splicing by antisense RNA annealing to exon sequences might be explained by a number of different models including (1) disruption of RNA secondary structure, (2) blocking of specific binding sites, (3) interference with scanning of 3′ exons, and (4) disruption of RNP assembly. Present results favor the latter of these possibilities. Further work is in progress to characterize the assembly of spliceosomes and hnRNP complexes in the presence of antisense RNA inhibitors in order to determine the mechanism of this inhibition and to use antisense RNA as a tool for dissecting the assembly of functionally important ribonucleoprotein structure on the pre-mRNA substrate.

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PROTEIN CHEMISTRY

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

The members of the Protein Chemistry Laboratory conduct research on the structure and function of proteins that are involved in normal and abnormal cell growth. The biochemical analysis of proteins includes their purification, primary structure determination, and analysis of modifications. We employ advanced technology for automated chemical analyses and structural studies. Understanding the relationship between structure and function in proteins is fundamental to developing an understanding of the mechanisms of cellular growth in normal and cancer cells.

Our research projects are oriented around four major areas: (1) modifications of the oncogene products, including adenovirus E1A, *myc*, *fos*, and SV40 T antigen; (2) purification and characterization of the mitogenic factor associated with the adenovirus E1A 12S gene product; (3) the role of protein kinases in cell proliferation and transformation; and (4) development of new methods for protein structural analysis and peptide synthesis. Our investigations are designed to use quantitative biochemical procedures to uncover the structure and function relationships in proteins. These approaches complement the genetic and cell biological strategies to unraveling mechanisms of cell growth.

Modification of Oncogene Products

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

We have used peptide-mapping procedures on high-performance liquid chromatography (HPLC) and developed several techniques in order to identify the sites of phosphorylation on adenovirus E1A proteins and SV40 T antigen. Amino acid analysis of phosphorylated amino acids has been developed using enzymatic hydrolysis, derivatization of the products with phenylisothiocyanate, and separation of the derivatives by reversed-phase HPLC. In this protocol, phosphoserine, phosphothreonine, and phosphotyrosine are separated from the corresponding unphosphorylated amino acids completely. Amounts of the amino acids can be quantitated to 1–5 pmoles. Enzymatic hydrolysis is limited, however, by two parameters. First, using sequential digestion with aminopeptidase M and carboxypeptidase Y, complete digestion is not always achieved. Second, there appears to be residual phosphohydrolase activity in commercial preparations of these enzymes, leading to some dephosphorylation during treatment. The method is useful when phosphorylation sites are at either end of a short peptide. We are currently trying to improve the method with more highly purified sources of enzymes for digestion. To sequence phosphorylation sites on the peptides, we have used β-elimination of phosphoserine with hydroxide, followed by reaction of the product, dehydroalanine, with ethanethiol. This results in the stoichiometric conversion of phosphoserine to ethylcysteine. During automated sequence analysis, ethylcysteine is stable, and the phenylthiohydantoin derivative elutes near diphenylthiourea in standard HPLC separations. Thus, the kinetics and extent of phosphorylation of serine can be quantitated accurately.

SV40 T antigen (TAg) was expressed in HeLa cells using an adenovirus construct developed by Y. Gluzman (Molecular Biology of SV40) and was purified from [^{32}P]PO_4-labeled HeLa cell extracts by immunoaffinity chromatography on immobilized antibody 419. Mapping of phosphorylation sites was accomplished by tryptic digestion of the protein and separation of the fragments by reversed-phase HPLC on a Vydac C_{18} column (300 Å pore size) using 0.1% trifluoroacetic acid and increasing proportions of acetonitrile. Two major peptides account for >90% of all the radioactivity incorporated. The carboxy-terminal peptide contained 30% of the radioactivity and was resistant to hydrolysis by alkaline phosphatase from calf intestine. This result (as well as amino acid analysis and sequence analysis) suggests that Thr-701 is the phosphorylated residue in this region. The second peptide nearer the amino terminus contained 50–70% of the radioactivity, and variable amounts of radioactivity was labile to alkaline phosphatase. Preliminary experiments have been conducted to subdigest this tryptic peptide with V8 protease and to analyze the smaller fragments by sequence and amino acid analysis. The majority of the

phosphate appears to be on serine, although some phosphorylation probably occurs at Thr-121 and/or Thr-123 as well.

Adenovirus E1A (13S) has been overexpressed in *Escherichia coli* by E. Harlow (Protein Immunochemistry) using a T7 expression system. We have purified this protein from the *E. coli* lysate-soluble fraction. The protocol consists of (1) DEAE-Sephadex chromatography, (2) phenyl-Sepharose chromatography, and (3) Sephadex G-75 chromatography. The resulting protein is >90% homogeneous as analyzed by gel electrophoresis. We are using this protein as substrate for various protein kinases in vitro. We are currently establishing peptide maps of the unlabeled protein and the phosphorylated protein. Corresponding maps of E1A labeled in vivo will then be evaluated by comparison to these standard maps. As described below, it will be essential to obtain in vivo maps at various time points after infection, since the phosphorylation patterns are likely to change, based on observations with various protein kinases.

Adenovirus E1A (12S)-induced Mitogenic Activity

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

Purification of the mitogenic activity from cells infected with adenovirus 5 (Ad5)-12S depends on the scaling up of production of conditioned media from infected cells. M. Quinlan has established a line of baby rat kidney epithelial cells, BRK 12-1, that can be infected with Ad5-12S and produces active conditioned media as described previously for primary BRK cells. We have increased production of this conditioned media to 2400 ml per week. The media is centrifuged at low speed, filtered to remove cell debris, and then concentrated using a Minitan tangential flow apparatus and a 100,000-molecular-weight cutoff filter. The activity is retained by the filter in a volume of 30 ml. Using this concentrated media, we have established a time course of dose-response curves for the activity by monitoring [^{3}H]thymidine incorporation into DNA for a 24-hour pulse. The activity is optimal at 72–96 hours incubation with the conditoned media concentrate. Half-maximal response of the activity occurs at approximately 0.6–1.5 µg/ml of protein. Thus, the amount of growth factor is roughly 1 µg/liter of conditioned media. Our current experiments are using concentrated conditioned media for column chromatography on ion exchange, hydrophobic interaction, and gel filtration. Preliminary data indicate that hydrophobic interaction chromatography on phenyl-Sepharose and affinity-based adsorption chromatography on immobilized dyes produce up to a 100-fold purification of the activity.

Protein Kinases in Cell Proliferation and Transformation

D. Carroll, N. Santoro [in collaboration with E. Moran, Cold Spring Harbor Laboratory]

We have examined the protein kinase activities from bovine liver using synthetic peptide that are specific substrates for different kinases. In parallel, we have monitored the phosphorylation of a peptide from E1A and the corresponding homologous site on TAg. The activity that phosphorylates the E1A and T-antigen peptides copurifies with the enzyme casein kinase II (CK-II) throughout the purification. The sites on TAg and E1A are serines residues, followed by several acidic amino acids, which is similar to that found for substrates for CK-II. We searched a protein sequence database for oncogene products and found that a concensus sequence for the phosphorylation site appears in the nuclear oncoproteins E1A, TAg, *myc*, *myb*, *fos*, and p53, but not in the cytoplasmic oncoproteins. To pursue this observation, we synthesized peptides corresponding to each of the sites on the nuclear oncoproteins and used them as substrates for purified CK-II. Most of these peptides are good substrates for CK-II and are not phosphorylated by cAMP-dependent protein kinase, phosphorylase kinase, myosin light-chain kinase, protein kinase C, and Ca^{++}/calmodulin-dependent protein kinase. Detailed kinetics have been done for all the peptides. We have also attempted to construct peptide analogs that might act as inhibitors of the enzyme.

To determine CK-II peptide kinetics rapidly, we have developed an assay using reversed-phase thin-layer chromatography (RP-TLC). The assay mixtures are applied to the RP-TLC plates and initially eluted with 0.1% phosphoric acid, 0.25% trifluoroacetic acid, and 10% acetonitrile to elute the nucleotides. The second elution is in the same solvent containing 50% acetonitrile to move the peptide away from the origin. This assay is rapid, simple, and boosts the signal/noise ratio considerably. Our current

studies are aimed at identifying the phosphorylated and unphosphorylated CK-II substrates in vivo. To this end, we are preparing monoclonal and polyclonal antibodies to phosphorylated and unphosphorylated peptides to use as reagents in cell biological experiments.

The identification of CK-II as a potential regulatory modifier of E1A and TAg led us to study its role in signal transduction during the process of adenovirus infection, changes in cell cycle, and transformation of cells. Wild-type Ad5 was infected in 7-day-old BRK cells, and the levels of CK-II and cAMP-dependent protein kinase were measured at various time points for 48 hours. Similar experiments were performed with Ad5 *dl*312, a virus whose E1A region is deleted. Both wild-type and *dl*312 viruses cause a biphasic increase in CK-II activity; the first phase is a 10-fold activation occurring at 15 minutes after application of the virus to the cells, and the second phase is a further 10-fold activation (20-fold total) appearing at 2 hours. In contrast, the cAMP-dependent protein kinase levels fall approximately 20-fold from basal levels in the first 15-minute period. The initial phase of CK-II induction is not blocked by cycloheximide, a protein synthesis inhibitor. Experiments to determine if the second phase requires protein synthesis are in progress. These results suggest that there are two modes for activation of CK-II, possibly from different intracellular pools or by different transduction mechanisms. We plan to study this by subcellular fractionation of cells during the same time course of CK-II induction by adenovirus infection. Overall, we believe that these results indicate that CK-II induction is rapid, does not require E1A expression, does not require protein synthesis, and is complementary to a decrease in cAMP-dependent protein kinase. Preliminary studies indicate that the initial phase of CK-II induction is stimulated by the adenovirus coat protein interacting with a host-cell receptor protein.

Experiments were conducted to study CK-II induction during the cell cycle. WI38 cells, a human lung fibroblast cell line, were serum-starved to synchronize in G_0. After serum stimulation, CK-II levels and cAMP-dependent protein kinase activities were measured over a 48-hour time course. The results were similar to those obtained for adenovirus infection of BRK cells. CK-II was induced by 15 minutes with a corresponding decrease in cAMP-dependent protein kinase activity. At 16 hours, when thymidine incorporation into DNA began, cAMP-dependent protein kinase levels recovered to near basal levels, whereas CK-II showed a recovery followed by a second phase of induction. In other preliminary experiments, phorbol esters stimulated CK-II levels, presumably through a protein-kinase-C-mediated event. Our current investigations in this area are to test whether CK-II induction is a general phenomenon that parallels stimulation of proliferation in cells. We will test growth factors and other stimuli and measure their effects on the kinetics and the level of CK-II stimulation.

Structural Analysis and Synthesis of Proteins

G. Binns, D. Marshak, N. Santoro

We provide protein chemistry support to all of the laboratories at Cold Spring Harbor. During the past year, we have synthesized, purified, and characterized 12 peptides that have been used as substrates or inhibitors of protein kinases. We obtained a preparative HPLC that permits purification of several hundred milligrams of peptide in one run, at flow rates of up to 75 ml/min. This greatly facilitates peptide purification. We have prepared over 30 peptide maps using a microbore HPLC with diode array detector for on-line monitoring of absorption spectra. In sequence analysis, we have made preliminary sequencer determinations on various proteins from other laboratories at Cold Spring Harbor, including transcription factors, protein kinases, and cell-cycle-related proteins.

In the past few months, we have optimized a new method for electroblotting proteins from polyacrylamide gels on to polyvinylidene difluoride membranes (PVDF). These membranes can be stained with Coomassie blue, and they are stable to sequencing chemistry. Thus, the spots appearing on these blots can be placed directly in the sequencer for analysis. This method has been applied to high-resolution two-dimensional gels prepared in the Quest Laboratory under the direction of J. Garrels. Successful sequencing of spots identified on these gels allows us to assign chemical structures to previously unidentified proteins.

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PROTEIN IMMUNOCHEMISTRY

E. Harlow C. Bautista, K. Buchkovich, M. Delannoy, L. Duffy, M. Falkowski, S. Koehler-Peck, A. Lele, M. Raybuck, C. Stephens, P. Whyte, N. Williamson

Work in the Protein Immunochemistry Laboratory has continued to focus on understanding the mode of action of nuclear oncogenes. Our research over the last year has concentrated on the adenovirus E1A proteins and in particular on two subjects: (1) the function of the protein/protein complexes formed between the E1A proteins and various host-cell polypeptides and (2) on the origin and function of the multiple E1A protein species. Our work has identified three cellular proteins with relative molecular weights of 300K, 107K, and 105K as potential targets for E1A-mediated transformation. One of these proteins, the 105K, has recently been identified as the product of the retinoblastoma gene. The identity of the 107K and 300K proteins has not been determined. The heterogeneity of the E1A proteins was first demonstrated by immunoprecipitation of these proteins and analysis on two-dimensional gels. These studies have shown that the heterogeneity arises from the production of at least four mRNAs by differential splicing and by extensive posttranslational modification of the primary translation products. We are currently trying to determine which of these modifications contribute to the function of the E1A proteins.

In addition to the research work in the Protein Immunochemistry Laboratory, a separate group prepares monoclonal antibodies for the Cold Spring Harbor Laboratory staff. The monoclonal antibody facility is run by Carmelita Bautista and is staffed by Margaret Falkowski, Susan Koehler-Peck, and Michael Delannoy.

Cellular Targets for Transformation by the Adenovirus E1A Proteins

P. Whyte, N. Williamson, E. Harlow

In cells infected or transformed by adenovirus, the viral proteins encoded by the early region 1A (E1A) form stable protein/protein complexes with a series of host-cell polypeptides. These proteins are known by their relative molecular weights of 28K, 40K, 50K, 60K, 80K, 90K, 105K, 107K, 130K, and 300K. The three most abundant of these proteins are the 105K, 107K, and 300K polypeptides, and physical and genetic studies have shown that all three of these proteins bind directly to E1A. As an initial strategy to determine the possible functions of these complexes, we compared the regions of E1A required for binding to the cellular proteins with the regions necessary for various E1A activities. A series of E1A deletion mutants were constructed and screened for their ability to induce foci in primary baby rat kidney cells when cotransfected with an activated Ha-*ras* gene. Two regions of the E1A proteins, amino acids 1–76 and 120–127, were shown to be required for cooperation with *ras*. Amino acids outside these sequences could be deleted without destroying the transforming ability of E1A. Any mutation that resulted in a deletion within one of two regions of the E1A proteins destroyed the focus-forming activity. Therefore, these mutations have defined the minimal regions of the E1A proteins essential for transformation.

The mutant E1A proteins were next screened for their ability to bind the cellular proteins. By infecting HeLa cells with recombinant adenoviruses containing the deletion mutations and then immunoprecipitating the E1A products and any bound cellular proteins, we have mapped the binding sites of the 105K, 107K, and 300K proteins. The binding sites of the 300K and 107K proteins are amino acids 1–76 and 120–127, respectively, whereas the 105K protein requires the regions between amino acids 30–60 and 120–127. Interestingly, the boundaries of these binding sites coincided precisely with the boundaries of the transforming regions. Every E1A mutant that failed to bind one of these proteins also failed to transform cells efficiently, and, conversely, every mutant capable of binding all three of these proteins was transformation-competent. This result led us to hypothesize that these three proteins are cellular targets for E1A-mediated transformation.

Monoclonal Antibodies Specific for the Cellular Proteins That Bind to the Adenovirus E1A Polypeptides

P. Whyte

To study the cellular proteins that bind to the adenovirus E1A proteins directly, we have prepared a series of monoclonal antibodies against these polypeptides. The M73 monoclonal antibody, which is specific for an epitope near the carboxyl terminus of the E1A proteins, was purified and coupled to agarose beads using cyanogen bromide. Protein lysates from 293 cells that constitutively express the adenovirus E1A and E1B proteins were added to the M73 beads. After the E1A proteins and their associated proteins were bound to the antibody beads, the extraneous proteins were removed by washing, and the bound proteins were eluted by treating with acetic acid. The purified proteins were used to immunize mice and prepare monoclonal antibodies. The hybridomas were screened by immunoprecipitation against lysates from radiolabeled 293 cells. Twenty hybridomas were identified that secreted antibodies specific for 293 proteins. Among these monoclonal antibodies were several that specifically bound to the E1A-associated proteins. The best characterized of these antibodies are the C36 antibody, which is specific for the 105K protein, and C160, which is specific for the 60K protein. The remaining antibodies are currently being characterized.

The Adenovirus E1A Proteins Bind to the Retinoblastoma Gene Product

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

Among the cellular polypeptides that bind to the adenovirus E1A gene products is a nuclear phosphoprotein of 105K. The 105K E1A-associated protein took on increasing interest when it became apparent that it shared several characteristics with the polypeptide product of the retinoblastoma (Rb) gene, a gene first identified as a locus responsible for a predisposition to retinal tumors. The Rb gene product was first identified by Lee and his colleagues (*Nature 329:* 642 [1987]) as an approximately 110K nuclear phosphoprotein. In addition to having similar molecular weights and similar subcellular distributions, both the 105K and Rb proteins are absent from several Rb tumor cell lines.

To determine if these two proteins were identical, we first confirmed that their molecular weights were the same. The protein immunoprecipitated with a monoclonal antibody specific for the E1A-bound 105K protein, and the protein immunoprecipitated with rabbit antisera raised against synthetic peptides deduced from the Rb cDNA sequence comigrated on SDS-polyacrylamide gels. Next, we compared these immunoprecipitated proteins by subjecting them to partial proteolysis with either *Staphylococcus aureus* V8 protease or *N*-chlorosuccinimide (NCS). V8 protease enzymatically cleaves proteins after acidic residues (aspartic acid or glutamic acid), whereas NCS chemically cleaves after tryptophan residues. Each reagent yielded an identical proteolysis pattern for both proteins, demonstrating that the positions of accessible aspartic acid, glutamic acid, and tryptophan residues are identical in the two polypeptides.

Finally, we performed two types of experiments to prove that these proteins were recognized directly by the anti-105K and anti-Rb antibodies and were not simply spurious cross-reacting proteins or proteins immunoprecipitated via another antigen. In one set of experiments, the monoclonal antibody specific for 105K was shown to immunoprecipitate polypeptides produced by in vitro translations of cRNA transcribed directly from the cloned Rb cDNA. In complementary immunoblotting experiments, the E1A-associated 105K was recognized by two antipep-

tide antibodies raised against different regions of the Rb proteins. By proving that 105K is recognized directly by more than one anti-Rb antibody, this last experiment demonstrated that 105K shares at least two epitopes with the Rb proteins. These data demonstrate that the E1A-associated 105K and the Rb proteins are products of the same gene.

The disruption of both copies of the Rb gene has been linked to the appearance of retinoblastomas and other genetically related tumors, suggesting that the Rb gene product may be a component of a regulatory pathway responsible for inhibiting cell proliferation. Since the inactivation of genes such as Rb leads to tumor growth, these proteins have been described as tumor suppressors or anti-oncogenes. The demonstration of the E1A/105K-Rb complex is the first example of an association between an oncogene and an anti-oncogene. The existence of this oncogene/anti-oncogene complex unifies two fields of research that were thought to be unrelated.

What is the role of the complex? One model suggests that anti-oncogene proteins like 105K are elements of a pathway that allows cells to respond to environmental signals. These signals might be differentiation inducers, cell-cycle regulators, or other factors that carry an inhibitory cue to the cell. The loss of the Rb gene and subsequent loss of the Rb protein would remove or block the signal. As a consequence, the cell would lose its ability to respond normally to the inhibitory signal while retaining its proliferative ability. In this model, by interacting with 105K, the E1A proteins may block the passage of the inhibitory signal and serve as an indirect stimulator of cell proliferation.

Monoclonal Antibodies Specific for the Adenovirus Type-3 and Type-12 E1A Proteins

A. Lele, E. Harlow

To study the possible role of the E1A/host-cell protein complexes isolated from cells that are infected or transformed with adenovirus serotypes other than our standard laboratory strains of adenovirus type 2 or 5, we have begun the preparation of antibodies specific for the E1A proteins isolated from these serotypes. cDNA clones for the 13S mRNA from types 3 and 12 were inserted into a derivative of the T7 expression system developed by W. Studier (Brookhaven National Laboratory). E1A proteins were isolated from *Escherichia coli* and used to prepare monoclonal antibodies. A series of monoclonal antibodies specific for the E1A proteins from adenovirus type 3 have been prepared. These antibodies are currently being characterized.

The Heterogeneity of the Adenovirus E1A Proteins

C. Stephens

The adenovirus E1A proteins migrate on SDS-polyacrylamide gels as a heterogeneous group of polypeptides. This heterogeneity is due to the existence of multiple E1A mRNAs that arise from differential splicing of the primary E1A transcript and to posttranslational modification of the multiple E1A polypeptides. Our interests are to understand the roles of the multiple E1A polypeptides.

The primary E1A transcript is differentially spliced to yield five mRNAs that have been designated the 13S, 12S, 11S, 10S, and 9S mRNAs. The 13S and 12S mRNAs are synthesized at both early and late times during the viral lytic cycle, whereas the 11S, 10S, and 9S mRNAs are produced at late times in infection. Two of these late mRNAs, the 10S and 11S species, were recently identified and cloned in both our laboratory and that of G. Akusjarvi. Functional analyses of the E1A proteins have shown that 13S protein product is essential for viral growth because of its role in the activation of the other adenovirus early genes. The 12S protein product is necessary for lytic infection of quiescent, serum-starved cells. An analysis of what function the "late" E1A mRNAs might have for viral growth was initiated by construction of mutant viruses that could only synthesize the 10S or 11S mRNA. These viruses were found to be defective, suggesting that the sequences removed in the generation of these mRNAs were essential for viral growth.

Another virus, designated 5-1, was constructed that removed the splice donor for all three late mRNAs (11S, 10S, and 9S) and therefore could not synthesize the late proteins. The 5-1 mutant virus was first tested for its ability to grow on HeLa cells and was found to grow to wild-type levels. The only phenotype that could be attributed to this virus was a slight cytopathic phenotype that caused the infected cells to round up and come up off the plate at earlier times in infection than wild-type virus-infected cells. Since this is a characteristic of E1B

19K mutants, the splicing mutant virus was further analyzed to determine whether it shared any of the other properties attributed to the E1B 19K mutants. These included accelerated growth in quiescent, serum-starved cells and the degradation phenotype, which results from nuclease activity on cellular DNA. When tested, the 5-1 virus was found to possess neither of these properties, but rather to behave similarly to the wild-type control virus.

Since the consensus splice donor and acceptor sequences used to form the late E1A mRNAs have been conserved between the different adenovirus serotypes, it appears that there must be a selective growth advantage in producing these late mRNAs, but we have been unable to determined what this advantage might be. It is possible that experiments done in tissue culture are sufficiently different from the normal growth environment of the virus that we will be unable to answer this question.

In addition to differential splicing, the heterogeneity of the E1A proteins is also generated by extensive posttranslational modification. Pulse-chase experiments have shown that modified polypeptides migrate more slowly and at more acidic pI values than unmodified forms. Phosphatase treatment removes most, if not all, of the modifications found on the E1A proteins, indicating that phosphorylation accounts for the majority of modification. To determine what residues in E1A are modified and whether these modifications are important for E1A function, all of the serine and threonine residues in the region encoded by the first exon of the 12S mRNA were mutated to alanine. Although these residues do not account for all of the serine and threonine residues found in the entire E1A, we chose to concentrate on mutations in the first exon for a number of reasons. First, these sequences contain two regions that are conserved between different adenovirus serotypes, and mutations in either of these two regions result in E1A proteins with altered biological properties. Second, deletion mutants in these regions eliminate many of the slower-migrating forms of the E1A proteins that are observed on SDS-polyacrylamide gels.

The serine and threonine mutants have been constructed by site-directed mutagenesis, and these mutant DNAs are being used to construct recombinant adenoviruses. Once viruses are constructed, the E1A polypeptides will be analyzed on two-dimensional gels, and the viruses will be tested for their ability to grow in HeLa cells. The mutated E1A proteins will also be tested for their ability to function in cotransformation assays with the *ras* oncogene.

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PROTEIN SYNTHESIS

M.B. Mathews A.P. Rice, E. Moran, M. Kostura, M. Laspia, A. Maran, G.F. Morris, C. Herrmann, K. Mellits, M. Corrigan, H. Goodrich, A. Kamenick, L. Manche, R. Packer, P.A. Wendel

Gene expression in higher cells is regulated at several levels, and viruses employ a diverse array of strategies in coopting cellular processes to their own ends. During 1987, we continued to exploit adenovirus as a means to study several of these regulatory events, with the dual purpose of understanding the normal processes and the means by which they are subverted. Taking advantage of the extensive knowledge of adenovirus molecular biology accumulated over the years in many laboratories, we have also begun to explore the functioning of *tat*, one of the regulatory genes of the AIDS virus, HIV-1.

VA RNA, DAI, and Translational Control

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

Adenovirus encodes two small RNAs, the virus-associated (VA) RNAs, transcribed by RNA polymerase III. These RNAs are approximately 160 nucleotides long and accumulate to high concentrations in the cytoplasm of adenovirus-infected cells. In the absence of VA RNA_I, protein synthesis in adenovirus-infected HeLa cells fails because of defective polypeptide chain initiation. The defect results from phosphorylation of the initiation factor eIF-2 on its α-subunit. Earlier work showed that the protein kinase responsible is DAI, the double-stranded RNA (dsRNA)-activated inhibitor of protein synthesis. This enzyme, which is present at a basal level in uninfected cells and at elevated levels in cells treated with interferon, is activated during the late phase of infection with the VA RNA_I-lacking mutant virus, Ad5*dl*331. During the year, we have worked on the nature of the enzyme activator, the relationship between the structure of VA RNA_I and its function, and the purification and properties of DAI.

DAI ACTIVATOR

We showed previously that an activator of DAI is present in cytoplasmic RNA from *dl*331-infected cells and suspected from the characteristic bell-shaped activation curve (low concentrations activate, higher concentrations do not) that the activator might be dsRNA. To identify the activator more definitively, we fractionated the deproteinized RNA prepared from cytoplasmic extracts of *dl*331-infected cells according to its secondary structure, using a Franklin column (a cellulose column that provides a method to separate various nucleic acids with graded concentrations of ethanol). Several lines of evidence indicate that the activator is RNA, and dsRNA in particular. First, the activator has chromatographic properties similar to that of dsRNA. Second, it is destroyed by a dsRNA-specific nuclease, RNase III, and it is at least partly resistant to RNases A and T_1 which are specific for single-stranded RNA. Third, the activator is heat-sensitive and regains its activity upon reannealing. Fourth, a bell-shaped activation curve similar to that observed with authentic dsRNA is obtained with the activator.

Previous work had shown that DAI is only activated in cells that have progressed into the late phase of infection, and we have now found that the dsRNA from *dl*331-infected cells contains viral sequences that hybridize to an extensive region in the center of the adenovirus genome. Thus, it seems that the activator of DAI present in *dl*331-infected cells includes viral dsRNA originating from symmetrical transcription of the viral genome. Its site of origin on the viral genome and shortness (only 100–200 nucleotide pairs, based on gel electrophoresis and gel filtration analyses) hint that it may represent fragments of discarded intron sequences. Further work demonstrated the presence of dsRNA in wild-type adenovirus-infected cells, as expected from the proposal that VA RNA is required to counter the effects of dsRNA generated during viral infection. Less expected was the detection of dsRNA in uninfected cell extracts. This observation raises several questions.

It is not clear whether dsRNA acts as an activator of DAI in uninfected cells. If it does, it may be that its effect is antagonized by small RNAs or by other factors present in uninfected cells. To date, we have not detected any RNA in the cytoplasm of uninfected cells that blocks DAI activation, but an inhibitory activity capable of this function has been found in the ribosomal salt-wash (as described below).

VA RNA STRUCTURE AND FUNCTION

The exact mechanism of VA RNA_I action is unknown, but it has long been thought that its secondary structure is important because VA RNA_I has extensive duplex regions and it is known that the kinase interacts with dsRNA. To test this idea, we constructed mutant VA RNA_I genes that would change proposed stem or loop regions. These alterations include a series of nested deletions and a series of linker scanning mutations. In a transient expression assay, 12 of 14 mutants exhibited drastically decreased VA RNA_I function. The remaining two mutants retained wild-type function. Many of the mutant RNAs also exhibited dramatically altered electrophoretic mobilities. We have therefore begun to examine the secondary structures of wild-type and four mutant VA RNAs in solution, using the nuclease-sensitivity method. We derived a structure for wild-type VA RNA_I that approximates models generated in other laboratories but differs from them in significant ways. As shown in Figure 1A, it includes regions of short, perfectly duplexed RNA and longer, imperfectly duplexed RNA, both of which are known to be able to inhibit DAI. Broadly speaking, results from the transient expression assay correlate with structural data: Mutant RNAs that behave as wild type in the transient assay preserve the same basic conformation as wild-type VA RNA_I, whereas mutant RNAs with decreased function have structures in which some or most of the duplexed regions of the molecule are destroyed (see Fig. 1B). The disruption often affects structures at a considerable distance from the mutation, suggesting that the conformation of the molecule is probably quite intricate. Its functionality is surprisingly sensitive to changes that alter its secondary structure, but not all modifications in regions of secondary structure destroy its function. Our current hypothesis is that certain stem-loop structures are more critical than extended duplex regions. Current plans include the generation of point mutations to allow more sensitive probing of VA RNA_I structure and function and the application of additional techniques to the determination of the higher-order structure of the molecule.

PURIFICATION AND PROPERTIES OF DAI

DAI activity is associated with the phosphorylation of an ~70-kD protein as well as of the α-subunit of the protein synthesis initiation factor eIF-2. Because of its ability to phosphorylate eIF-2, DAI could potentially exert a pronounced effect on the translation of both cellular and viral mRNAs. Furthermore, the induction of DAI synthesis probably plays a role in the antiproliferative effects of interferon. Adenovirus and other viruses that generate dsRNA during their life cycles have developed mechanisms to thwart the activity of this enzyme. To characterize its physical and enzymatic properties, we undertook the task of purifying the enzyme. Through a series of chromatographic columns, we have now obtained nearly homogeneous preparations of the kinase from human 293 cells. Final purification is accomplished by sedimentation through a glycerol gradient. Our data indicate that the ~70K protein is indeed DAI, as previously suggested by other investigators, and that no other polypeptides are required for its activities. This is evidenced by the copurification of the ~70K protein with protein kinase (autophosphorylation) activity, with eIF-2 kinase activity, with the ability to bind dsRNA (based on nitrocellulose filtration assays using dsRNA synthesized in vitro), and with the ability to bind VA RNA. Interestingly, VA RNA is able to block the binding of dsRNA, suggesting a rather simple mechanism for its inhibitory action.

During the course of the purification, we discovered a potent inhibitor of the kinase in the ribosomal salt wash of interferon-treated cells. This inhibitor is not a phosphatase and does not function to inhibit the ability of the activated enzyme to phosphorylate eIF-2. Indeed, it appears to function much like VA RNA_I, in that it can prevent the activation of the enzyme in the presence of dsRNA. The nature of this inhibitory factor and its possible relationship to the dsRNA found in uninfected cells are being explored.

Present work with the purified kinase is directed toward defining its interactions with activating dsRNA, on the one hand, and with VA RNA_I, on the other. Preliminary data suggest that the interactions may not, in fact, be simple ones. We have asked whether the activation reaction is intramolecular or intermolecular in nature. Dilution of the enzyme at

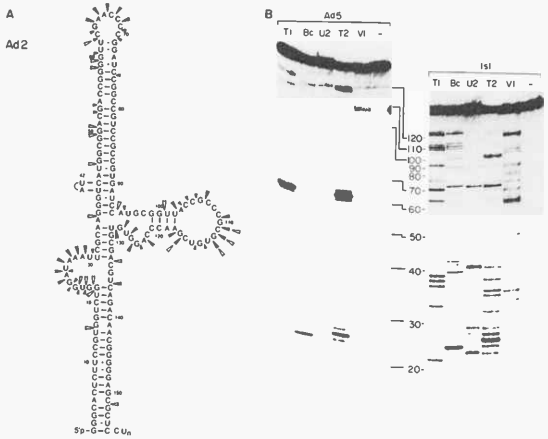

FIGURE 1 Secondary structure of adenovirus VA RNA_I. (*A*) A likely structural model for adenovirus-2 VA RNA_I, based on probing end-labeled molecules with nucleases specific for paired or unpaired nucleotides. Closed arrowheads denote sites sensitive to nucleases specific for single-stranded regions; open arrowheads denote sites sensitive to a nuclease specific for duplex regions and stacked bases. The large arrowheads mark the most preferred cleavage sites. (*B*) An example of the nuclease-sensitivity data derived from a wild-type adenovirus 5 molecule (*left*) and a mutant adenovirus 2 molecule (*right*). (Reproduced from Mellits and Mathews 1988.)

constant dsRNA levels revealed that the rate of DAI autophosphorylation is second order with respect to protein concentration, implying that autophosphorylation of DAI proceeds through an intermolecular phosphate transfer. Additional experiments that involved mixing of preactivated enzyme with latent DAI in the presence of VA RNA indicated that the substrate of the active enzyme is eIF-2 and that the activated enzyme cannot phosphorylate the latent form of the kinase. These results suggest that activation (autophosphorylation) of DAI occurs when two molecules of the latent kinase form a complex on dsRNA, resulting in the phosphorylation of one or both of the kinase proteins. The data also imply that there are two separable kinase activities contained in DAI, since the phosphorylated kinase molecule no longer uses latent kinase as substrate but instead phosphorylates eIF-2 quite readily. This latter event is not inhibitable by VA RNA and provides a useful method for discriminating between the two kinase activities intrinsic to DAI.

Activation of Human Immunodeficiency (AIDS) Virus Gene Expression

A.P. Rice, M. Laspia, H. Goodrich, M.B. Mathews

Transcription of human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), is directed by its long terminal repeat (LTR) sequences. The HIV-1 LTR is composed of several distinct regulatory elements: a negative regulatory element (NRE, located between nucleotides −340 and −185, upstream of the cap site), a core enhancer (−105 to −80), three binding sites for the transcription factor SP1 (−76 to −48), a TATA box (−26 to −22), and an element termed TAR (*trans*-activation response element). The TAR element confers responsitivity to the HIV-1-encoded *trans*-activator known as *tat* and appears to represent a novel type of genetic regulatory element. The TAR sequences encompass 17 nucleotides upstream and some 44 nucleotides downstream from

the site of transcription initiation. Unlike an enhancer, TAR function is orientation- and position-dependent: When inserted between a heterologous promoter and a reporter gene, the TAR element does not respond to *trans*-activation by *tat* in the reversed orientation or when removed to a location far upstream or downstream from the site of transcription initiation.

The mechanism by which *tat trans*-activates HIV-1 LTR-directed gene expression is unclear and controversial. Some studies suggested that an increase in mRNA accumulation directed by the HIV-1 LTR can fully explain the action of *tat*. Other studies presented contradictory evidence, indicating that mRNA levels increase little, if at all, and that the increase in mRNA accumulation can only partially explain *trans*-activation by *tat*. Consequently, it has been suggested that posttranscriptional mechanisms, perhaps translational control, are also involved. To analyze *trans*-activation of the HIV-1 LTR, we have developed an adenovirus vector system that offers some advantages over the plasmid DNA transfection techniques used previously by other investigators. It allows the highly efficient introduction of the HIV-1 LTR into a variety of cell types of rodent, simian, and human origin and allows experiments to be scaled to preparative levels for biochemical analysis.

We inserted the HIV-1 LTR, fused to the chloramphenicol acetyltransferase (*cat*) gene as a reporter, into adenovirus in place of the E1 region. As a control, a similar recombinant adenovirus, containing the Rous sarcoma virus (RSV) LTR fused to *cat*, was also constructed (Fig. 2). The HIV-1 LTR expressed from this adenovirus responds to *tat trans*-activation in a HeLa cell line constitutively expressing the *tat* protein. The RSV-LTR is not sensitive to *tat*. The mechanism of *trans*-activation in this system involves an increase in the transcription rate of the HIV-LTR and dramatically increased accumulation of mRNA encoding *cat*. On the other hand, the translational efficiency of the *cat* mRNA in the cell is unaffected by the presence of *tat* in this adenovirus system. At least in this system, it is evident that *tat* regulation operates on transcriptional processes but not translational processes.

We are continuing to use this adenovirus vector to analyze the transcriptional regulation of the HIV-1 LTR by *tat*. The two recombinant adenoviruses, HIV-1CAT-ad and RSVCAT-ad, lack most of the adenovirus E1 region and do not produce any of the multiple protein products of the E1A and E1B genes, including the 13S E1A protein, which itself is a powerful transcriptional activator. Consequently, expression of the adenovirus genes is extremely inefficient in HIV-1CAT-ad and RSVCAT-ad infections. To explore possible interactions between adenovirus gene product(s) and the HIV-1 LTR, we performed coinfection experiments with HIV-1CAT-ad and wild-type adenovirus. We found that wild-type adenovirus greatly increased *cat* expression in HeLa cells with HIV-1CAT-ad. This *trans*-activation of the HIV-1 LTR does not require DNA replication, as it occurs in coinfections when DNA synthesis is blocked by the drug cytosine arabinoside, indicating that an early adenovirus gene product is involved in activation of the HIV-1 LTR. Coinfections were also carried out with adenovirus mutants capable of expressing only one of the individual E1A products, 13S, 12S, and 9S. In HeLa cells, expression of the 13S protein is required for *trans*-activation of the HIV-1 LTR contained in adenovirus; neither the 12S nor the 9S protein is competent for *trans*-activation. Similarly, in experiments involving co-transfection of plasmid DNAs, we observed that the 13S protein, but not the 12S protein, is able to activate the HIV-1 LTR in HeLa cells, indicating that no other adenovirus products are required for this response. The 13S effect also increases expression from RSV-*cat*. This observation, together with the finding that the 13S E1A and *tat* proteins are able to work synergistically to "super-*trans*-activate" the HIV-1 LTR, suggests that *tat* and the 13S E1A protein act by different mechanisms, both largely, if not entirely, at the transcriptional level. We have constructed a number of additional recombinant adenoviruses containing various deletions of the HIV-1 LTR fused to the *cat* gene and will use these viruses, as well as adenoviruses with mutations within the 13S E1A protein, to investigate the interaction of the 13S E1A and *tat* proteins in regulating transcription of the HIV-1 LTR.

Structure-Function Relationships in the Adenovirus E1A Gene

E. Moran, M. Corrigan

We are continuing to study how the structural features of the adenovirus E1A gene products determine their biological activities, particularly with reference to the ability of these products to induce proliferation of quiescent cells. By last year, we had shown that the E1A gene is an exceptionally interesting

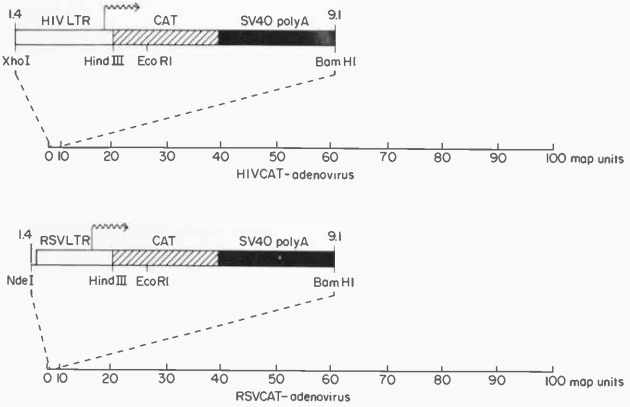

FIGURE 2 Structure of recombinant adenoviruses. Shown are HIVCAT-adenovirus (HIVCAT-ad) and RSVCAT-adenovirus (RSVCAT-ad). These recombinant adenoviruses contain a deletion of adenovirus sequences from 1.4 to 9.1 map units of wild-type adenovirus 5, comprising all of the coding region for the E1A genes and all but the last 60 amino acids of the E1B genes. The site of initiation and direction of transcription from the HIV and RSV LTRs are indicated by arrows. Also shown are the RSV and HIV LTRs (open box), *cat* reporter gene coding sequence (hatched box), and SV40 polyadenylation sequences (closed box). (Reproduced from Rice and Mathews 1988b.)

model for studying structure-function relationships in a eukaryotic cell-cycle control protein because its polypeptide products contain readily distinguishable functional domains (conserved domains 1, 2, and 3) that have been highly conserved in the evolution of various adenovirus serotypes (for review, see Moran and Mathews, *Cell* **48**:177 [1987]).

Recently, a mammalian homolog of the *Schizosaccharomyces pombe cdc2*$^+$ gene and its 34-kD protein product have been described (see Cell-cycle Control in Yeast and Vertebrates in the Genetics Section). This yeast gene is of particular interest because its activity is required for progression through both the G_1 and G_2 phases of the cell cycle, and a variety of evidence suggest that it acts as a regulator of the G_1/S and G_2/M transitions. To gain more information about the pathways by which the E1A products affect the cell cycle, in collaboration with G. Draetta and D. Beach (Genetics Section), we have investigated the expression of the p34 product during adenovirus-induced proliferation of baby rat kidney (BRK) cells. Adenovirus-mediated activation of BRK cell growth caused rapid induction of p34 synthesis. Induction was dependent on the E1A gene, did not require the presence of the E1B gene, and, like proliferating cell nuclear antigen (PCNA) induction, did not require 13S-specific activity. Neither p34 nor PCNA induction was affected by inhibition of cellular DNA synthesis with hydroxyurea. Increased synthesis of both products was due, at least in part, to an increased abundance of translatable mRNA. Preliminary results suggest that p34 induction in BRK cells, like induction of PCNA, is also relatively unaffected by the functional absence of E1A domain 2, consistent with previous observations that regions outside conserved domain 1 are not essential for induction of G_1 and progression to S phase in E1A-activated BRK cells (although domain 2 is required for progression beyond S phase). The similarity between PCNA and p34 induction supports the suggestion that E1A-mediated activation of the cell-growth cycle involves activation of a set of host products responding to a different regulatory function of E1A than that carried by the 13S product.

To explore the possibility that regions of similar sequence in other cell-growth-controlling proteins may be functionally analogous to E1A domains, we substituted a small region from SV40 large T antigen for domain 2 of E1A and found that this chimera has much of the biological activity of the

authentic E1A product. In particular, it is able to induce DNA synthesis and cell proliferation in quiescent BRK cells, to establish them, and to cooperate with the *ras* oncogene in transforming them. These results suggest that a functional substitution can indeed be made between a region of large T antigen and E1A domain 2, even though the two regions share less than 50% amino acid identity (Fig. 3). In contrast, an E1A derivative (pm928) containing a single amino acid substitution in one of the residues conserved in both adenoviruses and papovaviruses (Cys-124→Gly in Ad5 E1A) is nearly as defective as a mutant in which domain 2 is deleted entirely.

A molecular assay of the function of the chimeric protein involves the ability of the E1A proteins to bind to cellular products. In addition to the E1A proteins themselves, monoclonal antibodies specific for these polypeptides precipitate a series of cellular proteins from adenovirus-infected HeLa cells (see Protein Immunochemistry section). Coprecipitation of two of the most prominent of these, the 105K and 107K proteins, depends on the presence of domain-2 sequences in the E1A polypeptides. The pattern of coprecipitating bands seen upon immunoprecipitation of the E1A/SV40 chimeric products appears identical to that seen after immunoprecipitation of the 12S wild-type E1A products. With the recent identification of the 105K cellular protein with the product of the retinoblastoma oncogene by E. Harlow and his colleagues, it seems highly likely that these interactions are significant for the cell-cycle-regulating functions of the E1A products. Our evidence that a similar functional unit exists in the transforming proteins of both SV40 and adenovirus suggests that the SV40 T antigen may exert its cell-growth-regulating effects through a similar mechanism.

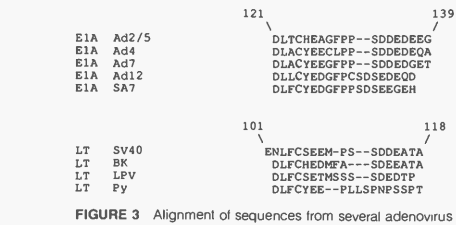

FIGURE 3 Alignment of sequences from several adenovirus E1A proteins and papovavirus large T antigens. Domain-2 sequences from human adenovirus serotypes 2/5, 4, 7, and 12 and simian adenovirus SA7 are aligned with homologous papovavirus sequences from SV40, BK, lymphotropic papovavirus (LPV), and mouse polyomavirus (Py). Gaps have been introduced to maximize the homology. The position numbers of the first and last amino acids of the Ad2/5 E1A and SV40 large T antigen sequences are indicated above the amino acid symbols.

At the biochemical level, domain 2 also exhibits features related to a cellular protein kinase, casein kinase II (CK-II). The carboxy-terminal half of domain 2 consists of a serine residue followed by several acidic amino acid residues (Fig. 3), a motif that constitutes a recognition site for CK-II. A similar sequence occurs in several viral and cellular nuclear oncogenes, including SV40 large T antigen. Experiments conducted with D. Carroll and D. Marshak (Protein Chemistry section) showed that peptides containing the E1A or SV40 T antigen CK-II motifs are excellent in vitro substrates for CK-II phosphorylation. Using these peptides as substrates, we assayed the activity of CK-II during adenovirus infection of BRK cells. Primary BRK cells are quiescent even in the presence of serum, but when infected with adenovirus, they are induced to proliferate. Uninfected BRK cells have a low basal level of CK-II activity, but within 15 minutes after infection, the activity of this enzyme increases severalfold. Simultaneously, the activity of cAMP-dependent kinase drops sharply and then rises, in a manner similar to the response that has been observed for this activity following serum stimulation of established serum-dependent cell lines. Interestingly, these responses do not appear to require expression of the E1A products. They occur well before E1A expression can be detected and are induced even after infection with a mutant virus from which E1A is deleted entirely. In fact, exposure of the cells to the purified adenovirus capsid protein, fiber, which mediates viral attachment to the cell membrane, is sufficient to induce at least the CK-II response. These effects suggest that viral attachment to the cell membrane may be able to stimulate initial membrane signal transduction events in a manner analogous to stimulation by serum growth factors.

To investigate these effects further in a system in which the responses to virus infection and serum stimulation can be compared directly, we are studying the activity of these enzymes during cell-cycle induction of quiescent WI38 cells, which can be stimulated to reenter the growth cycle by either serum addition or virus infection. Like primary BRK cells, WI38 cells have a low basal level of CK-II activity. Within the first 24–30 hours after serum stimulation of WI38 cells (the approximate length of a single cell cycle), CK-II activity exhibits three dis-

tinct peaks. Cellular DNA synthesis becomes detectable following the second peak of CK-II activity. These distinct peaks of CK-II activity are in clear contrast to the pattern of cAMP-dependent kinase activity, which stays constant after an initial early fall and rise. The finding that a kinase activity related to functional domains in transforming proteins exhibits specific peaks of activity during cell-cycle progression should provide a means for defining specific steps in this process and help distinguish the pathways by which extracellular events signal growth changes within the cell. We are now comparing cell-cycle progression induced by adenovirus infection with that induced by serum growth factors and exploring the role played by the E1A products in this process.

Regulation of Gene Expression by E1B

C. Herrmann, M.B. Mathews

The E1B 19K tumor antigen is the smaller of the two major proteins encoded by the E1B gene and is required in cooperation with the E1A gene for the complete transformation of primary rodent cells. As we reported last year, the E1B protein, like E1A proteins, can regulate expression of viral genes. Using a HeLa cell transient expression system in which the activity of the various adenovirus promoters was monitored by an enzymatic assay for the reporter gene *cat*, we have shown that all the adenovirus early promoters are stimulated by the E1B 19K protein. These promoters, which also respond to *trans*-activation by a product of the E1A gene, are stimulated to a higher degree by the combination of E1A and E1B genes than by either the E1A or E1B gene alone, suggesting that products of the E1A and E1B genes can cooperate in regulating viral gene expression. The activity of the promoters expressed at late times during virus infection is not detectably affected by either the E1A or the E1B gene when present alone, but the E1A and E1B genes together are clearly capable of increasing expression from the major late promoter. In addition to regulating expression of the adenovirus genes, the E1B 19K protein also stimulates expression from the SV40 early promoter linked to its enhancer, as well as the promoter for the cellular 70K heat-shock protein.

Our interest is to learn about the mechanism of action of the 19K tumor antigen in stimulating viral and cellular gene expression. One approach we have taken has been to construct an adenovirus vector that contains the E1A-*cat* chimeric gene in place of the E1A and E1B genes. By coinfecting the recombinant E1A-*cat* virus with the 9S virus (which contains a wild-type E1B gene but an inert E1A 9S cDNA in place of the genomic E1A gene) or the 9S.R2 virus (which is the same as the 9S virus except that it contains a point mutation within the coding region of the 19K protein), we can study the effect of the 19K protein in the absence of E1A products which can regulate viral gene transcription both positively and negatively. E. White (DNA Synthesis section) has reported that in the presence of E1A, the 19K protein exerts a negative effect on viral gene expression. We have shown that in the absence of E1A, the 19K protein has a positive effect during virus infection, consistent with our transfection results. We found higher steady-state levels of E1A-directed *cat* mRNA in cells coinfected with E1A-*cat* and the 9S virus than in cells coinfected with E1A-*cat* and the 19K mutant 9S.R2 virus. Nuclear run-on transcription assays revealed an increased rate of transcription from the E1A promoter, as well as from several other early and late promoters, in the presence of a wild-type 19K protein, suggesting that the 19K protein acts at the transcriptional level.

A second approach that we are taking to study the action of the 19K protein is to construct HeLa cell lines capable of expressing the E1B 19K protein. We have obtained several positive clones and, using the technique of immunofluorescence, we have detected the 19K protein in the nuclear envelope of the 19K-transformed cell lines, consistent with its location during lytic infection. Preliminary results indicate that these cell lines complement the *cyt* and *deg* phenotypes exhibited by 19K mutant viruses. When transfected with the E1A-*cat* plasmid, the 19K cell lines produced much higher levels of *cat* activity than control cell lines. However, we believe that at least some of the increased expression is due to a higher level of DNA template, which we have observed in cells expressing the 19K protein. We are currently conducting experiments to determine whether the E1B 19K protein acts by stabilizing the DNA template or by enhancing the rate of transcription, or by both mechanisms.

Regulation of PCNA

G.F. Morris, M.B. Mathews

PCNA, also known as cyclin and the auxiliary protein for DNA polymerase δ, is required for replica-

tion of the leading strand of SV40 DNA in vitro and it presumably performs an analogous function in the replication of cellular DNA in vivo (see DNA Synthesis section). The direct involvement of PCNA in cellular DNA replication, coupled with its detection by immunofluorescence only during S phase, led us to investigate the expression of PCNA during the cell cycle. Toward that end, we fractionated randomly growing HeLa cells into populations representing the various stages of the cell cycle. This was accomplished by a procedure known as centrifugal elutriation. Surprisingly, and in contrast with the results obtained with immunofluorescence, the amount of PCNA did not fluctuate by more than twofold through the cell cycle when assayed by Western blots of protein prepared from these synchronous cell populations.

While this work was in progress, a resolution of this paradox was provided by an observation made by Bravo and MacDonald-Bravo (*J. Cell Biol. 105:* 1549 [1987]). They reported that the pattern of PCNA detected by immunofluorescence depends on the fixation method used. The commonly employed methanol fixation method reveals only a population of PCNA that is tightly associated with the nucleus: This population is subject to cell-cycle regulation, in concert with DNA synthesis. An alternative method involving fixation with formaldehyde reveals diffuse nucleoplasmic staining of PCNA throughout the cell cycle. We have examined nuclei from synchronous cell populations for the presence of a tightly bound population of PCNA. The PCNA tightly associated with the nucleus increases dramatically as the cells enter S phase and remains at high levels until DNA synthesis discontinues, whereupon it declines dramatically. Presumably, the loosely bound population, which is a large fraction of the total PCNA population, provides a reservoir from which PCNA is recruited when DNA synthesis commences. Unless only a small increment in PCNA concentration can tip the balance, this picture implies that the availability of PCNA is unlikely to trigger replication. The situation may be different, however, when quiescent cells are stimulated by mitogens (serum, E1A, etc.) to re-enter the cell cycle. Such nongrowing cells have significantly reduced levels of PCNA and rates of PCNA synthesis, and it is reasonable to suppose that PCNA synthesis is a prerequisite for replication under these circumstances.

These findings notwithstanding, the rate of PCNA synthesis does vary during the cell cycle, as shown by pulse-labeling synchronous populations of HeLa cells. It seems that the alterations in the rate of synthesis of the protein during the cell cycle can be accounted for by changes in the level of PCNA mRNA. We are currently determining the mechanisms involved in the changes of PCNA mRNA levels during the cell cycle. Since PCNA is a relatively stable

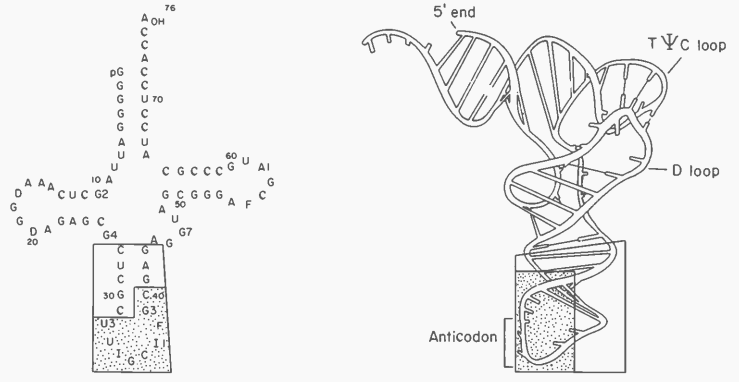

FIGURE 4 Localization of the autoreactive epitope on human $tRNA_1^{Ala}$. The antibody-binding site (stippled box) and antibody protected region (entire box) are superimposed on the $tRNA_1^{Ala}$ cloverleaf structure (*left*) and a model representing the tRNA tertiary structure (*right*). (Reproduced from Bunn and Mathews 1987a.)

protein, the fluctuation of PCNA synthesis does not correlate with a constant level of the protein throughout the cell cycle. We are also examining the possibility that PCNA stability is regulated in a cell-cycle-dependent fashion.

Autoimmunity

A. Maran, L. Manche, M.B. Mathews

Autoantibodies directed against cellular antigens are frequently found in the connective tissue diseases. Three antibody systems that involve transfer RNA occur in patients with myositis, an inflammatory muscle disease. In two of these systems, Jo-1 and PL-7, the antigenic components are protein molecules, the aminoacyl-tRNA synthetases specific for histidine (Jo-1) and threonine (PL-7). In the third system, PL-12, patients' sera contain antibody recognizing alanyl-tRNA synthetase, together with antibody that directly recognizes alanine tRNA. As reported last year and shown in Figure 4, the antigenic site on the tRNA molecule is the anticodon loop.

We have now set about the more difficult task of determining the antigenic sites recognized on the synthetases. Presently, attention is focused on the Jo-1 antigen. F. Tsui (Toronto Western Hospital) has isolated a full-length cDNA clone for human histidyl-tRNA synthetase and constructed mutants carrying insertions and deletions at various sites in the coding region. In collaboration with Dr. Tsui, we are currently using the mutant proteins, produced in a transient expression system, to map the epitopes reacting with various Jo-1 antisera. The information obtained in this way should shed light on the origin of the autoantibodies and, we hope, of the disease.

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TRANSCRIPTIONAL CONTROL

W. Herr R. Aurora, T. Baumruker, J. Clarke, L. Gloss, E. Mathews, B. Ondek, R. Sturm, M. Tanaka

Eukaryotic transcriptional regulation is manifested by a complex combination of positive and negative signals. The DNA sequences responsible for regulation of transcriptional initiation (i.e., the promoter) are a patchwork of *cis*-acting elements that combine with a multitude of *trans*-acting factors. To dissect these complex promoter structures, we have used the small DNA tumor virus SV40 as a probe. This relatively simple virus can be used to perform genetic selections in which deleterious promoter mutations are overcome by selection for viral revertants that have restored growth potential. Our studies have been focused on the structure and function of the SV40 enhancer region. Enhancers are promoter elements that can activate transcription over large distances from either downstream or upstream of the transcriptional start site. We have shown in previous years that the SV40 enhancer contains three 15- to 20-bp-long enhancer elements, called A, B, and C, which can compensate for loss of function in the remaining two elements by duplication. Studies with synthetic multimerized enhancer elements showed that these three elements possess different patterns of cell-specific enhancer activity. Experiments during the past year indicate that these elements are themselves bipartite, being composed of subunits we call enhansons. Enhansons combine in pairs to form enhancer elements. This creates a two-tiered enhancer substructure that allows for a much more complex pattern of combinatorial regulation.

To study the factors that regulate SV40 enhancer function, we have been purifying HeLa cell proteins that bind to the SV40 enhancer. These experiments have led to the purification of a 100-kD nuclear protein that binds to the octamer sequence ATGCAAAT. This sequence motif is found to be associated with a number of enhancers and promoters. To our surprise, we found that the purified protein can bind to two adjacent sites within the SV40 enhancer, one of which is a poor match to the octamer concensus. It is not evident, however, whether this 100-kD octamer-binding protein, which we call OBP100, is involved in SV40 enhancer function or whether it is involved in SV40 DNA replication or late transcription.

SV40 as a Vector to Identify Elements in Heterologous Enhancers

W. Herr, J. Clarke

Last year, we described the construction of an SV40 enhancer replacement vector in which the SV40 enhancer region had been deleted and could be replaced by heterologous enhancers. When the SV40 enhancer was replaced by two separate regions (called A and B) from the polyomavirus enhancer, we were able to obtain revertant viruses from the A, but not the B, enhancer replacement virus. The A enhancer revertants contained duplications that all spanned a 28-bp element within the polyoma A enhancer. To extend the studies of the polyoma B enhancer region, we have replaced the SV40 enhancer with the polyoma B enhancer host-range mutant F9.1.

Polyomavirus does not normally replicate in undifferentiated murine embryonal carcinoma (EC) cells, but polyomavirus host-range mutants capable of replication in EC cells can be isolated after repeated passage in these cells. When host-range mutants have been isolated in the embryonal carcinoma cell line F9, they frequently contain a specific A→G point mutation within the polyoma B enhancer (called F9.1), and this mutation improves enhancer function in a variety of cell lines (Herbomel et al., *Cell 39:* 653 [1984]). When the SV40 enhancer was replaced by the F9.1 point mutation polyoma B enhancer, we were able to isolate viable virus in CV-1 cells. This result is consistent with the F9.1 point mutation creating a generally active enhancer element. Analysis of the enhancer region from the viable viruses gave a surprising result. As expected, the region carrying the F9.1 point mutation was duplicated in all but two out of ten revertants. Surprisingly, however, the polyoma B enhancer/SV40 recombinants also carry variably sized deletions or, in one case, point mutations within a specific region of the B enhancer sequences. The pattern suggests that a negative element is being inactivated to allow growth in CV-1 cells. We are currently testing the deletions to determine whether they inactivate a negative element with enhancer-like properties (i.e., a silencer). This

is the first time we have observed such a consistent deletion pattern and suggests that the SV40 genetic selections can be used to identify both positive and negative elements.

Cell-type-specific SV40 Enhancer Elements

M. Tanaka, E. Mathews, W. Herr

We have taken two approaches to investigate the cell-specific activity of individual SV40 enhancer elements. First, we have extended to new cell lines the analysis of the activity of synthetic enhancers containing multimerized SV40 enhancer elements, and second, we have constructed an "enhancer replacement" polyomavirus vector that allows genetic selection of SV40 enhancer rearrangements in murine cell lines. This latter approach allows revertant analysis of the SV40 enhancer in cells other than African green monkey kidney cells. The analyses of cell-type-specific enhancer activities using synthetic enhancers have focused on the B element in the murine lymphoid cell line NS-1 and on the C element in differentiated and undifferentiated embryonal carcinoma F9 cells.

The location and sequence of the SV40 enhancer A, B, and C elements are shown in Figure 1. The 22-bp B element contains an imperfect direct 9-bp repeat AAG$^{C}/_{T}$ATGCA, which has been referred to as the sph motifs. The junction formed by these two repeats creates a sequence similar to the octamer motif (ATGCAAAT; see Fig. 1) found in a variety of upstream promoter and enhancer regions including the immunoglobulin enhancer. We have shown previously (Ondek et al., *EMBO J. 6:* 1017 [1987]) that multimers of a 17-bp fragment (6×B17), which when multimerized create the 18-bp sph motif repeat, are active as an enhancer. In these previous studies, the same construct containing the two mutations (Fig. 1) was inactive in all cell lines tested including lymphocytes. These results indicate that the SV40 octamer-like sequence is insufficient for enhancer activity. We have extended these studies by analyzing mutations within the octamer-related sequence. Two mutations *dpm*7 (AT*TT*AAAG) and *dpm*8 (A*AGG*AAAG) have been introduced into the B17 constructs and assayed for enhancer activity. The *dpm*8 mutant is inactive in all cell lines tested (CV-1, HeLa, and NS-1, a lymphocyte cell line) but the *dpm*7 mutation only has an effect in the lymphoid cell line NS-1. These results suggest that there are two functionally distinguishable overlapping elements within the SV40 enhancer B element. We are currently mapping the sequences responsible for the lymphoid-specific activity.

Another example of overlapping elements is emerging from the experiments using F9 cells. The embryonal carcinoma cell line F9 can be induced to differentiate in vitro by treatments with retinoic acid. We have tested the activity of the A, B, and C synthetic oligomer enhancers in both undifferentiated and differentiated F9 cells. The A and B enhancer constructs are active in both undifferentiated and differentiated cells, whereas the C enhancer showed little or no activity in undifferentiated F9 cells but became the most active among the the three elements upon differentiation. The inactivity of the C element in undifferentiated cells was surprising because, of the three sets of point mutations *dpm*1, *dpm*2, and *dpm*6, the *dpm*6 mutations within the C element (see Fig. 1) have the most detrimental effect on SV40 enhancer function in undifferentiated F9 cells. These results can be explained if there is another overlapping SV40 enhancer element that functions in undifferentiated F9 cells and is sensitive to the *dpm*6 mutation. To define this putative overlapping element, we isolated growth revertants in undifferentiated F9 cells of SV40 enhancer/polyomaviruses carrying either the wild-type SV40 enhancer or the *dpm*12 mutant in which the A and B elements are inactive. In general, these growth revertants contain simple duplications of the SV40 enhancer sequences, but the duplication pattern differs from that seen when *dpm*12 revertants are isolated in CV-1 cells, because the C element is not consistently duplicated. This pattern further establishes the inactivity of the C element in undifferentiated F9 cells. We are currently using synthetic oligomer constructs to identify the enhancer element that is active in undifferentiated F9 cells and overlaps the C element.

The SV40 Enhancer Contains Two Distinct Levels of Organization: Enhancer Elements and Enhansons

B. Ondek, L. Gloss, W. Herr

The SV40 enhancer is characteristic of many enhancers in that it contains multiple short (8–10-bp) sequence motifs. Many of these are shown in Figure 1 and include the coreA and coreC motifs within the

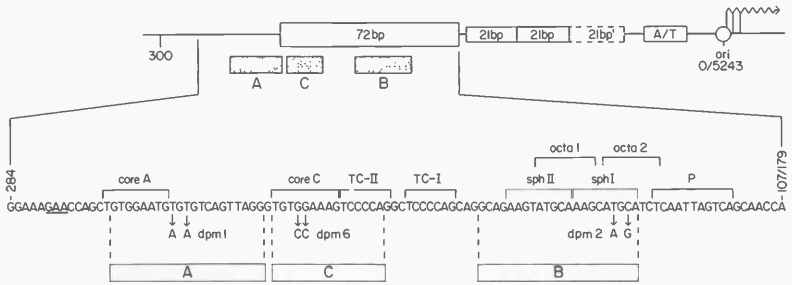

FIGURE 1 SV40 early promoter showing the location of enhancer elements and sequence motifs. The diagram of the SV40 early promoter shows, from right to left, the early transcriptional start sites, origin of replication, AT-rich TATA-like element, one imperfect (dashed box) and two perfect 21-bp repeats, and a single copy of the wild-type 72-bp repeat. The A, B, and C enhancer elements (stippled boxes) are shown below their corresponding position within the early promoter. The nucleotide sequence of the enhancer region (nucleotides 179–284) is shown below with sequence motifs bracketed above the sequence, and the *dpm*1, *dpm*2, and *dpm*6 point mutations are identified below the sequence along with the location of the A, B, and C elements. (Reprinted, with permission, from Ondek et al. 1988.)

A and C elements and the sphI and sphII motifs in the B element. Studies by Chambon and colleagues have shown that these motifs are important for enhancer function and interact with distinct factors (Zenke et al., *EMBO J. 5:* 387 [1986]).

Nevertheless, our studies with wild-type and mutant A, B, and C element synthetic enhancers indicate that these motifs are insufficient for enhancer function because inactive mutant elements can still contain wild-type motifs. During the past year, we discovered that the A and B enhancer elements are bipartite, containing subunits that correlate with the core and sph sequence motifs. These subunits are called enhansons because they appear to be the basic units of enhancer structure. Enhansons can be duplicated or combined with a heterologous enhanson to create new enhancer elements, but unlike enhancer elements, they are very sensitive to changes in spacing. These results lead to a picture of the SV40 enhancer as a composite of many subunits that are organized in a hierarchical structure, first creating enhancer elements and subsequently an enhancer.

This two-tiered enhancer substructure became apparent because of the complex revertant double-duplication patterns that arose when all three of the SV40 A, B, and C elements were inactivated by point mutation. For example, a small 9-bp duplication of the coreA motif (Fig. 1) arose in three independent revertants of the triple A, B, and C mutants, and this short duplication was subsequently duplicated itself. The double-duplication pattern suggested that the initial coreA motif duplication was creating a new enhancer element. To test this hypothesis, we made a synthetic enhancer with multiple copies of the 18-bp coreA repeat. This construct is exceptionally active as an enhancer, but point mutations in either coreA repeat inactivate the enhancer, showing that both coreA motifs are required for activity. Similar analyses of the 9-bp sph motifs in the B element showed that the sphI and sphII motifs, which differ at a single position, are not equivalent (the sphI motif is much "weaker" than the sphII motif), but they can be inverted with respect to one another and still function as an enhancer element. Furthermore, a combination of the sphII and coreA motifs or enhansons was active, which showed that enhansons from separate elements can be combined to create new enhancer elements. The activities of enhansons and enhancer elements are differentiated by their sensitivity to spacing. Enhansons must be positioned near one another to form an enhancer element, whereas enhancer elements can be separated by up to about 100 bp and still cooperate with one another to enhance transcription.

The bipartite structure of enhancer elements suggests that two *trans*-acting factors bind to adjacent enhansons to form a complex that can enhance transcription in combination with another enhancer element complex. Figure 2A shows a hypothetical series of productive (1a, 1b, and 4) and nonproductive

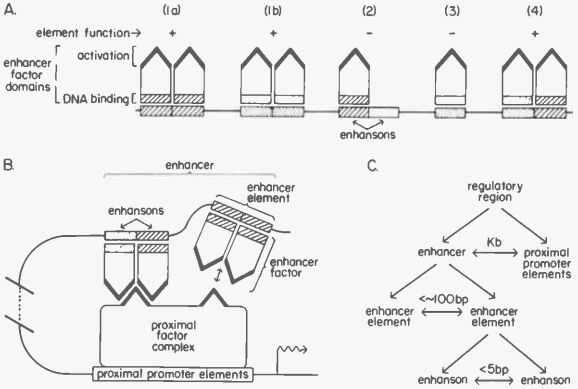

FIGURE 2 Enhancer, enhancer element, and enhanson organization. (*A*) Putative functional and nonfunctional complexes between *trans*-acting factors and enhansons. Enhansons of different sequences are shown as differently shaded boxes along a DNA molecule (line). *trans*-Acting factors are shown above with (1) shaded portions indicating DNA-binding domains that correspond to a specific enhanson sequence, and (2) blackened Λ shape, indicating a half-activation domain. (*B*) Illustration of two functional enhancer element-factor complexes interacting with a proximal factor complex bound to proximal promoter elements. (*C*) Three levels of binary organization within a regulatory region can be functionally distinguished by their differential response to spacing. See text for details. (Reprinted, with permission, from Ondek et al. 1988.)

(2 and 3) complexes between enhancer factors and enhansons. In the productive complexes, juxtaposed factors are shown creating identical activation domains (ΛΛ) because enhancer elements (and thus factors) can functionally compensate for one another. Complexes 2 and 3 represent solitary, nonproductive interactions between enhansons and enhancer factors. Complex 2 is inactive because the transcription factor specific for the unoccupied enhanson is absent, and complex 3 simply represents a solitary enhanson. These hypothetical nonproductive complexes show how enhancer motifs could serve as binding sites for *trans*-acting factors but not display activity in vivo.

The relatively strict spacing requirements between paired enhansons can be explained by a need for close protein-protein contacts to form a functional enhancer element complex. It is not clear, however, how enhancer elements display less-strict spacing constraints. As shown in Figure 2B, we have suggested that enhancer elements do not interact directly with one another but instead associate with factors bound to proximal elements. If these interactions are broken and reformed during transcription, the long-range enhancer/promoter interaction can be maintained by breakage and reassociation of the separate enhancer element complexes at different times.

This enhancer substructure emphasizes multiple levels of binary organization, in which each level is differentiated from the other by the flexibility in spacing between its component pairs of subunits. In Figure 2C, we show a regulatory region divided into three levels of binary organization, with each level exhibiting increased sensitivity to spacing between its components. At the first level, a regulatory region is divided into enhancers and proximal promoter elements (e.g., CCAAT and TATA boxes); these two control elements are capable of activating transcription when separated by distances as large as 10 kb. The enhancer is, in turn, composed of elements that can enhance transcription when separated by roughly up to 100 bp, whereas a pair of enhansons must be in close proximity to create a functional enhancer element. An enhancer structure such as this with multiple levels of binary organization permits a greater degree of transcriptional regulation with a limited number of transcription factors.

The increased complexity generated by two levels

of binary organizations may also serve to buffer regulatory regions from the spontaneous appearance of new enhancers during evolution. Unlike proximal elements, enhancers can activate transcription over very large distances from the transcriptional start site. This high degree of positional freedom creates a much larger target size within which mutations can create new enhancers. Thus, if enhancers were structurally simple, they might evolve frequently, creating chaotic patterns of gene expression.

Replacement of the Wild-type SV40 Enhancer with Multimerized Synthetic Enhancers Creates Stable Viable Viruses

B. Ondek

Previous experiments showed that multiple (six) synthetic copies of the three SV40 enhancer elements, A, B, and C, possess intrinsic enhancer activity when assayed in the SV40 host cell line CV-1. To determine whether these synthetic enhancer constructions are capable of supporting SV40 growth and replication in the absence of the wild-type viral enhancer, the wild-type enhancer region was replaced by each of the A, B, and C synthetic enhancers. These recombinant viruses are viable when tested by plaque assay, although the viability is decreased 100–1000-fold compared to wild-type SV40. As expected, viruses containing inactive mutant enhancer elements are defective for viral growth. When virus stocks were passaged and then tested for rearrangements within the enhancer region, the recombinant viruses were stable. This contrasts with the rapid appearance of rearrangements after passage of the enhancer point mutants (e.g., *dpm*12). These results indicate that the active synthetic enhancer constructions can substitute for the wild-type SV40 enhancer and that there is no distinct activity within the enhancer region, other than enhancer activity, that is required for growth and replication of a stable virus.

Determination of Late Start Sites in SV40 Containing Synthetic Enhancer Elements

R. Aurora

The SV40 early and late promoters overlap one another but are oppositely oriented. The late promoter initiation sites are scattered throughout the regulatory region, and there are no obvious TATA boxes to position the initiation sites. It is not understood how initiation sites are selected in the absence of a TATA element. The frequency of initiation site usage could be determined by sequences immediately adjacent to the start site or by a global effect of distant sequences. To address this question, the pattern of initiation sites in SV40 carrying multiple tandem copies of the individual A, B, or C enhancer elements was analyzed. These recombinant viruses are viable and replicate stably (see above). Because these viruses contain six reiterated copies of the enhancer element sequence, it is possible to distinguish local from global sequence effects on late start-site selection. If local sequences dominate the selection, then six-fold reiteration of an initiation site should create six reiterated start sites. But, if global sequences are responsible for determining start sites, then an even reiteration of start sites would not likely occur.

Late initiation sites in the different multimerized enhancer recombinant viruses were mapped by primer extension and nuclease protection of total RNA isolated from CV-1 cells after infection. The results indicate that start sites are reiterated with each synthetic repeat and that, when present, the nucleotides used to initiate in each repeat are approximately the same as in the wild-type virus. Nevertheless, initiation occurs more frequently at the repeats proximal to the 21-bp repeats. This gradient of start-site usage is observed regardless of the synthetic element or its orientation. These results indicate that the selection of initiation sites by RNA polymerase II in the SV40 late promoter is determined by local sequences, whereas the frequency of use is determined by global elements.

A 100-kD HeLa Cell Octamer-binding Protein (OBP100) Interacts Differently with Two Separate Octamer-related Sequences within the SV40 Enhancer

R. Sturm, T. Baumruker, W. Herr [in collaboration with B.R. Franza, Jr., Cold Spring Harbor Laboratory]

Numerous eukaryotic upstream promoter and enhancer regions contain a functional octamer sequence ATGCAAAT. We have purified a 100-kD HeLa cell octamer-binding protein, called OBP100, and studied its interactions with the SV40 enhancer. We were surprised to find that, although only one

octamer-related sequence (ATGCAAAG, see Fig. 1) had been identified by sequence comparison, OBP100 binds to two adjacent sites within the SV40 enhancer. Before purifying OBP100, we characterized its binding sites using a gel-retardation assay in combination with mutagenesis of the binding sites and chemical interference.

Figure 3A shows the nucleotide sequence of the SV40 enhancer containing the OBP100-binding sites. This region contains two sets of repeated sequences. The B element (labeled B17) contains the imperfect 9-bp direct repeats called sphI and sphII (see Fig. 1). The junction formed by the sph motifs creates the octamer-related sequence ATGCAAAG, which we refer to as Octa1. A second octamer-related sequence ATGCATCT is called Octa2. Figure 3B shows a gel-retardation assay in which radiolabeled wild-type or mutant SV40 enhancer fragments were allowed to bind to a partially purified preparation of OBP100 and separated by electrophoresis on nondenaturing polyacrylamide gels. Complex formation between DNA and protein(s) results in a retarded mobility of the labeled fragment during electrophoresis. Two complexes (CI and CII) form when the wild-type SV40 enhancer is used as probe. The *dpm*2 mutations within the sphI motif, which inactivate the B element, reduce the amount of CII complex, but not the amount of the CI complex. Likewise, mutations called *dpm*8 in the sphII motif also have a large effect on CII formation. The combination of these two sets of point mutations in *dpm*28 eliminates not only CII formation, but also CI formation, indicating that the *dpm*2 and *dpm*8 mutations affect different interactions.

To characterize these interactions, we assayed the effects of chemical modification of the wild-type and mutant sites on complex formation. In general, modification of bases that are important contact points for protein binding will disrupt complex formation, and thus these modifications will be underrepresented in the complexed probe DNA. We have used both dimethylsulfate (DMS) and diethylpyrocarbonate (DEPC) as modifying agents. Results of this analysis revealed that chemical modifications over a 10-bp sequence spanning the Octa1 site affect binding, whereas modifications over a 13-bp sequence spanning the Octa2 site prevent binding. These results indicated to us that the two octamer-related sequences and not the sph motifs are responsible for formation of complexes CI and CII and thus are the result of interaction with an octamer-binding protein. This conclusion was confirmed by showing

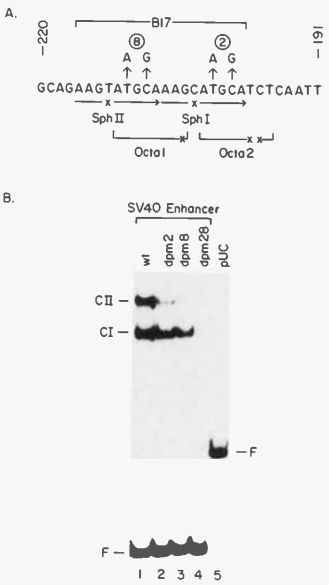

FIGURE 3 Gel retardation with mutated SV40 enhancer fragments and a partially purified HeLa cell nuclear extract. (*A*) Nucleotide sequence of the region surrounding the SV40 enhancer B element is shown with point mutations (*dpm*2 and *dpm*8) and sequence motifs described in the text shown above and below the sequence, respectively. The Xs in the brackets identifying the Octa1 and Octa2 sequences identify the bases that differ from the octamer consensus ATGCAAAT. (*B*) Wild-type (lane *1*), *dpm*2 (lane *2*), *dpm*8 (lane *3*), and *dpm*28 (lane *4*) SV40 enhancer fragments and a nonspecific fragment (lane *5*) were assayed by gel retardation using a HeLa cell nuclear extract that had been purified by heparin-agarose column chromatography. (Reprinted, with permission, from Sturm et al. 1987.)

that the same activity binds to the consensus octamer sequence in the immunoglobulin heavy-chain (IgH) enhancer.

This octamer-binding activity was extensively purified in a three-step purification procedure from a HeLa cell nuclear extract. The activity was first fractionated by heparin-agarose chromatography and then greatly purified by DNA affinity-precipitation (Franza et al., *Nature 330:* 391 [1987]). In the DNA affinity precipitation procedure, fractions enriched for the octamer-binding protein were incubated with

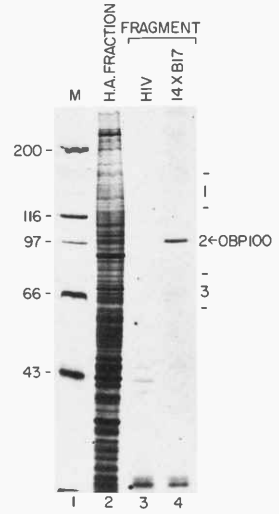

FIGURE 4 Purification of OBP100. OBP100 was purified by DNA-affinity precipitation as described in the text. The figure shows a silver-stained 8% SDS-polyacrylamide gel. (Lane *1*) Bio-Rad high-molecular-weight markers; (lane *2*) 1/250 of the starting material used in the OBP100 purification; (lanes *3* and *4*) protein specifically precipitated by the negative control HIV enhancer fragment or the Octa1-containing 14×B17 fragment, respectively. (Reprinted, with permission, from Sturm et al. 1987.)

a biotinylated DNA fragment containing 14 copies of the B17 sequence (see Fig. 3A), which contains the Octa1 sequence but not the Octa2 sequence. The proteins able to interact specifically with this sequence were purified by subsequent binding of the DNA to streptavidin agarose beads, which can be easily removed from the mixture by centrifugation. After several washes, the bound protein was eluted and identified on an SDS-polyacrylamide gel as shown in Figure 4. This assay identified OBP100, a 100-kD *o*ctamer-*b*inding-*p*rotein. OBP100 was shown to be responsible for the formation of the SV40 enhancer CI and CII complexes by elution of the protein from the SDS gel and renaturation of the DNA-binding activity. The renatured protein interacts with the Octa1 and Octa2 sites and the IgH enhancer sequence, showing that OBP100, which was purified to the Octa1 sequence, can bind to dissimilar sequences.

Although OBP100 interacts with the SV40 enhancer B element and the *dpm*2 and *dpm*8 mutations that affect its binding also inactivate the B element (see above), the protein may not be responsible for the enhancer activity of the B element. This is because the 6×B17 synthetic enhancer, which contains multiple copies of the Octa1 site but not the Octa2 site, is inactivated by the *dpm*2 mutations that have no discernible effect on OBP100 binding to the Octa1 site. We are presently testing whether the Octa1- and Octa2-binding sites in combination can function to enhance transcription. These OBP100-binding sites may instead be involved in some other process, including DNA replication or late transcription, because the SV40 enhancer is within a complex regulatory region that includes the bidirectional early and late promoters and the origin of replication.

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MOLECULAR GENETICS OF EUKARYOTIC CELLS

This section encompasses laboratories studying the mechanism of the control of gene expression and the ways that alterations in gene expression lead to alterations in cellular proliferation. The laboratory of M. Wigler focuses on the genes that control cell proliferation. Mammalian oncogenes were identified by methods of gene transfer, and these oncogenes are studied in great detail. Studies of the *ras* oncogene have led to studies of growth regulation in yeast. The *ros* oncogene encodes a transmembrane tyrosine kinase. The *mas* oncogene encodes a novel member of a class of transforming proteins, with structural homology with the family of receptors that contain multiple membrane spanning regions. The laboratory of M. Gilman focuses on the c-*fos* oncogene. Activation of the expression of this oncogene is perhaps the earliest event in the response of cells to a large number of external signals. Dr. Gilman and co-workers have identified serum response and cAMP *cis*-response elements and have shown that these are distinct. They are in the process of purifying and characterizing the protein factors that bind to these elements. The laboratory of N. Hernandez studies the expression of the small nuclear RNA (snRNA) genes, which are involved in a variety of RNA processing events. Molecular analysis reveals that the transcription units of three of these genes, U1, U2, and U6, combine features of classic RNA polymerase II and III transcription units. The laboratories of D. Hanahan and L. Field employ the transfer of genes into the germ line of laboratory mice to explore a wide variety of biological problems: tumor formation induced by various tumor virus genes, secondary events associated with tumor progression, essential hypertension and autoimmune disease, and, finally, the embryological origin of endocrine cells.

GENETICS OF CELL PROLIFERATION

M. Wigler C. Birchmeier, D. Broek, S. Powers, T. Toda, R. Ballester, G. Bolger, J. Colicelli, K. Ferguson, J. Field, T. Michalei, J. Nikawa, P. Sass, S. Sharma, D. Young, I. Wieland, S. Cameron, H. Ping, L. Rodgers, K. O'Neill, M. Riggs, R. Elsin, B. Shenko

During the past year, our laboratory has continued its investigations into oncogenes and growth control. The central focus of the laboratory has been on the *RAS* genes, which may play a critical role in a large portion of human cancers. We mainly have been studying the function of the *RAS* genes of the model eukaryotic organism, *Saccharomyces cerevisiae*. As a consequence, we also have been examining growth control in this organism. In addition to these studies, we have been studying two new human oncogenes, the *MAS* and *ROS* oncogenes.

Function of the *RAS* Oncogenes

S. Powers, D. Broek, T. Toda, J. Field, J. Nikawa, S. Cameron, P. Sass, T. Michaeli, J. Colicelli, R. Ballester, B. Shenko

Three basic questions about the *RAS* gene products remain unanswered: What controls their activity? What biochemical activities do they control? How does activation of the *RAS*-controlled pathways lead to tumorigenicity? We have explored these questions by using as a model system the *S. cerevisiae RAS*

genes, which encode proteins that regulate yeast adenylate cyclase. This is to date the only *RAS*-coupled effector system known. Although *RAS* proteins probably do not control adenylate cyclase in vertebrates, this yeast system can be used to yield insights into *RAS* function.

Four points of significance have emerged from our yeast *RAS* studies during the past year. First, we discovered that the product of the *CDC25* gene appears to regulate the yeast *RAS1* and *RAS2* proteins, which was the first evidence that *RAS* activity is controlled by other proteins (Broek et al., *Cell 48:* 789 [1987]). *RAS* proteins in cells lacking the *CDC25* gene act as though they are not in an active state: They fail to stimulate adenylate cyclase, although they can be induced to do so by incubation with GTP. Cells containing a $RAS2^{val19}$ gene, a mutant form of *RAS2* that is "activated," are virtually oblivious to the absence of *CDC25*, which suggests that *CDC25* may be involved in maintaining *RAS* proteins in the GTP-bound state. We do not yet know the mechanism of this control, nor whether an analogous mechanism exists in mammals. The normal Ha-*ras* protein when expressed in yeast does not require the *CDC25* gene product to function, but we do have preliminary genetic evidence that the *CDC25* gene product can interact with the mammalian Ha-*ras* protein. A mutant form of *RAS2* can block *CDC25* function, and the analogous Ha-*ras* mutation appears able to do likewise. These results may indicate a direct interaction between the protein products of these genes.

Second, we have developed a novel genetic/biochemical method for purifying the yeast adenylate cyclase complex from cells (Field et al., *Mol. Cell. Biol.* [1988] in press). The method entails the construction and expression of the desired gene product as an epitope fusion protein which is then purified by immunoaffinity chromatography and eluted with a synthetic peptide epitope. This method yields a several hundredfold purification of adenylate cyclase from yeast. The purified adenylate complex is about 600 kD and probably contains two molecules of the 210-kD adenylate cyclase and some number of a 70-kD component. The complex is fully responsive to *RAS* proteins and requires the continued presence of *RAS* proteins to remain active. Analysis of the responsiveness of the purified complex demonstrates that *RAS* proteins have an almost absolute requirement for GTP in order to activate their effector. We are currently investigating the role of the 70-kD component of the complex in *RAS* responsiveness.

Third, we have evidence from genetic experiments that there must be a second *RAS* effector system in yeast (Toda et al., in *Oncogenes and cancer*, Japan Scientific Societies Press, Tokyo, Japan [1987]; Toda et al., *Cell 50:* 277 [1987]). Two observations support this idea. First, although the major effects of *RAS* in yeast are mediated through its effects on adenylate cyclase, deletion of the adenylate cyclase gene is not uniformly lethal in haploid spores, whereas deletion of both *RAS1* and *RAS2* genes is. Second, the slow growth phenotype of adenylate cyclase deletion mutants is fully suppressed by high-copy plasmids expressing a variety of protein kinases, although these same plasmids suppress deletion mutants in *RAS* poorly and in a temperature-sensitive manner. We have demonstrated that the alternate function of *RAS* is also dependent on *CDC25* function. We are currently testing whether human Ha-*ras* can supply this function in yeast and are trying by genetic means to isolate genes encoding components of the alternate *RAS* pathway. These results raise important new questions. Do *RAS* proteins have more than one function in mammalian cells? If so, which are important in malignant transformation?

Fourth, we have discovered "interfering" mutants of the *RAS* genes. These are mutants that either block normal *RAS* function or block "activated" or "oncogenic" *RAS* in a dominant way. We first discovered these mutants during suppressor studies of yeast. A dominant temperature-sensitive *RAS2* mutant was isolated that blocks the function of *CDC25*. A similar mutation in Ha-*ras* also appears to block *CDC25* function, and we are currently testing the ability of this Ha-*ras* mutant to block Ha-*ras* function in mammalian cells. Upon realizing the potential value of such dominant interfering mutants, we deliberately sought mutants of Ha-*ras* in mutagenized pools of Ha-*ras* genes that could block activated $RAS2^{val19}$ function. These were indeed found, and they may be useful tools for exploring *RAS* function, particularly in mammalian cells. Preliminary analysis indicates that these mutants compete for at least one of the *RAS* effectors.

Growth Regulation in *S. cerevisiae*

T. Toda, S. Cameron, J. Nikawa, S. Powers, P. Sass, K. Ferguson, J. Colicelli, K. O'Neill, M. Riggs

Our attempts to understand *RAS* function in yeast have led us to explore in great detail the cAMP-

signaling system and its role in growth regulation. First, we isolated *BCY1*, the gene encoding the regulatory subunit of the cAMP kinases (Toda et al., *Mol. Cell. Biol. 7:* 1371 [1987]). We also isolated three genes, *TPK1*, *TPK2*, and *TPK3*, that encode the catalytic subunits (Toda et al., *Cell 50:* 277 [1987]). Cells lacking *BCY1* display a cluster of phenotypes, including a failure to sporulate, accumulate glycogen, resist starvation, or become heat-shock-resistant. These are the responses of a normal cell to nutrient limitation and can be thought of as the yeast equivalent of entering the G_0 state of the cell cycle. These defective phenotypes appear to be mediated by the unbridled action of the catalytic subunits of the cAMP-dependent protein kinases, since mutant, attenuated *TPK* genes can reverse these phenotypes. These results suggest that the only function of the regulatory subunit is to control the activity of the cAMP-dependent protein kinases. Moreover, cells lacking *BCY1* but containing attenuated *TPK* genes can dynamically regulate responses to nutritional limitation in an apparently normal manner, entering and leaving the G_0 state appropriately (Cameron et al., *Cell* [1988] in press). In such cells, it is even possible to delete the entire ensemble of genes encoding the cAMP-generating and -regulatory machinery (*CDC25*, *RAS*, and *CYR1*) and still retain normal growth regulation. These results support the idea that, although the cAMP signaling system may regulate entry and exit from the G_0 state, there must be cAMP-independent mechanisms for regulating this growth pathway. The "simple" eukaryote, *S. cerevisiae*, may resemble mammalian cells in the complexity of its overlapping signaling pathways.

In the course of our work, we also isolated the cAMP phosphodiesterase genes of yeast. There are two, *PDE1* and *PDE2*, that encode proteins of low and high affinities for cAMP, respectively (Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]). Deletion of these genes only modestly elevates cAMP levels in yeast, a surprising result that suggested the existence of feedback control of cAMP levels in that organism. This idea received dramatic confirmation when we measured cAMP levels in yeast strains lacking *PDE* genes but containing the $RAS2^{val19}$ mutation (Nikawa et al., *Genes Dev. 1:* 931 [1987]). Such cells had cAMP levels that were nearly a thousandfold elevated when compared to normal cells. Cells containing $RAS2^{val19}$ but possessing *PDE* genes have only modestly elevated cAMP levels. These results indicate that there is feedback control of cAMP levels, that normal *RAS* proteins are part of that feedback pathway, and that the mutant form of *RAS* is not responsive to that feedback. The source of the feedback is almost certainly the activity of the cAMP kinase itself. Cells lacking the regulatory subunit have almost unmeasurable levels of cAMP, whereas cells with attenuated catalytic subunits have dramatically elevated levels of cAMP. Mammalian cells also have feedback systems that modulate cAMP levels, and in this regard, they may resemble yeast cells. However, in neither organism are the pathways of feedback understood, and the extent of the similarity of mechanism cannot presently be assessed.

During our effort to clone the *CDC25* gene by complementation analysis, we isolated a number of yeast genes all possessing the ability to suppress the temperature-sensitive defects of a *CDC25* mutant. Among these were the *TPK* genes and a gene we called *SCH9*. Sequence analysis of *SCH9* revealed that it encodes a protein with a carboxy-terminal domain strikingly similar to that of the catalytic subunits of the cAMP-dependent kinases (Toda et al., *Genes Dev.* [1988] in press). *SCH9* also encodes an amino-terminal domain similar in size to the regulatory domains of protein kinases C and the cGMP-dependent kinases, but without sequence homology. The suppression of *CDC25* by *SCH9* suggested that *SCH9* might have functions that are redundant with the cAMP-dependent protein kinase. Further genetic analysis bears out this conclusion. High-copy plasmids containing the *SCH9* gene can suppress the effects of loss of *RAS*, *CYR1*, and *TPK* genes. Conversely, although loss of the *SCH9* gene is not lethal, it does lead to a slow-growth phenotype, which can, in turn, be suppressed by activating the cAMP pathway. These results indicate that *SCH9* might encode a protein that is a component of a cAMP-independent signaling pathway in yeast controlling growth regulation.

Mammalian Oncogenes

C. Birchmeier, D. Young, S. Sharma, K. Ferguson, L. Rodgers, K. O'Neill, M. Riggs

We have been studying two human oncogenes, *MAS* and *ROS*, which we first isolated using a cotransfer and tumorigenicity assay (Birchmeier et al., *Cold Spring Harbor Symp. Quant. Biol. 51:* 993 [1987]). In both cases, the activation of these genes required a rearrangement occurring during gene transfer. Nevertheless, both genes are of considerable interest. We find that the *ROS* gene is expressed almost

exclusively in tumors of glial origin, but not in normal brain or in tumors of other origins (Birchmeier et al., *Proc. Natl. Acad. Sci. 84:* 9270 [1987]). We are in the midst of cloning and sequencing a full-length copy of *ROS* cDNA and have raised antibodies to its product, which is a membrane spanning tyrosine protein kinase. Sequence analysis indicates a significant homology with the *Drosophila sevenless* gene product, both in the external and internal domains. The gene product is large, perhaps 270,000 daltons, and we estimate the mRNA length to be 9 kb, of which 7 kb may be coding. The full-length product appears to be expressed in many glial tumor cells, with the exception of one glioblastoma cell line in which the *ROS* gene appears to have suffered a rearrangement and now produces a truncated product that has lost its transmembrane domain but still retains tyrosine kinase activity. Our studies suggest a possible role for *ROS* in the etiology of glial tumors, and we wish to test this possibility in an experimental model system. Our findings may provide a useful diagnostic marker for glial tumors.

The *MAS* gene represents a new class of oncogene. Its sequence indicates that it encodes a protein in the same class as the multiple membrane-spanning hormone receptors that activate G proteins (Birchmeier et al., *Cold Spring Harbor Symp. Quant Biol. 51:* 993 [1987]). It is normally expressed at high levels in the cerebral cortex and especially in the hippocampus (D. Young et al., Submitted). Thus, it is likely that *MAS* encodes a neurotransmitter receptor. We do not find it expressed in other tissues or in over 40 different human tumor cell lines we have examined. Activation of the gene during DNA transfer appears to have resulted from rearrangement of the promoter sequences, since merely forcing expression of a normal *MAS* product in NIH-3T3 cells is sufficient to transform these cells. The phenotype produced in NIH-3T3 cells by *MAS* is rather unusual (D. Young et al., Submitted). Such cells are highly tumorigenic and grow to high cell density in culture, but they appear to be morphologically normal and do not grow in agar suspension. *MAS* has an even more limited effect on other rodent cell lines. Our interest in *MAS*, as it relates to oncogenesis, is the biochemical pathway that it activates.

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NUCLEAR ONCOGENES AND SIGNAL TRANSDUCTION

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

Our goal is to understand how extracellular signals are communicated to the nucleus. Our efforts are focused on the c-*fos* proto-oncogene, which appears to represent a critical junction in the pathways through which growth signals are relayed to nuclear targets. Transcription of the c-*fos* gene is induced rapidly and dramatically within minutes of exposure to a broad collection of growth factors. Induction is a direct consequence of intracellular signals triggered by growth factors and does not require the synthesis of new cellular proteins. Our experiments are primarily aimed at identifying and following the several intracellular routes through which these signals travel from the cell surface to the c-*fos* gene. Our strategy is to identify the *cis*-acting DNA sequences in the c-*fos* gene required for response to signals and to use these DNA sequences to identify cellular proteins that bind to these DNA elements. Our assumption is that such proteins constitute important intermediates in these signaling pathways. We hope that these proteins will lead us further back along each signaling pathway so that we may eventually reach a complete molecular description of how signaling information from outside the cell reaches the genome and influences the pattern of gene expression.

c-*fos* Sequences Required for Growth Factor Induction

M. Gilman, R. Graham

To identify cellular signaling pathways through which growth factor signals travel to the c-*fos* gene and the c-*fos* sequences through which they act, our strategy is to use site-directed mutagenesis to inactivate c-*fos* regulatory elements and return these mutant genes to cells. The mutants are tested for their ability to respond to a variety of signals. A critical element for the response of c-*fos* to growth factors is the serum response element (SRE), located 300 bp upstream of the gene. This 20-bp element is necessary and sufficient for the response of c-*fos* to whole serum, which is a complex mixture of growth factors. Because the growth factors in serum activate several characterized and uncharacterized intracellular signaling pathways, it is not known which signals act on c-*fos* through the SRE and which use other sequences. Therefore, we have mutated the SRE and asked to what signals the mutant c-*fos* genes can still respond. As expected, these mutations reduce induction of the c-*fos* gene by serum as well as by a number of purified growth factors. In addition, the mutations abolish the response to phorbol esters, pharmacological agents that activate a critical intracellular signaling intermediate, protein kinase C. This observation shows that the protein kinase C pathway communicates with c-*fos* via the SRE.

Growth factors also trigger signals that do not involve protein kinase C. To determine whether the SRE is required for these protein-kinase-C-independent pathways, we have transfected c-*fos* genes into cells made temporarily deficient in protein kinase C. Under these conditions, the transfected wild-type gene still responds to serum and to purified growth factors, suggesting that the gene carries sequences responsive to protein-kinase-C-independent pathways. Transfected genes carrying mutations in the SRE no longer respond under these conditions, suggesting that the SRE is also required for this response. Our results therefore show that the SRE is the target for at least two distinct intracellular signaling pathways.

In contrast, the SRE is not required for response to agents that elevate the intracellular concentration

of cAMP, another important signaling molecule in the cell. c-*fos* genes carrying mutations in the SRE that abolish reponse to serum still respond fully to cAMP. Thus, cAMP constitutes a third signal that reaches the c-*fos* gene, and, in contrast to the others, this signal does not operate through the SRE.

There may be still more signals that act through distinct c-*fos* regulatory sequences. We find, for example, that certain SRE mutations that abolish c-*fos* induction by serum and phorbol esters do not fully ablate response to platelet-derived growth factor (PDGF). c-*fos* response to PDGF is not fully independent of the SRE, however, because more extensive SRE mutations do prevent PDGF induction. Experiments are currently under way to clarify the role of the SRE and other c-*fos* sequences in the response to PDGF.

Induction of c-*fos* by cAMP

L. Berkowitz, M. Gilman

Our studies with c-*fos* SRE mutants indicate that the SRE is not required for c-*fos* induction by cAMP. We are therefore trying to identify the c-*fos* sequences required for this response. Simple 5′-deletion analysis suggests that an element located approximately 60 bp upstream of the transcriptional start site of the c-*fos* gene is required for induction of the gene by cAMP in fibroblasts. However, deletion of this element almost completely abolishes c-*fos* promoter function. Therefore, it is difficult to prove that this element is indeed cAMP-regulated and not simply the last constitutive element in the promoter, which, when deleted, prevents any cAMP induction from being detected. To address this problem, we have placed several other regulatory elements immediately upstream of the −60-bp site in either a wild-type or mutant configuration. Our goal is to increase the activity of the promoter in the absence of the −60-bp element so that we can determine clearly whether mutation of this element leaves a promoter detectably active but no longer regulated by cAMP. Preliminary results suggest that when multiple copies of the SRE are inserted upstream of position −71, the resulting gene responds to both serum and cAMP, but when mutations are introduced into the −60 element, response to cAMP but not serum is lost. Therefore, we believe that this element is indeed cAMP-responsive.

When the same point mutations are introduced into a c-*fos* promoter carrying sequences through position −356, cAMP responsiveness is not lost. Therefore, in addition to the element at −60, other cAMP-responsive sequences lie between positions −356 and −71. Again, the SRE does not appear to be cAMP-responsive because a double mutant with an inactive SRE and an inactive −60 element behaves similarly to a mutant in which only the −60 mutant is inactive. By comparing a set of promoter mutants carrying the −60 mutations and various amounts of upstream sequence, we believe that we have mapped an additional cAMP-responsive element to the sequence between positions −110 and −71. There are probably still others farther upstream.

Cellular Proteins That Bind to c-*fos* Regulatory Sequences

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

Cellular proteins that bind to the c-*fos* SRE and other regulatory elements are potential intermediates in the pathways through which signals travel to the c-*fos* gene. We are therefore identifying cellular proteins that interact specifically with these sites. Using the mobility-shift DNA-binding assay, we and other investigators detect what appears to be a single DNA-binding activity in nuclear and whole-cell extracts of a variety of cell types. Fractionation of extracts by heparin-agarose chromatography results in approximately tenfold purification of this activity but also yields a DNA-protein complex with faster mobility in polyacrylamide gels. Mixing these active DNA-binding fractions with fractions that flow through the heparin-agarose column and contain no detectable SRE-binding activity reconstitutes the complex with the mobility seen in crude fractions. Therefore, we believe that the protein-DNA complex that forms on the SRE in vitro is at least tripartite, consisting of DNA, the primary DNA-binding protein, and an unidentified third protein (or complex of proteins). We are currently further purifying this heparin-agarose flow-through activity to learn more about it.

Although only a single SRE-binding activity is detectable using the mobility-shift assay, the presence of this activity does not appear to be sufficient to

account for the transcriptional state of the c-*fos* gene, because many cells that do not express c-*fos* contain this activity and some cells expressing c-*fos* at high levels lack the activity. Therefore, we have used a different assay to determine if there are additional SRE-binding proteins in the cell that are not detected using the mobility-shift assay. Using a microscale DNA-affinity assay to recover cellular proteins that bind to the SRE, we find several proteins that are specifically recovered with a wild-type SRE oligonucleotide and not with a mutant. These are new candidate SRE-binding proteins. Our current efforts are focused on determining which of these proteins corresponds to the mobility-shift activity we detect in the same extracts and to examine these proteins in extracts of mitogen-stimulated cells by high-resolution, two-dimensional gel electrophoresis.

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EXPRESSION OF SMALL NUCLEAR RNA GENES

N. Hernandez S. Lobo P. Reinagel
R. Lucito

Small nuclear RNA (snRNA) genes encode the snRNAs U1 to U7. These RNAs are complexed with a set of proteins to form small nuclear ribonucleoprotein particles (snRNPs). The functions of several snRNPs have been elucidated recently. The snRNPs U1, U2, U4, U5, and U6 are involved in pre-mRNA splicing, whereas U7 is involved in processing of the 3′ end of histone mRNAs. The role of U3, which is localized in the nucleolus, is unknown.

We are studying the mechanisms that govern the synthesis of the human snRNAs U1, U2, and U6. The human U6 gene is transcribed by RNA polymerase III, whereas the human U1 and U2 snRNA genes are transcribed by RNA polymerase II. These transcription units, however, differ in several respects from the majority of polymerase III and polymerase II genes. Thus, the U6 gene is characterized by a promoter located entirely in the 5′-flanking sequence of the coding region, in contrast to most polymerase III promoters described to date that are at least in part located within the RNA-coding region. The U6 promoter contains several sequence motifs also found in polymerase II promoters: (1) an octamer motif centered around position −215, (2) a motif also found in the proximal element of the U1 and U2 genes and located between positions −66 and −49, and (3) a TATA box centered around position −27. Deletion analyses have shown that the distal part of the promoter, which contains the octamer motif, serves to enhance the efficiency of transcription. Upon deletion to position −43, promoter activity is lost.

The U1 and U2 promoters are bipartite, consisting of a distal element and a proximal element. The distal element contains an Sp1-binding site adjacent to an octamer motif and has some of the functional characteristics of an enhancer. The proximal element is located between the cap site and position −62. In this region, a sequence motif is conserved between the U1 and U2 genes; as mentioned above, this sequence motif is also found in the U6 promoter region and in two additional polymerase III promoters, the 7SK and *myc* polymerase III promoters.

The U1 and U2 snRNAs are not polyadenylated, and their 3′ ends are formed by a unique process. The longest U1 and U2 precursors that can be detected in the nucleus are five to ten nucleotides longer at the 3′ end than the mature RNAs. Within minutes

after synthesis, these precursors are exported into the cytoplasm. There, the RNAs are packaged into snRNPs and shortened to the mature size, probably by an exonuclease. The snRNPs are then translocated back into the nucleus. The reaction that forms the 3′ ends of the U1 and U2 precursors is dependent on a signal, the 3′ box, located 10–19 nucleotides downstream from the mature 3′ end of the RNAs. Surprisingly, the reaction also requires promoter elements. Thus, if the U1 or U2 promoters are replaced by other polymerase II promoters derived from mRNA-encoding transcription units, 3′-end formation at the 3′ box is abolished and the RNAs become polyadenylated at a polyadenylation site inserted downstream. This observation demonstrates that 3′-end formation of U1 and U2 is coupled to transcription and therefore differs fundamentally from the processing reactions that form the 3′ ends of polyadenylated and nonpolyadenylated mRNAs. The 3′ ends of U1 and U2 could be formed by termination of transcription or by a processing reaction coupled with transcription.

U2 Promoter Sequences Involved in Initiation of Transcription and 3′-end Formation

N. Hernandez, R. Lucito

The promoter region of the U2 snRNA gene is required for correct 3′-end formation at the 3′ box. To define precisely the sequences involved in initiation of transcription and U2 3′-end formation, we have extensively mutated the U2 promoter region. The different constructs were transiently transfected into HeLa cells, and the RNA was analyzed by RNase T1 mapping. The first series of mutations, 5′–3′ deletions, are shown in Figure 1A. Promoter sequences can be deleted in a 5′–3′ direction up to position −240 upstream of the cap site without effect on initiation of transcription. Thus, the SP1-binding site located just downstream from position −270 is dispensable. Upon deletion to position −198, the efficiency of transcription drops by 80%, confirming that the Sp1 and adjacent octamer motifs serve to enhance transcription from the U2 cap site. Deletion to position −62 reduces transcription another 10%. In the Minipr and Minipr+4 constructs, the Sp1 and octamer motifs were placed just upstream of position −62, with an insertion of 4 bp in Minipr+4. These "minipromoters" are quite active, although the efficiency of transcription varies with the spacing and/or sequence separating the proximal region of the promoter and U2 enhancer.

The proximal region of the promoter was analyzed by linker-scanning mutations, as shown in Figure 1B. The two substitutions (LS −51/−60 and LS −44/−53) that modify the motif conserved in the U1, U2, and U6 genes (and thereafter referred to as the proximal element) reduce transcription to barely detectable levels. Interestingly, the next two substitutions (LS −33/−42 and LS −28/−37), which fall outside of the conserved motif, also reduce transcription drastically and therefore define another promoter element. The other substitutions had little effect on transcription.

In the last series of mutations (Fig. 1C), the U2 enhancer was replaced by artificial enhancers (6×B17, 6×A21, and 6×C17; obtained by B. Ondek and W. Herr [Tumor Viruses Section]), which consist of six tandem copies of the A, B, or C element of the SV40 enhancer (Ondek et al., *EMBO J. 6:* 1017 [1987]). The B element of the SV40 enhancer consists of two Sph repeats (indicated by arrows under the sequence in Fig. 1C), the junction of which forms an octamer motif (boxed) similar to the octamer motif found in the natural U2 enhancer. The double point mutation *dpm*2 affects the second Sph repeat and destroys the enhancer activity of the element when tested downstream from the β-globin promoter (Ondek et al., *EMBO J. 6:* 1017 [1987]), whereas *dpm*7 destroys the octamer motif and has no effect on enhancer activity on the β-globin promoter (see 1986 CSH Annual Report, p.75). Interestingly, the situation is reversed when these artificial enhancers are tested upstream of the U2 proximal element; 6×B17/*dpm*2 is as active as 6×B17 or the U2 natural enhancer, whereas 6×B17/*dpm*7 is inactive. The octamer motif in the B element therefore seems to be essential for activation of transcription from the U2 promoter, but not from the β-globin promoter. Similarly, 6×A21 and 6×C17, but very active enhancers when tested downstream from the β-globin gene (Ondek et al., *EMBO J. 6:* 1017 [1987]), are completely inactive upstream of the U2 promoter. These results suggest that the factors that bind to the 6×B17/*dpm*7, 6×A21, and 6×C17 enhancers cannot cooperate with the U2 proximal element to enhance transcription. The U2 enhancer is thus a promoter-specific enhancer, which can only be replaced by enhancers such as 6×B17/*dpm*2, which presum-

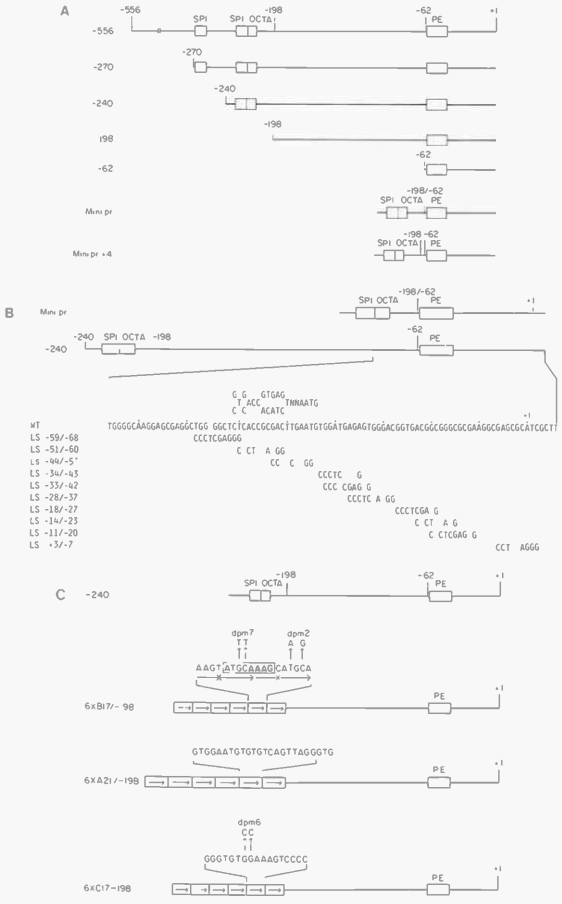

FIGURE 1 (*A*) Deletions in the U2 promoter region. Boxes indicate the proximal element (PE), the octamer sequence (octa), and sequences similar to S1-binding sites (SP1). The start site of transcription is labeled +1. (*B*) Linker-scanning mutations in the U2 promoter region. The upper two lines indicate the structures of the U2 promoter region in the "Minipr" construct and in the parent construct. The linker-scanning mutations indicated below the wild-type sequence were introduced into both of these constructs, except for the linker-scanning mutation LS −59/−68, which was introduced only in the parent construct, since it replaced a sequence absent in the Minipr construct. (*C*) Enhancer replacements in the U2 promoter. The region upstream of position −198 was replaced by different synthetic enhancers: 6×B17 containing the double point mutation *dpm7*, 6×B17 containing the double point mutation *dpm2*, 6×A21 and 6×C17.

ably bind the same factors as the wild-type U2 enhancer.

The deletions, linker-scanning mutations, and enhancer replacements were also analyzed for their effect on 3′-end formation. None of the deletions or linker-scanning mutations inhibited 3′-end formation. Replacement of the U2 enhancer by the SV40-derived artificial enhancers, however, had interesting effects. 6×B17/*dpm*2, which enhanced transcription from the U2 promoter, had no effect on the efficiency of correct 3′-end formation. In contrast, correct 3′-end formation was reduced five- to ten-fold when the U2 enhancer was replaced by the 6×B17/*dmp*7 and 6×A2I enhancers. 6×C17 had a very weak inhibitory effect. These results suggest that the introduction of foreign transcriptional elements in the U2 promoter, and hence presumably foreign transcription factors in the U2 transcription complex, is incompatible with 3′-end formation. The U1 and U2 promoters must, then, direct the formation of a specialized transcription complex different from the transcription complexes that form on promoters of mRNA-encoding genes and compatible with 3′-end formation at the 3′ box. We will learn more about this transcription complex as we identify its constituent proteins.

Biochemical and Functional Characterization of a Protein That Interacts with the U2 and U6 Proximal Elements

S. Lobo, N. Hernandez

The promoter regions of the U2 and the U6 genes share at least two sequence motifs: the octamer and U2 proximal element. These motifs may bind factors that participate in polymerase II and polymerase III transcription. Using the gel-mobility shift assay, we detected a protein that interacts specifically with the U2 and the U6 proximal elements. The precise binding sites on U2 and U6 were determined by diethyl pyrocarbonate interference and were very similar. We are mutating the DNA-protein contact points defined by the chemical interference analysis to correlate loss-of-factor binding in vitro and loss-of-promoter activity in vivo. We are also purifying the protein for in vitro transcription studies.

Factors Binding to the 3′ Box of the Human U1 and U2 Genes

P. Reinagel, N. Hernandez

The 3′ box of the U1 and U2 snRNA genes may constitute a binding site for a 3′-end formation factor. Because the mechanism of 3′-end formation at the 3′ box is completely unknown, it is unclear whether the 3′ box is recognized as a DNA signal or an RNA signal. It could even be recognized as a single-stranded DNA signal, if the reaction forming the 3′ end occurred concomitantly with the passage of the RNA polymerase. We have screened extracts and protein fractions for factors that form a complex with a double-stranded or single-stranded DNA fragment containing the 3′ box, but not with a DNA fragment in which the 3′ box was replaced by a *Sal*I linker. Formation of complexes was monitored by the gel-mobility shift assay and by DNase I footprinting. We have detected a factor that binds to single-stranded DNA and footprints over the 3′ box. This factor recognizes only the RNA strand of the DNA. We are now examining whether it also recognizes the 3′ box at the RNA level.

In Press, Submitted, and In Preparation

Hernandez, N. and R. Lucito. 1988. U2 5′-flanking sequences required to 3′ end formation cannot be separated from promoter sequences. (In preparation.)

TRANSGENIC MICE

D. Hanahan J. Alexander V. Bautch J. Hager J. Skowronski
L. Field A. Almeida S. Efrat N. Labella M. Steinhelper
S. Alpert S. Grant J. Moyse E. Wetzel

Research using gene transfer into the mouse germ line to produce stable lines or families of "transgenic" mice has continued to expand in scope both at Cold Spring Harbor Laboratory and in the scientific community at large. The current work of the mouse molecular genetics group described below illustrates the breath of possible applications of this powerful technique. Transgenic mice are being used to investigate mechanisms of tumorigenesis and of immunological tolerance, the requirements for tissue-specific gene regulation, and the physiological consequences of altering synthesis of hormones involved in maintaining blood pressure and, finally, to expand cells selectively from rare cell types and establish them in culture.

Characterization of Skin Tumors Induced by Bovine Papillomavirus in Transgenic Mice

E. Wetzel, J. Skowronski, D. Hanahan [in collaboration with M. Sippola-Thiele, V. Lindgren, and P. Howley, National Cancer Institute, Bethesda, Maryland]

Bovine papillomavirus type 1 (BPV-1) is a DNA tumor virus with a circular, double-stranded genome of 8 kb. In cattle, BPV-1 infects cutaneous tissue and induces benign fibropapillomas composed of proliferative epidermal cells and dermal fibroblasts. The viral genome replicates as an autonomous plasmid to a high copy number in tumors. Experimental introduction of viral DNA induces fibroblastic tumors in a variety of heterologous hosts, including hamsters, mice, and horses. The ability of BPV-1 to replicate and to morphologically transform murine cells in vitro has allowed molecular and genetic analysis of the viral genome, including the characterization of regions that are involved in cellular transformation. A subgenomic fragment comprising 69% of the genome is sufficient to induce in vitro transformation of selected murine cell lines and to maintain viral DNA as a free plasmid. Eight open reading frames (ORFs), designated E1 through E8, have been identified within the 69% subgenomic transforming segment. Two of these ORFs, called E5 and E6, have been shown to encode proteins with oncogenic activity.

To study papillomavirus-induced tumorigenesis, we have constructed a transgenic mouse lineage that harbors the BPV-1 genome integrated in the mouse germ line. The transgene, BPV-1.69, consists of a partial tandem duplication of the complete viral genome. This design provides an uninterrupted copy of the complete genome to allow transcription from all ORFs and to facilitate possible excision and extrachromosomal replication. About five copies of the BPV-1.69 plasmid are integrated in a head-to-tail tandem array on chromosome 15.

Transgenic mice harboring the BPV-1 genome develop skin tumors that have characteristics similar to those that arise after natural infection in cattle. Tumors induced by BPV-1 in this transgenic lineage do not appear until 8–9 months of age. Two classes of pathology arise: abnormal skin and protuberant tumors. Areas of abnormal skin, which appear first, are characterized by balding, thickening, and hardening of the skin. Histologically, these skin lesions are characterized by hyperplasia of the dermal fibroblasts. The protuberant tumors, which arise approximately 2 months later, are defined as fibrosarcomas, with densely packed fibroblasts. The skin tumors are locally invasive, as they do not have a capsule typical of benign tumors, but apparently are not metastatic.

Abundant extrachromosomal viral DNA and BPV-1-specific transcripts are found in abnormal skin and protuberant tumors. In contrast, neither BPV-1 episomes nor BPV-1-specific transcripts can be demonstrated in other tissues, including areas of normal skin. Thus, there is a complete correlation between the presence of viral episomes and transcriptional activity of BPV-1 in the two types of pathologies. The two clearly distinct pathologic phenotypes (abnormal skin and protuberant tumors) are indistinguishable in terms of BPV-1 extrachromosomal copy number and level of viral gene expression. Thus, although BPV-1 provides the animals with a predisposition to develop skin cancer, expression of BPV-1 information does not seem to be sufficient for tumor formation. Thus, cellular events are

likely to be required for the full neoplastic transformation. To study the molecular and genetic events occurring during the progression of the BPV-1-induced pathology, we have established cell cultures from the different skin lesions. Fibroblasts isolated from the three different conditions show stable differences in their in vitro phenotypes, which might be representative of the stage of tumor development from which they were removed. Fibroblasts isolated from normal skin are flat, grow slowly, and are contact-inhibited. Additionally, they do not form colonies in soft agar nor do they induce tumors in athymic or syngeneic mice. The BPV-1 genome is apparently latent with respect to both expression and extrachromosomal replication. Fibroblasts derived from abnormal skin and fibrosarcomas are spindel-shaped, grow rapidly, and form spontaneous, dome-shaped foci. They also exhibit anchorage-independent growth in soft agar and induce tumors in athymic or syngeneic mice. Expression of the BPV-1 genome is activated in these cells in that extrachromosomal BPV-1 DNA and BPV-1-specific transcripts are detectable in tissue biopsies and in all cell cultures derived from abnormal pathologies.

The chromosomes of cells isolated from mice of this lineage have been characterized in order to examine whether specific chromosomal abnormalities are associated with tumor formation. In general, the karyotypes of tumors and tumor cell lines are more complex than those of abnormal skin, which are essentially diploid. When the various tumor karyotypes are compared, it is apparent that two nonrandom changes correlate with the fibrosarcomas; namely, abnormalities of chromosomes 8 and 14 are characteristically associated with tumor formation. Chromosome 8, or a portion of it, is frequently duplicated (70%). Chromosome 14 is lost with similar frequency (60%). All fibrosarcomas have one or both of these chromosomal changes. The BPV-1 transgene is integrated on chromosome 15; thus, the commonly observed rearrangements of chromosomes 8 and 14 are not likely to result from rearrangements of viral DNA.

Several known genes that are implicated in neoplastic transformation are located on chromosomes 8 and 14 and therefore might be important for tumorigenesis in this model system. We are currently investigating two of them. One candidate that maps to chromosome 8 is the tissue plasminogen activator (tPA) gene. tPA is thought to play an important role in cell migration, tissue remodeling, and metastasis. However, preliminary results do not suggest any consistent correlation between tPA activity and specific stages of tumorigenesis. The retinoblastoma (*Rb*) gene, which is located on chromosome 14, is another cellular gene that might be involved in tumor progression in this transgenic lineage. *Rb* is a recessive oncogene that is thought to have anti-oncogenic effects, such that the loss of the *Rb* gene results in tumorigenicity. We are currently analyzing the transcriptional activity of the *Rb* gene and the levels of *Rb* gene product in the three different types of fibroblastic cultures. In addition, we are beginning to study expression of proto-oncogenes, growth factors, and receptors in tissues and in cell lines isolated from either normal skin or skin lesions. It is our expectation that these types of approaches will increase our understanding of the nature of tumor progression and the types of molecular changes that accompany the development of fibrosarcomas.

Proliferation, Senescence, and Neoplastic Progression of β Cells in Hyperplastic Pancreatic Islets

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

Tissue hyperplasia can result from the generalized proliferation of most cells in the population (or of a special subclass of those cells), and the hyperplasia can be either preneoplastic or not. We have characterized three different conditions of pancreatic β-cell hyperplasia in mice. Two of these, the transient increase in β-cell number during pregnancy and the localized proliferation in some islets of the mutant ob/ob mouse, do not lead to tumor formation. The third case, that of β cells expressing the SV40 large T oncoprotein, does result in progression to neoplasia.

The latter condition is achieved in transgenic mice, called RIP1-Tag2, which carry a hybrid gene composed of the 5′ regulatory sequences of the rat insulin II gene linked to the protein-coding information for the oncogene, SV40 large T antigen (Tag). The islets are normal in neonatal mice and become hyperplastic in young adult mice, and a fraction progress into highly vascularized β-cell tumors in adult mice. The contrasting cases, of transient β-cell hyperplasia and regression in pregnant mice (Green et al., *J. Endocrinol. 88:* 219 [1981]) and of localized hyperplasia of some but not all of the islets in the ob/ob mouse (Herberg and Coleman, *Metabolism 26:* 59

[1977]), appear to involve normal β cells responding to external stimuli.

Our initial observations demonstrated that hyperplastic islets of transgenic mice contain a large number of β cells that coexpress insulin and tyrosine hydroxylase (TH). In contrast, normal islets of adult mice contain only a few TH-insulin cells (Teitelman and Lee, *Dev. Biol. 121:* 454 [1987]). These findings suggested that the TH-insulin cells could be islet cell precursors that proliferate and increase in number, thereby effecting islet growth. Alternatively, islet hyperplasia could result from the proliferation of another subclass of β cells, and the appearance of large numbers of TH-insulin cells in islets might only be a consequence of the general increase in β cells.

Pregnant and ob/ob mutant mice were examined for the expression of TH by immunocytochemistry. In both models, hyperplasia was accompanied by an increase in the number of TH-containing cells. Virtually all of the TH cells in normal and hyperplastic islets express insulin and none of the other pancreatic hormones. Thus, the increase in the number of TH cells in these two systems results from an increase in the number of β cells that coexpress insulin and TH and is not due to the appearance of TH in another cell type.

To determine if the rare TH-insulin cell present in nonhyperplastic islets divides to give rise to the larger number of these cells present in the hyperplastic islets, pancreata of ob/ob and pregnant mice were examined to determine which populations of endocrine cells were proliferating. Cells that contain insulin did proliferate, whereas TH-containing cells did not. Thus, although "insulin-only" cells can divide, cells containing TH-insulin have withdrawn from the cell cycle. Experiments conducted to determine the fate of the TH-insulin cells revealed that these cells disappear from the islets. Either these cells lose TH and re-enter the cell cycle or they may be postmitotic cells destined to die. The possibility that the TH-insulin cells are on a pathway toward elimination is supported by the observation that in normal pregnancy, the number of β cells doubles, but then subsequently returns to the original level, which must involve the elimination of β cells.

A distinct condition of β-cell proliferation is that of tumorigenesis under the direct influence of an oncogene(s) expressed in the β cells. One might predict that such transformed cells would not undergo normal senescence. Since TH apparently marks β cells in a pathway toward senescence and elimination, its expression has been examined during the heritable oncogenesis of pancreatic β cells, which occurs in the RIP-Tag2 line of transgenic mice (Hanahan, *Nature 325:* 155 [1985]). In agreement with previous findings (Hanahan, *Nature 325:* 155 [1985]), it was observed that the increase in islet size was due to an increase in the number of insulin-containing cells.

The proportion of TH cells in pancreatic islets of young transgenic mice was significantly higher than that in controls, and it continued to increase during postnatal development. Certain islets in young adult mice contained a majority of cells that expressed the enzyme. However, the pancreas of adult transgenic mice contained many large islets that completely lack TH cells. When sections of pancreas were processed for immunocytochemical localization of two antigens, it was found that TH cells always coexpressed insulin. Therefore, the increase in the proportion of TH cells during tumor progression reflects an increase in the proportion of TH-insulin cells. When experiments were conducted to determine which populations of β cells were dividing, it was found that, in contrast to normal and mutant ob/ob mice, the TH-insulin cells and insulin-only cells do proliferate in RIP1-Tag 2 mice.

The observation that both insulin-only cells and TH-insulin cells proliferate in islets in transgenic mice raises the possibility that different stages of tumor progression could be marked by the populations of β cells that comprise the islets. Thus, pancreata from RIP-Tag2 mice at several stages of postnatal life were sectioned and processed to determine the mitotic rate and the number of β cells that contained TH. Several classes of islets were detected, and a change in the distribution of islet types was noted during postnatal life. It is possible that as an islet becomes a tumor, it undergoes a gradual progression through the various classes until it becomes a rapidly dividing tumor. As not all islets become tumors, the progression in islet cell types is likely to be accompanied by an additional, relatively infrequent event, such as the induction of angiogenesis, which converts a rapidly proliferating islet into a tumor. Thus, TH, an enzyme involved in catecholamine biosynthesis, has been shown to mark a particular class of pancreatic β cells. TH is expressed in nonmitotic β cells following periods of proliferation in what appears to be a terminal differentiation pathway to cellular senescence and is also expressed during transformation of β cells into tumor cells. The implications of these findings are severalfold. First, TH can serve as a marker to study the life cycle of pancreatic β cells in vivo. Second, the possibility arises that the

TH-insulin cells may be distinct and perhaps physiologically deficient. The development of disease states could be related, in part, to the appearance of large numbers of this class of β cells. Third, in β cells containing an oncogene, the patterns of TH expression identify states in the preneoplastic period, where critical secondary events may occur. Although TH is not active in the catecholamine biosynthetic pathway in pancreatic β cells, it is possible that TH is performing some other function; perhaps, it is actively participating in the senescence pathway. Alternatively, its expression may relate to the regulatory pattern and cellular history of the β cells, therefore providing a marker for conditions of β cells that have heretofore been indistinguishable.

Hybrid Insulin Genes Reveal a Developmental Lineage for Pancreatic Endocrine Cells

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

The mouse pancreas develops as an outpocketing from the embryonic gut beginning on day 10 of gestation (e10) (Wessells and Evans, *Dev. Biol. 17:* 413 [1968]; Pictet and Rutter, *Handb. Physiol. 7:* 25 [1972]). Cells lining this evagination differentiate and segregate into exocrine and endocrine tissues. The endocrine cells initially appear adjacent to the nascent pancreatic duct and then are displaced from the duct as the islets of Langerhans are formed (Pictet and Rutter, *Handb. Physiol. 7:* 25 [1972]). The hormones produced by the endocrine pancreas appear sequentially during development: Glucagon (α cells) appears first, followed by insulin (β cells), somatostatin (δ cells), and finally pancreatic polypeptide (PP cells) (Pictet and Rutter, *Handb. Physiol. 7:* 25 [1972]).

We have analyzed pancreatic development in two lines of mice, each harboring a distinct transgene consisting of the regulatory information flanking the rat insulin II gene linked to the sequences encoding the SV40 large T antigen (Hanahan, *Nature 325:* 155 [1985]). T antigen is a nonsecreted nuclear antigen and therefore identifies the cells where it is being synthesized. During the pancreatic development of mice harboring the insulin/T antigen hybrid gene, many cells express the transgene product. T antigen can be found in cells expressing any of the pancreatic hormones at the time of onset of expression. Initially, T antigen is present in 100% of the cells that contain insulin, TH, and glucagon. At the onset of somatostatin and PP expression, a significant subpopulation of the cells (36% for somatostatin and 25% for PP) contain the transgene product. As development proceeds, the proportion of glucagon, somatostatin, and PP cells immunoreactive for T antigen decreases, whereas the number of insulin-containing cells that also produce T antigen remains at 100%. T antigen is never present in cells immunoreactive for amylase. Thus, the information contained in the transgene specifies transient expression of T antigen in all four endocrine cell types during prenatal development of the pancreas.

The observation that the hybrid insulin/T antigen gene is coexpressed with other islet hormones during embryogenesis motivated a similar evaluation of possible coexpression of the endogenous insulin genes with the other pancreatic markers in nontransgenic mice. When sections of CD-1 mouse pancreas were processed for the dual localization of insulin and glucagon, cells containing both hormones could be detected at all stages of embryonic development. The number of cells that produced both hormones decreased dramatically during postnatal life. These results suggest that throughout normal development, insulin can be coexpressed with glucagon. These results argue that the coexpression of the insulin/T antigen genes with glucagon in the same cells in transgenic mice is neither unprecedented nor artifactual. To determine if insulin colocalizes with other pancreatic hormones in the same cells, e17 pancreatic sections were processed for the localization of somatostatin and insulin, and p1 pancreatic sections were processed for the localization of insulin and PP. In both cases, significant numbers of cells could be found that contained both hormones. Thus, during development, both T antigen and insulin can be detected in a subpopulation of all of the other endocrine cell types.

Classically, lineage relationships have been analyzed through the removal of cell populations by ablation or microdissection, as well as by visualizing either endogenous markers (Le Douarin, in *Molecular Basis of Neural Development*, John Wiley and Sons, New York [1985]) or histological labels (see Jacobson, *Ann. Rev. Neurosci. 8:* 71 [1986]). More recent approaches have utilized retroviral integration (see, e.g., Price et al., *Proc. Natl. Acad. Sci. 84:* 156 [1987]) or cell-specific ablation using targeted expression of a toxin gene (Palmiter et al., *Cell 50:* 435 [1987]). The experiments described here demonstrate

that cell lineages can be specifically marked by hybrid gene expression in transgenic mice so as to provide insight into progenitor populations and to evaluate the regulation of genes during development.

Tolerance and Autoimmunity in Transgenic Mice

S. Alpert, J. Skowronski, D. Hanahan

Transgenic mice carry a new genetic element that has been experimentally transferred into the germ line. Different transgenes have been found to be expressed at different times during development, depending both on the regulatory sequences contained in the construct used to generate the mice and on the site of integration of the injected gene (Palmiter and Brinster, *Ann. Rev. Genet. 20:* 465 [1986]). In principle, transgenic mice can provide a new approach to the study of immunological tolerance and autoimmunity, as novel transgene products can be presented in a physiological manner, since they are part of the genetic makeup of the animals. Recently, the ability of mice to respond to transgenic products has been studied at a humoral level in several lines of mice carrying a hybrid insulin/T antigen transgene (Hanahan, *Nature 325:* 155 [1985]).

Immunohistochemical characterization of adult animals from four lineages of transgenic mice harboring hybrid insulin/T antigen genes show that T antigen is expressed in a tissue-specific manner in the β cells of the islets of Langerhans. However, more detailed analyses of the ontogeny of T-antigen expression has revealed that both timing of the onset and levels of T-antigen expression vary between different insulin/T antigen lineages. Individual mice in a given lineage show a consistent temporal pattern of expression of the transgene.

We identified two transgenic lineages with an early onset of expression of the transgene: the RIPI-Tag2 lineage, where T antigen is easily detected in all cells immunoreactive for insulin from embryonic day 10 (e10) of development through postnatal life, and the RIR-Tag2 lineage, where T antigen can first be detected at e10 and is present in most, if not all, β cells from e18 onward (Alpert et al., *Cell 53:* 295 [1988]). In two other lineages, RIPI-Tag3 and RIPI-Tag4, the onset of expression was considerably delayed when compared to that of the endogenous insulin gene. In these lines, immunoreactive T antigen was not detectable in the β cells prenatally, nor through the first 2–3 months of postnatal development. Rather, beginning at 9–12 weeks of age, a subset of the insulin-producing cells begin to express T antigen.

The availability of lines of transgenic mice that reproducibly show either prenatal or delayed onset of expression of a unique β-cell antigen presents an opportunity to examine the relationship between this expression and the establishment of tolerance (Adams et al., *Nature 325:* 223 [1987]). When RIPI-Tag2 (early onset of expression), RIPI-Tag3, and RIPI-Tag4 (late onset of expression) mice were immunized with exogenous large T antigen protein and analyzed for the production of a humoral response to T antigen, it was found that the transgenic lineages with delayed onset of expression could mount a readily detectable antibody response to T antigen. RIPI-Tag2 mice exhibited a very weak response; thus, the ability to respond to the transgenic protein is impaired in these mice. Quantitative assays on control, RIPI-Tag2, and RIR-Tag2 mice revealed that the titer of antibodies following immunization was two- or threefold lower than that of controls.

It thus appears that prenatal expression of T antigen has a tolerogenic effect. Second, the tolerizing effect of the prenatal expression of the transgene is quantitative in nature and leads to reduced, rather than abolished, responses upon exposure to the same antigen in adult life. The degree to which the response is affected correlates positively with levels and duration of expression of the transgenic antigen through the prenatal period of life.

Delayed onset of expression of T antigen does not affect the ability of RIPI-Tag3 and RIPI-Tag4 mice to respond to large T antigen. In addition, a systematic survey of sera revealed that antibodies against T antigen arise spontaneously at a high frequency. The frequency of this autoimmune response appears to be characteristic of the lineage. In addition to the humoral response noted in the lineages with delayed onset of expression, a cellular response was also noted. The islets of Langerhans of RIPI-Tag3 and RIPI-Tag4 mice frequently exhibited infiltration with lymphocytes (insulinitis) and physical disruption in the islet structure. This insulinitis is not focused on the tumors; rather, it appears to be present in the islets.

The observation that some but not all of the mice with delayed onset of transgene expression develop an autoimmune response to T antigen indicates that the delayed presentation of the antigen is necessary, but not sufficient, to elicit the response. The transgenic lineages generated with the insulin/large T an-

tigen hybrid gene were constructed in F2 hybrids derived from crossing two inbred strains of mice (C57BL/6J and DBA/2J) and maintained by backcrossing to C57BL/67 or by intercrossing. Thus, individual mice of the delayed-onset lineages have different contributions of genetic information originating from either of the parental inbred strains. It was therefore possible that there was a genetic contribution to the immune response.

When RIP1-Tag3 progeny were generated by crossing transgenic males to DBA/2J or C57BL/6J females, distinct differences in the frequency and dynamics of the humoral response were noted in the offspring. All of the progeny generated from the DBA crosses developed a humoral response in the first 6 months of life, whereas only 20% of the mice derived from the C57BL/6J crosses responded to T antigen during the first 8 months of life. Although this analysis does not unambiguously define the number and mode of action of loci responsible for the autoimmune reaction, since the transgenic parent is a mixture of DBA/2J and C57BL/6J, the pattern is not inconsistent with the idea that a single locus in the DBA/2J backround acts dominantly to promote the autoimmune response. The actual number and identity of the immunoregulary loci that control the response to T antigen are unknown.

Transgenic mice can thus provide a new approach to address the question as to how specific antigens of rare cell types interact with the immune system. This raises the prospect that a variety of immunologically or pharmacologically characterized proteins can be directed to a cell type such as the β cell, and the reaction of the immune system to this antigen can be assessed. The mechanisms by which tolerance is established for proteins specific for rare cells can thus be studied from the perspective of using a well-characterized antigen, with known temporal pattern and level of expression.

Transgenic Mice Carrying Two Viral Genes Show an Altered Tumor Phenotype

V. Bautch, S. Grant, D. Hanahan [in collaboration with I. Seidman, New York University Medical School, New York]

We are studying the cooperative effects of different oncogenes in transgenic mice. The paradigm of this concept is found in cultured primary cells that require an immortalization function found in one group of oncogenes and a transformation function found in a second group of oncogenes to become fully transformed in culture. The polyoma early region genes are well suited to this type of analysis because polyoma large T (PyLT) antigen is an immortalizing gene, whereas polyoma middle T (PyMT) antigen is a transforming gene. Polyoma small T (PyST) antigen has no well-defined role in transformation of cultured cells. The early region of the DNA tumor virus SV40 has one gene with both immortalizing and transforming functions (SV40 large T antigen [SV40 LT]) and another gene with no well-defined role in transformation (SV40 small T antigen [SV40 ST]). To test for cooperation among these viral genes in mouse tissues, we targeted their expression to the insulin-producing β cells in the pancreas using transgenic mice.

We generated transgenic mice carrying each of the polyoma early region genes (as cDNAs) linked to the rat insulin promoter. Mice carrying insPyLT developed pancreatic β-cell tumors, but with a latency of 1 year or more. Mice carrying insPyMT or insPyST did not develop any tumors or other abnormalities, although these genes could be expressed. These mice were bred with each other to generate mice carrying two different insulin-promoted transgenes. These combination mice carried either insPyLT and insPyMT or insPyLT and insPyST. None of these combination animals appear to develop β-cell or other tumors with kinetics different from that of the parental insPyLT. Thus, the paradigm of cooperating oncogenes does not appear to be operative for the polyoma genes in β cells.

We have also bred the insPy mice to mice carrying an insulin-promoted SV40 early region. The insSV40 mice have been described previously, and they develop pancreatic β-cell tumors that are fatal at 14–16 weeks of age. These mice on rare occasions also develop tumors at extrapancreatic sites that include the intestine, mesentery, and liver. Combination mice carrying insSV40 and insPyST, however, showed a much higher frequency of extrapancreatic tumor formation. For example, in littermates 12 weeks of age or older, insSV40 mice have a 20% (4 of 19) incidence of intestinal tumors, whereas combination mice (insSV40 and insPyST) have a 100% (20 of 20) incidence of intestinal tumors. Both classes of mice show the same kinetics of β-cell tumor development. Preliminary analysis indicates that SV40 LT, SV40 ST, and PyST are all expressed in tumors. These results suggest that the SV40 early region and PyST can cooperate to accelerate the de-

velopment of extrapancreatic tumors in mice carrying both gene regions linked to the insulin promoter.

Our current efforts include clarification of both the biological and the molecular aspects of this unpredicted cooperation between two transgenes. Because tumors at all sites have been classified as neuroendocrine, we are investigating whether the extrapancreatic tumors arise de novo or metastasize from other sites. We are attempting to reproduce this effect with different lineages of each parent. Finally, we will determine the relative contributions of SV40 LT and SV40 ST to this phenomenon.

Oncogenesis in Polyoma Transgenic Mice

V. Bautch, J. Moyse, K. Theiler, L. Gregorio, D. Hanahan [in collaboration with J. Hassell, McGill University, Montreal]

We are analyzing the effects of the early region of polyomavirus in transgenic mice. Ultimately, we would like to understand oncogenesis in vivo and, specifically, to relate the relatively well-understood process of transformation of cultured cells to the poorly understood process of tumor formation. The polyomavirus early region genes were chosen for this analysis because both the virus and the early gene products have been characterized. The permissive host for polyomavirus is the mouse, and inoculation of newborn mice with high titers of polyomavirus leads to the development of tumors in a variety of tissues starting at 3 months of age. Thus, polyomavirus can be tumorigenic in mice, and the tumor-forming potential of the virus resides in the early region. Three proteins are encoded by the early region: large T (100 kD), middle T (56 kD), and small T (22 kD) antigens. Polyoma large T (PyLT) antigen can immortalize primary cells to continuous growth in culture, and polyoma middle T (PyMT) antigen can morphologically transform established cultured cells, but not primary cells. These two proteins can cooperate with each other and with other oncogenes to transform primary cells. The function of polyoma small T antigen (PyST) in cultured cells is not well defined.

These analyses were initiated by creating transgenic mice carrying different polyoma early region genes linked to the polyoma early region promoter. Each mouse that scored positive for polyoma sequences was used to establish a lineage or family of mice with the same gene integrated in the same chromosomal position. Two lineages of mice carrying PyLT were generated, and until recently, these animals showed no overt phenotype. Recently, one of the two lineages has developed pituitary tumors with a latency of greater than 1 year. These results were unexpected because polyomavirus-infected mice do not develop pituitary tumors. Because only one of the two PyLT lineages develops these tumors, the phenotype is probably modulated by the position of transgene integration. However, these results do suggest that expression of an "immortalizing" gene can increase the probability that certain cell types will become tumorigenic, although expression is not sufficient for tumor formation.

As described in previous reports, animals carrying PyMT develop fatal hemangiomas, or tumors of the endothelial cells that line the blood vessels. This phenotype is an intrinsic property of the transgene because it is found in multiple lineages. This year, we have completed an extensive expression analysis of the transgene. We found that MT RNA and protein are expressed in both tumors and testes of these mice. The MT protein in both cases is found complexed to a cellular tyrosine kinase. In cultured cells, this complex is thought to be necessary but not sufficient for PyMT-mediated transformation. In mouse testes, expression of complexed MT protein does not appear to result in any visible or functional abnormalities, although expression correlates with tumor formation in endothelial cells. These results indicate that, despite the fact that PyMT can transform different types of cell lines in culture, the deleterious effects of this oncogene are cell-type-restricted in vivo. As demonstrated in cell culture, a complex between PyMT and a cellular tyrosine kinase appears to be necessary but not sufficient for tumorigenesis in mice.

This year, we have also developed three lineages of mice that carry the intact early region of polyomavirus, capable of expressing all three early region proteins. These mice show an expanded tumor spectrum relative to mice carrying PyMT alone. In addition to endothelial cell tumors, these mice develop bone abnormalities and rare spindle cell sarcomas. We are undertaking an expression analysis to determine why these mice show an expanded tumor spectrum relative to PyMT mice. One hypothesis is that coexpression of LT and/or ST with MT is necessary for tumor formation in bone cells. Because preliminary evidence suggests that the polyoma genes are coexpressed in the testes of these poly-

oma transgenic mice with no deleterious effect, cooperation between different polyoma oncogenes to form tumors may also be cell-type-specific.

To summarize, our studies to date indicate that some oncogenes show a cell-type specificity for tumor formation in mice that is not obvious in cultured cells. The cooperative effects of different oncogenes may also be cell-type-restricted. Finally, the requirements for a complex between PyMT and a cellular kinase appear to be similar in both cultured cells and in vivo.

Targeted Oncogenesis in the Cardiac Atria of Transgenic Mice

N. Labella, L. Field

We have generated transgenic mice that carry fusions between the putative transcriptional regulatory sequences of the atrial natriuretic factor (ANF) gene and those encoding the SV40 large T antigen. ANF is a peptide hormone that is intimately involved in the maintenance of blood pressure and electrolyte levels. The hormone is made and stored in the atria of the heart and is secreted in response to elevated blood-pressure levels. Once in the circulation, ANF modulates blood volume through both acute and chronic mechanisms. The goals of these experiments were threefold. First, we wanted to identify the ANF promoter sequences in an in vivo system. The second goal was to assess the capacity of the cardiac atria to generate tumors and to determine the pathological consequences of any tumors that may arise. Finally, if any atrial tumors were obtained, we would attempt to establish cell lines that retain ANF expression.

The fusion gene carried 500 bp of 5′-flanking ANF sequence and was constructed such that the ANF promoter would drive synthesis of T antigen. Eight independent transgenic mice have been generated that carry this construct, four of which successfully established lineages. Immunohistochemical analyses were performed on animals from each of the lineages to assess the specificity of transgene expression in cardiac tissue. T-antigen expression was observed in both left and right atrial muscle cells, with virtually no expression in ventricle tissue. This pattern of expression is identical to that observed for the endogenous ANF gene and is seen in all four transgenic lines. Western blot tissue surveys have shown that oncogene expression is specific for the cardiac atria. Thus, these experiments demonstrate that 500 bp of 5′-flanking sequence are sufficient to direct tissue-specific expression of the ANF gene in vivo.

Experiments were performed to assess the pathological consequences of atrial T-antigen expression. Fusion gene expression is observed in the first week of life. However, at this stage, no pathology is discernible by histological analyses. At 20 weeks of age, asymmetrical atrial hyperplasia is apparent; the right atrium has increased in mass and the left atrium remains relatively normal. At 25 weeks of age, the asymmetry is gross; the right atrium is five- to tenfold larger than the left atrium. Interestingly, light and electron microscopy analyses indicate that nuclear abnormalities are apparent in both left and right atria at this stage of pathology. At 30 weeks of age, the right atrium has grown to three to five times the size of the normal heart, which corresponds to a 50- to 100-fold increase in size compared to nontransgenic atria. Remarkably, the hyperplasia is restricted to the right atrium: Even animals with grossly enlarged right atria have relatively normal-sized left atria. Thus far, this pathology has been observed in three of the four established lines.

The asymmetrical nature of the atrial hyperplasia is intriguing. Both atria express T antigen at similar levels (although oncoprotein dose is slightly higher on the right side). Moreover, similar nuclear abnormalities are present in both atria, indicating that the oncoprotein is biologically active on both sides. However, only the right atrium exhibits a hyperplastic response. Logic dictates that this unilateral pathology is a consequence of (1) differential expression and/or activity of a requisite progression factor(s) in left versus right atria, (2) differences in the physiological cues or signals presented to left versus right atria, or (3) a combination of points 1 and 2. We are currently performing experiments aimed at distinguishing between these possibilities. Given the asymmetrical nature of the pathology, it would seem that these animals provide a unique model system in which to assess the steps required for a cell to commit to the hyperplastic state.

We have also started physiological analysis of the ANF-Tag mice to determine the consequences of right atrial hyperplasia on the blood pressure and electrolyte regulatory cascade, as well as the effect on cardiac function per se. Initial studies suggest that mice that carry the fusion gene show somewhat higher levels of urine production, as well as elevated

levels of circulating ANF. This observation is interesting as both protein and mRNA analyses indicate that ANF expression is greatly attenuated in the hyperplastic right atrium. Animals with gross hyperplasia exhibit normal blood-pressure levels, in the vicinity of 85–95 mm Hg systolic.

Electrocardiograms (ECGs) were performed in an effort to develop a noninvasive means with which to monitor the progression of atrial hyperplasia. This experiment was based on the assumption that the depolarization waves observed in ECG traces would increase proportionally with atrial mass. Our results indicate that ECGs can be used to monitor atrial hyperplasia in the transgenic animals. Moreover, the study has also shown that cardiac arrhythmias accompany the hyperplasia. The frequency and severity of these arrhythmias increase with atrial mass, suggesting a causative association. We are currently trying to establish the source of the arrhythmias, and we will determine if these animals can be useful for the screening of anti-arrhythmic pharmaceuticals. Finally, we have been trying to establish cell lines derived from the right hyperplastic atria. However, our initial attempts have met with little success. Given that fact that the hyperplastic material has significantly down-regulated ANF expression, it is likely that any lines derived from this material will be of limited value for the analysis of ANF gene expression.

Transgenic Models of Hypertension

M. Steinhelper, P. Weinberg, L. Field

Transgenic mice have been used to develop animal models of oncogenesis by many investigators. However, transgenic systems have not been used extensively to study other complex biological systems. We are interested in studying the molecular events associated with cardiovascular homeostasis. A potentially useful experimental tact involves generating animals that aberrantly express gene products which influence this complex regulatory system. To this end, we are producing transgenic mice that will overexpress proteins involved in blood pressure and electrolyte homeostasis. Since the transgenic lineages are being generated and maintained in inbred backgrounds, the only genetic difference between experimental and control mice will be the presence of the transgene.

Overexpression of vasoactive peptide hormones is being targeted to the peripheral circulation. The initial studies will once again utilize the ANF gene. Fusion genes have been constructed between the mouse albumin (ALB) transcriptional regulatory sequences and those encoding the mouse ANF gene. Orientation is such that transcripts which arise from the fusion gene will encode ANF. Studies performed by other investigators have shown that transgenic mice that carry ALB growth hormone fusion genes express and secrete the hormone from the liver. By analogy, expression of the ALB-ANF fusion gene should result in secretion of pro-ANF. Moreover, recent studies suggest that the ALB and ANF converting enzymes are identical; if so, then the secreted product may in fact be ANF (if not, sufficient converting enzyme exists in the circulation to process the pro-hormone). These mice should thus provide an animal system that exhibits chronicly elevated levels of ANF in the peripheral circulation. We have recently obtained our first ALB-ANF transgenic animals and will soon initiate the physiological analyses.

Many of the hormones that regulate blood pressure and electrolyte levels are found both in the peripheral circulation and in the cerebral spinal fluid (CSF). However, numerous studies suggest that these proteins do not readily cross the blood brain barrier, suggesting that synthesis must occur both peripherally and centrally. As such, it would be of interest to discriminate between the effects of central versus peripheral overexpression. Accordingly, we are constructing fusion genes between the transthyretin (TTR) promoter and the ANF structural gene. TTR is expressed at high levels in the choroid plexus and to a lesser extent in the liver and kidney. We have recently cloned the mouse TTR gene and will soon have the fusion gene completed and injected into mice. Expression of the fusion gene in the choroid plexus should result in secretion of ANF into the CSF, whereas expression in the kidney and liver will result in peripheral secretion. These animals will be compared to ALB-ANF mice which exhibit similar levels of peripheral ANF; any phenotypic differences will most likely be due to central expression. Should an interesting phenotype emerge, constructs that express exclusively in the central nervous system will be generated. A potential promoter for such constructs is that from the serotonin receptor gene.

Insulinoma Cell Lines Derived from Insulin/T Antigen Transgenic Mice

S. Efrat, D. Spector, M. Delannoy, S. Grant, D. Hanahan
[in collaboration with S. Baekkeskov, S. Linde, and H. Kofod, Hagedorn Research Laboratory, Gentofte, Denmark]

The ability to target expression of oncogenes to particular cells in transgenic mice, using cell-specific regulatory elements, offers, in principle, a method for immortalization of rare cell types. We have reported that transgenic mice harboring insulin/SV40 T antigen hybrid genes heritably develop tumors in β cells of the endocrine pancreas. Normal β cells are difficult to isolate, since they comprise only 1% of the pancreas and are dispersed in the pancreatic exocrine tissue, and they do not grow well in culture. In recent years, several cell lines of transformed β cells have been generated, such as RIN cells from X-ray-induced rat insulinoma, and HIT cells, developed by infecting hamster islets with SV40 virus. Although they have some properties of β cells, these cells show various abnormalities in expression of insulin and in regulation of insulin secretion. A more authentic β-cell line may be useful for studies on regulation of the insulin genes and on β-cell physiology, as well as for isolation of molecules that regulate insulin gene expression and those involved in the autoimmune response against β cells in diabetes.

We have established a β-cell line from an insulinoma derived from an insulin/T antigen transgenic mouse. The βTC1 (β tumor cell) line maintains the features of differentiated β cells after more than 60 passages in culture. The cells produce both proinsulin I and II and efficiently process each into mature insulin, in a manner comparable to that of normal β cells in isolated islets. Electron microscopy reveals typical β-type secretory granules in which insulin is stored, as is shown in Figure 1. The levels of insulin mRNA are much higher in βTC1 cells compared to that of the HIT and RIN cell lines.

Unlike the situation with other insulinoma cell lines, the availability of lineages of transgenic mice that heritably develop β-cell tumors allows for repeated derivation of cell lines from the original tumor material as necessary. So far, we have derived βTC lines from four different tumor-bearing mice.

These results demonstrate that the use of cell-specific control regions not only may provide unique access to dispersed, rare cells, but may as well present a selection for the maintenance of the differentiated phenotype of those cells in culture, given that the hybrid oncogene providing the proliferative signal requires the differentiated condition to be expressed.

Glucagon/T Antigen Transgenic Mice

S. Efrat, D. Hanahan [in collaboration with G. Teitelman, M. Anwar, and D. Ruggiero, Cornell University Medical School]

The preproglucagon gene is expressed in three cell types: α cells of the endocrine pancreas, located in the outer rim of the islets of Langerhans; L cells of the intestinal mucosa; and some neurons in the brain, particularly in a region of the brain stem. The preprohormone contains, in addition to glucagon, two glucagon-like peptides, GLP-I, and GLP-II. The proteolytic processing of the preprohormone gives rise in the pancreas primarily to glucagon and GLP-I, both involved in maintaining serum glucose levels and regulating carbohydrate metabolism, whereas in the intestine, the major products are glycentin, a larger form of glucagon, GLP-I and GLP-II. In the brain, the processing resembles the intestinal pattern.

The regulatory region of the rat glucagon gene was employed to direct expression of SV40 T antigen to these cell types and to compare the effects of the oncogene product on different cells in the same mouse. In the islets, this region is sufficient to direct correct onset of expression during development and cell-specific transformation of α cells. Immunohistochemical analysis of pancreas sections with antibodies against T antigen, glucagon, and GLP-I reveals specific expression of the transgene in α cells of mice from four independent lineages. T antigen can be first detected at day 10 of development, a time at which glucagon cells normally appear. In young transgenic mice, the pancreatic α cells are distributed in normal numbers around the periphery of the islets. Hyperplasia of α cells develops at about 5 months of age. This is manifested in a broad ring of cells coexpressing T antigen and glucagon, which are initially localized in the periphery of each islet, and later invade the core of the islets. By 9 months of age, a few islets (out of several hundred) have progressed into highly vascularized, solid tumors, which are composed of cells that continue to express glucagon, GLP-I, and T antigen, and contain very few of the other endocrine cells normally found in the islets.

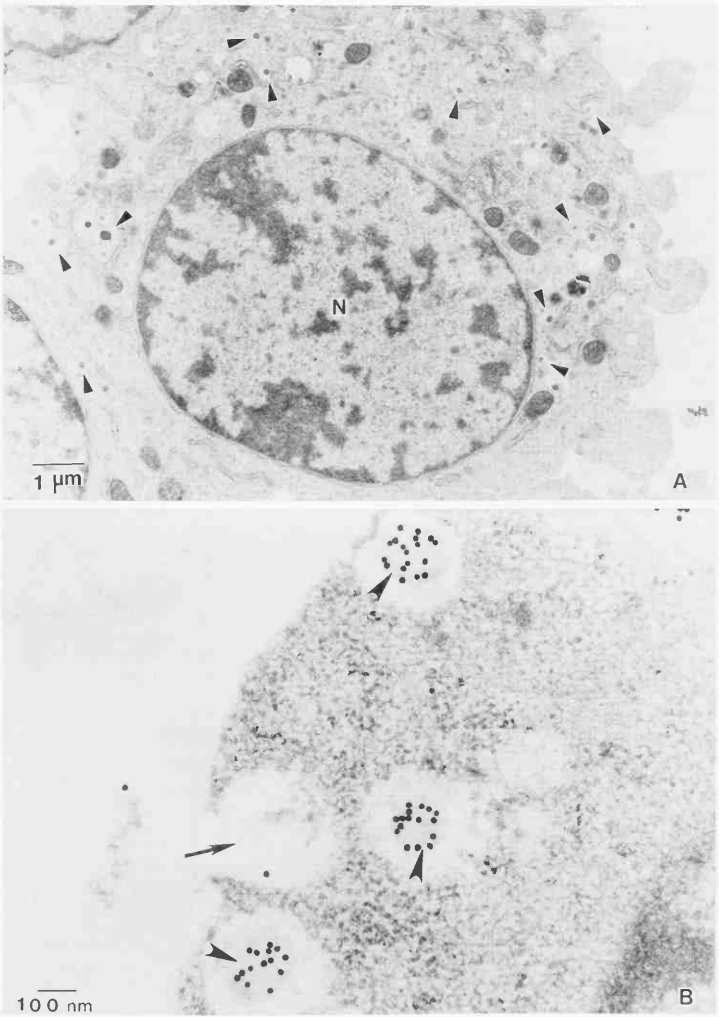

FIGURE 1 Electron microscopy analysis of βTC1 cells. (*A*) Negative staining; (N) nucleus. Arrows indicate insulin secretory granules. Magnification, 10,600×. (*B*) Immunoelectron microscopy with anti-insulin serum and protein A–gold particles. Specific signal is observed over insulin granules (arrowheads). One insulin granule appears to have secreted its content (arrow). Magnification, 80,000×.

A cell line has been derived from one of the glucagonomas, and preliminary characterization has revealed qualities consistent with its identification as immortalized α cells. The development of tumors is followed by premature death. These stages of progression are remarkably similar to those observed in insulin/T antigen transgenic mice and suggest a requirement for other events, in addition to expression of the oncogene, for the development of neoplasia.

The enteroendocrine cells of the intestinal mucosa that stain for GLP-1 do not seem to express T antigen in the transgenic mice, nor do tumors arise in the intestine. It may be that the hybrid gene lacks a regulatory element necessary for expression in the intestine, a possibility that will require additional transgenic experiments.

In contrast to the transformation of pancreatic α cells, expression of T antigen in the central nervous system does not induce any abnormality. T antigen is first detected in the hindbrain at day 12 of development, in proliferating cells of the germinal layer. Despite continuous expression of the oncogene through adult life, these cells follow their fate and withdraw from the cell cycle and remain postmitotic throughout life. [^{3}H]Thymidine labeling reveals that they are also not synthesizing DNA. This demonstrates that the cessation of cell division prevails over the proliferative effects of T antigen, which has been shown to transform almost all other cell types of transgenic mice in which it has been expressed.

Studies on the Insulin Gene Regulatory Region in Transgenic Mice

S. Efrat, D. Hanahan

Hybrid genes composed of the 5'-flanking region of the rat insulin II gene linked to the early region of SV40 direct expression of large T antigen to the insulin-producing β cells of the endocrine pancreas. A fragment of the insulin gene extending 450 bp upstream of the cap site, and including promoter and enhancer elements, is sufficient to elicit cell-specific expression in transgenic mice. A promoter element, located 255 bp upstream of the cap site on the opposite strand, can also direct specific expression of large T antigen to the β cells, by activating transcription in the opposite direction from the insulin-coding region. Bidirectional transcription is apparently an intrinsic quality of the insulin gene regulatory region.

Deletion analyses indicate that elements of the enhancer region, but not the insulin promoter, are necessary for reverse promoter activity in transgenic mice. Expression of T antigen in mice harboring two of these deletions, although remaining cell-specific, is markedly reduced, as determined by immunohistochemical analysis of pancreas sections with antibodies directed against large T antigen. The levels of the cellular protein p53, which is associated with large T antigen in cells transformed by SV40, are also reduced proportionally. The islets of Langerhans in adult mice harboring these constructs show hyperplasia of β cells, but no tumors have been observed in mice over 20 months of age. A possible interpretation of these results is that certain minimal levels of T antigen or p53 in the cells are necessary for the progression of some islets into solid tumors and that these mice express amounts that are below the threshold for transformation.

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GENETICS

The Genetics group at Cold Spring Harbor Laboratory a year ago experienced a large turnover where many of our colleagues moved to other reputed institutions. Although it took some doing to hire new investigators, we are very happy with the scientists who have since joined. The major exciting area is that of plant molecular genetics. Three colleagues, V. Sundaresan, S. Briggs, and T. Peterson, all specializing in understanding the behavior of movable genetic elements in corn, are on board. We have also continued our studies and made major advances in the field of cell-cycle and cell-type determination of both *Saccharomyces cerevisiae* and of *Schizosaccharomyces pombe* yeasts. An account of some of the work carried out by these subgroups follows.

YEAST GENETICS

A.J.S. Klar R. Cafferkey V. Sharma
M. Kelly J. Wood

During the past year, we have continued our studies on defining the molecular basis of cell-lineage determination and the DNA sequences required for switching of both *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Both yeasts switch their mating types in a nonrandom fashion. In *S. cerevisiae*, the older "mother" cell produces a pair of switched cells (designated **a** and α), whereas in *S. pombe*, only one in four grandchildren of a cell is switched between minus (M) and plus (P) types (Fig. 1). In last year's Annual Report, we presented evidence suggesting that the one-in-four rule of *S. pombe* is a consequence of DNA-strand-specific imprinting at the *mat1* locus of the grandparent cell. The main argument presented was that cells containing inverted duplications of the *mat1* locus cleave each cassette at one or the other cassette, but never at both. DNA cleavage is required for the recombination event. From this analysis, it is predicted that in strains containing inverted tandem duplication, one member of each pair of granddaughter cells should switch, i.e., two in four granddaughters should switch. We wanted to test this genetic prediction by phenotypically assaying the cell type in cell-lineage studies. Unfortunately, this was impossible because if we test the mating type of a cell by mating to another cell of known mating type, we essentially lose that cell to zygote formation. Thus, it is not possible to determine the phenotype of a cell in consecutive generations.

Diploid Pedigrees Allow Test of Both *mat1* Loci

A.J.S. Klar, J. Wood

Diploid strains that contain both *mat1-P*- and *mat1-M*-expressed cassettes are competent to undergo meiosis and sporulation. Thus, we can test the *mat1* contents of a cell by assaying for its capability to sporulate. An exciting result is that a diploid strain containing the constitution *mat1/h*$^{-L}$ allows us to test the pattern of switching of the single chromosome containing the *mat1* locus. The *h*$^{-L}$ allele is a fusion between *mat1* and *mat3*; it provides stable expressed *M* information and it does not switch. By following the ability of such cells to sporulate in a cell-lineage study, we find that the *mat1* allele can switch from *mat1-M* to *mat1-P* again in one in four cells (Fig. 2a). In addition, once a cell has become competent to switch, it can produce one switched cell and the other cell, which can again switch con-

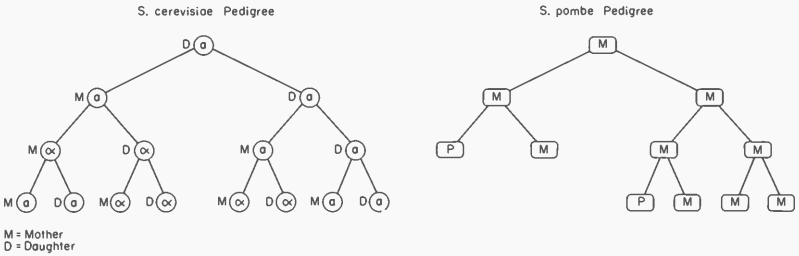

FIGURE 1 Comparison of the patterns of switching of budding and fission yeasts.

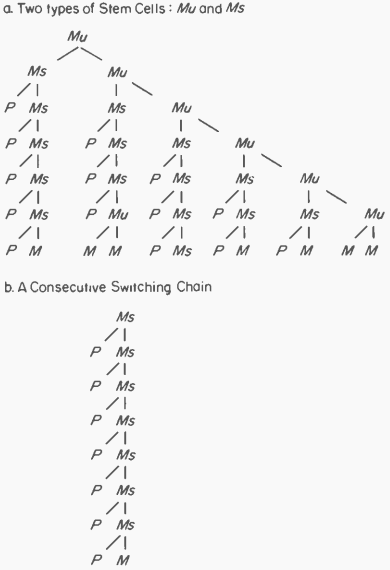

FIGURE 2 Pattern and efficiency of switching of *mat1* in *mat1/h*$^{-L}$ diploid cells in a cell lineage. The switch from *mat1-M* to *mat1-P* results in a cell that can sporulate (*P*). *Mu* is a cell that is unswitchable and produces a switchable (*Ms*) cell and an unswitchable (*Mu*) cell. *Ms* mostly produces a switched cell and another *Ms* cell.

tinuously in subsequent generations (Fig. 2b). As many as eight consecutive switches were found. Therefore, this procedure of assaying the contents of the *mat1* allele by assaying sporulation of the cells will be useful for testing strains with *mat1* duplication.

Sequence Determination of *swi3* and *swi1* Genes and a *mat1 cis*-Acting Mutation

R. Cafferkey, V. Sharma, A.J.S. Klar

We have previously defined the roles of the *swi1*, *swi3*, and *swi7* genes, which are required for efficient switching of *mat1*. Mutants in these genes are defective in switching because they are defective in catalyzing a break at the *mat1* locus. Clearly, these gene functions are required for the above-mentioned imprinting event and/or to form components of the hypothesized endonuclease that cleaves the imprinted substrate in vivo. To understand the details of this process, we have molecularly isolated the *swi3* gene. We are almost finished with the sequence determination of this gene, and we have subcloned the *swi1* gene for sequence determination. Knowing the sequence will allow us to make constructs for preparing large quantities of the coded protein for functional analysis.

Efficient *mat1* switching can also be reduced by mutations present at the *mat1* locus that act in a *cis*

manner to reduce the level of double-strand breaks at *mat1*. We have sequenced one such mutation (a deletion of 27 bases) that deletes 7 bp of the distal homology box H1 and a *mat1* distal sequence (Fig. 3). Interestingly, the deletion affects the ability of the chromosome to be cleaved at a site 50 bp away from the deletion.

Protection of Cut Ends In Vivo

A.J.S. Klar, R. Cafferkey, J. Wood

We found that 20–25% of the DNA is broken when it is isolated from cells from any phase of the cell cycle. These broken ends persist throughout the cell cycle and are presumably healed in the next cell cycle, resulting in a switch. We wondered whether the broken ends are protected from degradation in vivo and subsequently found that both 5′ ends generated are protected from λ-exonuclease digestion even in vitro (Fig. 4). One of the 3′ ends is protected and the other is sensitive to 3′-end-specific exonuclease III digestion. Even more interesting is the result that the exonuclease-III-sensitive strand has an exonuclease digestion block about 150–200 bases away from the 3′ end. Whether the block constitutes the imprinted event is the major question to address in the near future.

The Mother-Daughter Mating-type Switching Asymmetry of Budding Yeast Is Not Conferred by the Segregation of Parental *HO* Gene DNA Strands

A.J.S. Klar

The fundamental issue to address in developmental biology is to determine why a given cell division generates two developmentally unequivalent daughter cells. As shown in Figure 1 in *S. cerevisiae*, only the mother cell switches, whereas the newly born bud cell never does. It is known that the mother cell expresses the *HO* gene (which encodes the site-specific endonuclease) required for initiating recombination, whereas in the daughter cell, it is transcriptionally unexpressed. In light of the discussion on *S. pombe*, we may imagine that the *HO* gene is expressed in a mother cell because the DNA strand containing the transcriptionally competent gene is segregated

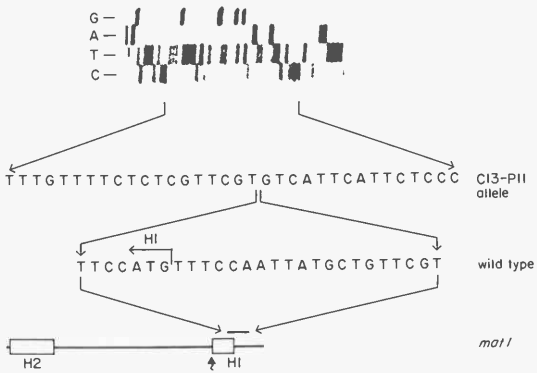

FIGURE 3 C13-P11 is a 27-bp deletion mutation, ~50 bp away from the cut site.

to the mother cell; the incompetent chromatid is segregated to the daughter cell. A critical test of the strand-segregation model is that the daughter cells should switch in those strains containing an inverted *HO* gene, whereas the mother cells should not. We find that in such a strain, only mothers switch. Therefore, this simple test rules out the strand-segregation model for *HO* gene expression.

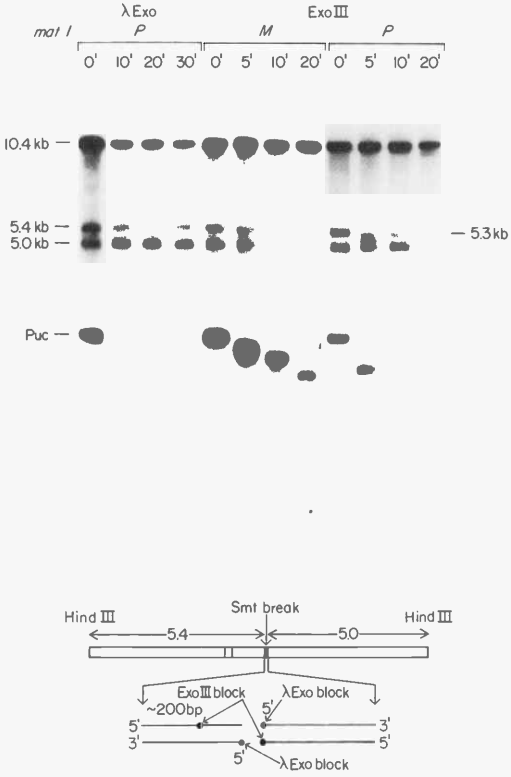

FIGURE 4 Protection of double-stranded DNA cut ends at *mat1* in vitro to digestion with λ-exonuclease and exonuclease III. The total yeast DNA is treated with the indicated enzyme for varying lengths of time. Then, after the nucleolytic activity is removed, the DNA is digested with *Hin*dIII, run on agarose gels, transferred to a nitrocellulose filter, and probed with *mat1* sequences. The 10.4-kb fragment results from an intact *mat1*-containing fragment, whereas the 5.4-kb and 5.0-kb fragments result from a break at the *mat1* locus. *P* and *M* indicate whether the DNA is derived from the *mat1-P* or *mat1-M* strain. Puc indicates *Hin*dIII-cut pUC118 plasmid fragment added in each reaction. The interpretation of the result is diagramed at the bottom.

Isolates of *Schizosaccharomyces kompucha*

A.J.S. Klar, J. Wood

kompucha is regarded as a "divine fungus" of heavenly origin and has been used since its introduction by the Chinese emperors of the Tsin dynasty in the 3rd century B.C. It is used to make a fermented drink called "Tshe" from sugar and tea, which is claimed to provide a remedy against aging, improved health and strength, and revitalization of the whole body. Mr. Joseph Licata, a curious neighbor of Cold Spring Harbor Laboratory, brought this culture to our attention when he inquired about its contents. We noted that it is composed primarily of one kind of rod-shaped bacteria and primarily one kind of fungus. The fungus is fission yeast!

Among ten isolates tested, six are homothallic, since they switch mating type, and the rest seem to be heterothallic. At least one of the latter class is capable of mating with the laboratory strains of *S. pombe*. Comparison of the *Hin*dIII digestion pattern of mDNA of the laboratory stock with that of the *kompucha* yeast shows that four fragments are of identical size and the other four differ (Fig. 5). The similarity clearly shows that the new yeast is related to *S. pombe*. The restriction site polymorphism has also been found at *mat1* and *mat2* but not at *mat3*. The polymorphism should be useful for future studies should this yeast successfully cross with the laboratory stocks. Nevertheless, the existence of fission yeast in the culture suggests that the wonderful health-related effects of the Tshe drink could possibly be ascribed to alcohol.

FIGURE 5 Comparison of the ethidium-bromide-staining pattern of *Hin*dIII-digested *S. kompucha* yeast DNA (*a–e* lanes) with that of *S. pombe* (lane *f*). The strong-staining bands are of mitochondrial DNA. Mitochondrial genes exist in about 100 copies per genome and thus can be easily visualized by staining.

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CELL-CYCLE CONTROL IN YEAST AND VERTEBRATES

D. Beach G. Draetta, L. Brizuela, M. Stein, M. McLeod, L. Molz, S. Dembski, R. Booher

Our research has continued to focus on the mechanism of regulation of the eukaryotic cell cycle. As in previous years, we have made use of the fission yeast for genetic investigations, but we have now considerably expanded our work on the molecular basis of cell-cycle control in mammalian cells. We have

also initiated one entirely new research project, in collaboration with the laboratory of Dr. John Newport (University of California, San Diego), to test the possibility that elements of the mitotic control pathway in *Schizosaccharomyces pombe* might be the so-called maturation promoting factor identified in *Xenopus*. During the past year, we have been joined by two visiting scientists, Dr. Jeremy Hyams (University College, London) and Dr. Paul Young (Queens University, Ontario).

Cell Cycle of Fission Yeast

G. Draetta, R. Booher, L. Brizuela, L. Molz, D. Beach

The *cdc2*$^+$ gene product plays a unique role in the regulation of the cell cycle of *Schizosaccharomyces pombe*. This protein kinase is required for traverse of both the G_1/S and the G_2/M boundaries. Our major objective is to identify those genes and their protein products that regulate *cdc2*$^+$ and that act as the critical substrates of the protein kinase in G_1 and G_2.

During the past year, we have been able to identify a protein, the product of the *suc1*$^+$ gene, that forms a complex with the *cdc2*$^+$ gene product. *suc1*$^+$ was initially identified as a plasmid-borne gene that could rescue certain temperature-sensitive alleles of *cdc2*. p13^{suc1} was expressed in *Escherichia coli*, and antibodies raised against the protein purified from this source recognized the yeast protein. We were able to show that p13^{suc1} is not a substrate of the *cdc2*$^+$ protein kinase either in vitro or in vivo, but nevertheless forms a stable complex with p34^{cdc2}. This complex has *cdc2*$^+$-dependent protein kinase activity that is lost as cells enter the stationary phase under adverse nutritional conditions. At present, the precise role of p13^{suc1} is unclear, but it is known to be essential for cell-cycle progression and may act to allow the *cdc2*$^+$ protein kinase to interact with its substrates.

Since *cdc2*$^+$ is required for the initiation of both DNA replication and mitosis, there is considerable interest in establishing whether the protein kinase phosphorylates an identical set of substrates in G_1 and G_2 or whether its G_1 and G_2 roles can be differentiated. At present, no physiologically relevant substrates of the *cdc2*$^+$ protein kinase have been biochemically identified. A genetic analysis of revertants of temperature-sensitive *cdc2* mutants has given us the first indication that the G_1 and G_2 roles of *cdc2*$^+$ can be discriminated. A cold-sensitive allele of *cdc2*$^+$ was rescued by a compensating mutation in *cdc13*$^+$, a gene that been had previously thought not to interact with *cdc2*$^+$. This result is of particular interest because *cdc13*$^+$ is required for the initiation of mitosis. *cdc13* mutants arrest with condensed chromosomes analogous to the metaphase state of higher eukaryotic cells. A variety of genetic evidence leads us to believe that the *cdc13*$^+$ gene product is a G_2-specific substrate of the *cdc2*$^+$ protein kinase. We have recently isolated the *cdc13*$^+$ gene, expressed its 55-kD product in *E. coli*, and are currently testing by direct biochemical experiment whether this protein is indeed a substrate of *cdc2*$^+$.

In addition to *suc1*$^+$ and *cdc13*$^+$, we have identified other genes that interact with *cdc2*$^+$ in either G_1 or G_2. Use has been made of the observation that a certain dominant allele of *cdc2* (*cdc2-3w*) is lethal in combination with a loss-of-function *wee1* mutation. This phenomenon is not well understood but has been termed mitotic catastrophe. We have isolated mutants that suppress the mitotic catastrophe lethality. They define six new genes (*m*itotic catastrophe *s*uppressor, *mcs* 1 through 6). Among these, two appear to interact very closely with *cdc2*$^+$ and might encode substrates. The others appear to interact more closely with the *wee1*$^+$ protein kinase. These possibilities will be further elaborated by isolation of the relevant *mcs* genes.

Mammalian Cell-cycle Control

G. Draetta, L. Brizuela, D. Beach

During the course of our studies with the *cdc2*$^+$ gene product, we prepared both poly- and monospecific antibodies against the protein. Two monoclonal antibodies were obtained that recognized not only p34^{cdc2}, but also p36^{CDC28} of *Saccharomyces cerevisiae*. These antibodies were also able to immunoprecipitate a 34-kD protein from HeLa cells. Peptide mapping revealed a remarkable degree of evolutionary conservation between the yeast and human proteins. Human p34 was also shown to be complexed with a homolog of p13^{suc1}. We have continued to study mammalian p34. The tissue distribution of the protein in rat organs was first determined. In general, p34 was more abundant in tissues that contain a sub-

stantial fraction of proliferating cells, with the notable exception of the brain. Brain tissue has high levels of p34. This leads us to suppose that mammalian p34 plays some role in nonproliferative cells.

One organ that contains very low levels of p34 is the kidney. In collaboration with E. Moran (Tumor Viruses Section), we tested whether kidney cells induced to divide by infection with adenovirus expressed higher levels of p34 and found that this was indeed the case. Adenovirus infection induced rapid synthesis of p34, and the protein accumulated at a higher level than in uninfected cells.

As a more direct test of the role of p34 in the cell cycle, we have tested whether the activity of the protein kinase varies during the cell cycle. We found that p34 phosphorylates a protein of 62 kD that is coimmunoprecipitated with p34. In HeLa cells synchronized at various cell-cycle phases by elutriation centrifugation, p62 phosphorylation was barely detectable in G_1 but rose dramatically during G_2/M. p34 also phosphorylated an artificial substrate (casein) much more actively in G_2 than in G_1. We further found that p34 itself was more highly phosphorylated at this stage of the cell cycle (Fig. 1). These observations are provocative and suggest that the human p34 protein kinase may play a role in the regulation of mitosis.

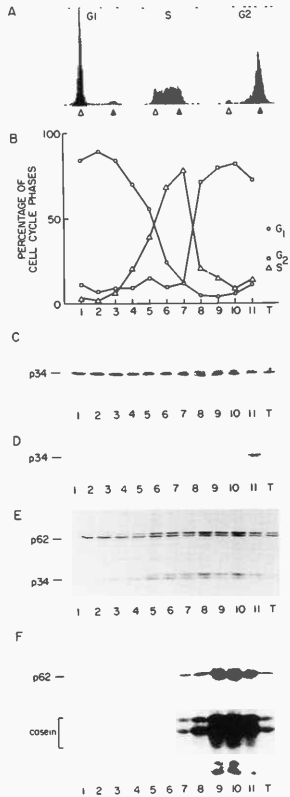

FIGURE 1 Cell-cycle regulation of p34 protein kinase. (*A*) Flow-cytometric profiles of HeLa cells separated by elutriation; (*B*) percentage of cells in G_1 (circles), S phase (triangles), and G_2 (squares) in each elutriated fraction; (*C*) immunoblot of each elutriation fraction probed with anti-p34 serum; (*D*) incorporation of $^{32}PO_4$ into p34; (*E*) association between p34 and p62 during the cell cycle; (*F*) protein kinase activity of p34 with respect to p62 and casein substrates during the cell cycle.

Relationship between p34 and MPF

L. Brizuela, D. Beach [in collaboration with W. Dunphy and J. Newport, University of California, San Diego]

For many years, it has been known that the cytoplasm of fertilized *Xenopus* eggs is able to induce meiotic maturation upon injection in oocytes. The activity responsible for this effect has been termed maturation-promoting activity (MPF). It is now known to be a mitosis-inducing factor. Many properties of MPF are known, but it has proved very difficult to purify the factor. We were interested in determining whether p34 might be a component of MPF.

Since $p34^{cdc2}$ is complexed with $p13^{suc1}$ in yeast and the same complex can be found human cells, we assumed the same situation would prevail in *Xenopus*. Since native p13 protein had previously been purified from *E. coli*, we tested whether this polypeptide might interfere with MPF activity. We found that microinjection of p13 into oocytes, in conjunction with MPF, abolished the activity of the latter. This effect was also apparent in an in vitro assay of MPF. Furthermore, it was discovered that a p13 affinity column totally retained MPF activity and that p34 was one of the components of the initial cellular extract that bound to the column. These ex-

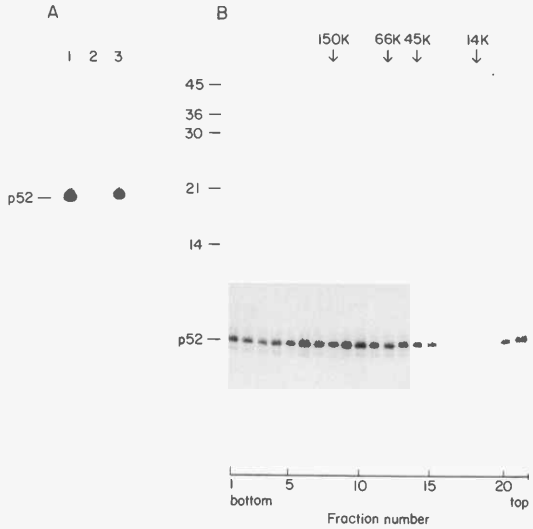

FIGURE 2 Inhibition of p52^{ran1} by p21^{mei3}. (*A*) Lysate of fission yeast was immunoprecipitated with anti-p52 serum. Prior to addition of ATP to the kinase reaction, either no material (lane *1*), 0.5 μg of bacterial lysate containing p21^{mei3} (lane *2*), or 0.5 μg of bacterial lysate without p21^{mei3} was added to the immunocomplexes. (*B*) Sedimentation of purified p21^{mei3} analyzed by SDS-PAGE (*top*) and p52^{ran1} protein kinase inhibitory activity (*bottom*) through a glycerol gradient. The numbers marked by arrows indicate the position of molecular-weight markers in the gradient.

periments do not prove that p34 is MPF, but they indicate that the p34-p13 complex is probably at least a component of the activity. These studies will be pursued further, particularly making use of substrates of p34 that are formally identified in *Schizosaccharomyces pombe*.

Meiotic Control in Fission Yeast

M. McLeod, D. Beach

For the last 5 years, we have been using fission yeast as a model system in which to investigate the molecular basis of the transition from mitosis to meiosis. The last year has seen considerable progress with the first biochemical studies of the *ran1*$^{+}$ protein kinase.

In *S. pombe*, meiosis is ultimately regulated by the genes of the mating-type locus. Only h^{+}/h^{-} diploids that express all four mating-type genes are meiotically competent. The primary meiotic role of the *mat* genes is to induce transcription of *mei3*$^{+}$. Expression of this gene alone in a vegetative cell is sufficient to cause the full transition from mitotic division to meiosis. We have proposed previously that the role of the *mei3*$^{+}$ gene product might be to inhibit *ran1*$^{+}$. The *ran1*$^{+}$ gene acts as a critical negative regulator of meiosis. Temperature-sensitive *ran1* mutants undergo haploid meiosis and sporulation at the restrictive temperature.

We have isolated both the *ran1*$^{+}$ and *mei3*$^{+}$ genes and have raised antibodies against their protein products. p52^{ran1} was predicted by homology to be a protein kinase and this turns out to be the case. p52^{ran1} displayed autophosphorylation activity in vitro.

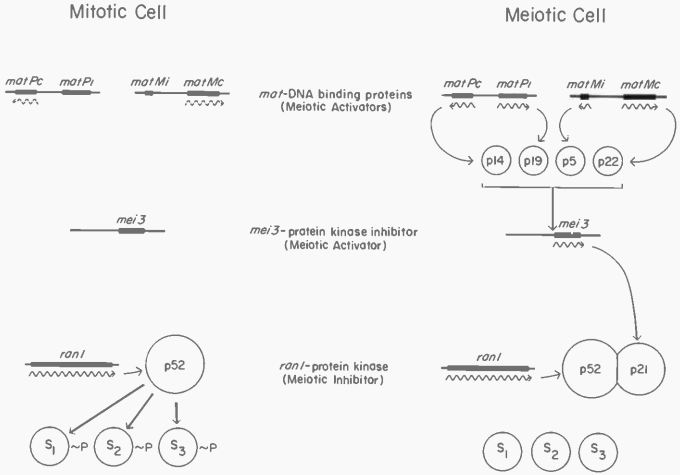

FIGURE 3 Model of meiotic control in fission yeast. (*Mitotic cell*) During vegetative growth of either haploid or diploid cells, the *mat-Pc* and *Mc* genes are weakly expressed, but *Pi* and *Mi* are not. Consequently, *mei3*$^+$ is not expressed. *ran1*$^+$ is expressed constitutively and encodes a 52-kD protein kinase. This is presumed to phosphorylate (heavy arrows) hypothetical substrates, S1, S2, and S3, which promote vegetative growth and inhibit meiosis. (*Meiotic cell*) In an h^+/h^- diploid, under nitrogen starvation conditions, each *mat* gene is induced and is presumed to be translated into proteins of the indicated molecular weights. The *mat* gene products induce transcription of *mei3*$^+$. The 21-kD *mei3*$^+$ gene product binds to the p52^{ran1} protein kinase and inactivates it. This leads to dephosphorylation of S1, S2, and S3 and thus to meiotic initiation.

In vegetative cells, p21^{mei3} is not expressed, but in meiotic cells, this protein is expressed and was found to be complexed with *ran1*$^+$ protein kinase. The protein kinase was strongly inhibited in the complex, suggesting that the role of p21^{mei3} might indeed be to inactivate *ran1*$^+$. This was tested further by purifying p21^{mei3} from *E. coli* and adding this material to a *ran1*$^+$ protein kinase assay. We were able to show that p21^{mei3} is a direct inhibitor of the *ran1*$^+$ protein kinase (Fig. 2). These results led to the following model for meiotic initiation (summarized in Fig. 3). In an h^+/h^- diploid cell, nutritional starvation causes transcriptional induction of each of the four genes. These in turn induce transcription of the *mei3*$^+$ gene. The product of *mei3*$^+$ acts to inhibit directly the activity of the *ran1*$^+$ protein kinase by direct protein-protein binding. The major objective of future studies will be to identify substrates of the *ran1*$^+$ protein kinase and thus to understand how it acts as the critical negative regulator of meiosis.

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PRE-mRNA SPLICING IN *SCHIZOSACCHAROMYCES POMBE*

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

This past year, we began our investigation of the mechanism of splicing of messenger RNA precursors (pre-mRNAs) in the fission yeast *Schizosaccharomyces pombe*. In eukaryotes, many, if not most, of the protein-coding genes are interrupted by intervening sequences (introns). The removal of these introns from the primary transcripts by the process of splicing is therefore essential for the expression of a large portion of the nuclear genome. This also applies to the simple eukaryotes such as yeast.

The splicing of mRNA precursors occurs in the nucleus by the concerted action of a number of small nuclear ribonucleoprotein particles (snRNPs) and other proteins that associate with the pre-mRNA in a large complex known as the spliceosome. The nucleic acid components of the snRNPs constitute a family of distinctly sized small RNAs. The sizes of these snRNAs in *S. pombe* are very similar to those in the higher eukaryotes. This is not the case, for example, in the budding yeast *Saccharomyces cerevisiae*. As more genes are cloned and sequenced in *S. pombe*, it is beginning to appear that the majority of the protein-coding genes possess introns. *S. pombe* shows no bias for the types of genes that contain introns or the position of the introns within the genes, and several examples of genes interrupted by multiple introns are known. This pattern is similar to that observed in higher eukaryotic genomes. In *S. cerevisiae*, on the other hand, introns are found primarily in the genes for the ribosomal proteins and are located near the extreme 5′ ends of the pre-mRNAs. Only one example of multiple introns has been described. These observations, as well as the genetically well-characterized simple genome of *S. pombe*, led us to choose this yeast as our experimental system for the study of pre-mRNA splicing.

Isolation of Pre-mRNA Splicing Mutants from *S. Pombe*

J. Potashkin, R. Li, D. Frendewey

One of the goals of our research is to identify genes that are involved in pre-mRNA splicing. Historically, this has been accomplished by isolating and characterizing mutants that are defective in pre-mRNA splicing. Our approach has been similar. First, we have produced a collection of mutants that grow poorly at an elevated temperature. We have then screened these temperature-sensitive (*ts*) mutants for defects in pre-mRNA splicing.

To create the initial *ts* bank, we exposed a wild-type strain of *S. pombe* to the chemical mutagens ethylmethanesulfonate and nitrosoguanidine. Survivors of the mutagenesis were grown at the permissive temperature (23°C) and then replica-plated at 23°C and the nonpermissive temperature (37°C). Those colonies that grew very poorly or not at all at 37°C were picked from the 23°C plate. After confirmation of the *ts* phenotype, the mutants were included in our *ts* bank. By using this procedure, we

have collected 350 mutants that exhibit growth deficiencies at 37°C.

We have worked out a method that allows us to screen the *ts* bank for mutants defective in pre-mRNA splicing by a Northern blot assay. The procedure consists of preparing whole-cell RNA from the *ts* mutants after a shift from growth at 23°C to 37°C, fractionating the RNA on formaldehyde agarose gels, blotting the RNA onto nylon membranes, and probing the blots with an oligodeoxynucleotide (oligo) that is complementary to a particular intron. The rationale for the screen is based on the assumption that a pre-mRNA splicing mutant will accumulate precursors and that these can be detected by the intron-specific probe. Thus, a positive signal on the Northern blot is indicative of a splicing defect. For the screen to work optimally, two conditions must be met: (1) an mRNA must be chosen that is sufficiently abundant to be detectable and (2) the steady-state level of pre-mRNA in a wild-type culture at 37°C must be too low to be detectable.

As our target RNA, we chose the mRNA for the *S. pombe* β-tubulin. This transcript is relatively abundant, and the pre-mRNA has five small introns whose combined length is approximately 300 nucleotides. Since introns in *S. pombe* are rather small (usually <100 nucleotides), the difference between the sizes of the pre-mRNA and mature mRNA for a single-intron pre-mRNA would be difficult to resolve on an agarose gel. The 300-nucleotide difference for β-tubulin, however, is large enough to detect. Although the ability to resolve the pre-mRNA and mRNA is not essential to the screen, it confirms our interpretation of the results. We designed two oligo probes. One is complementary to a 30-nucleotide stretch of intron 4 of β-tubulin and is specific for the pre-mRNA. The other is complementary to 15 nucleotides of exon 4 and 15 nucleotides of exon 5 that are contiguous and span the splice junction between these two exons in the mRNA. This exon oligo is specific for spliced mRNA under sufficiently stringent hybridization conditions. In preliminary experiments, we used the exon oligo to demonstrate that we could detect β-tubulin mRNA in total RNA from wild-type cells. The intron oligo gave no hybridization signal on the same RNA. Thus, the two conditions stated above, which are necessary for the screen to work, were satisfied.

The initial screen of the first 100 *ts* mutants with the intron oligo probe identified three mutants that accumulated pre-mRNA at the nonpermissive temperature: *ts32*, *ts46*, and *ts50*. These three were subsequently confirmed by repeated blotting and probing. In addition, the same blots were stripped of the intron oligo and then reprobed with the exon oligo. This probe produced a positive signal in all the *ts* mutants except *ts32*, *ts46*, and *ts50*, indicating that the accumulation of pre-mRNA in the three was due to an essentially complete block in splicing. Repeated analysis has indicated that *ts46* is slightly leaky; a small amount of mRNA is detected at 37°C.

To investigate the temperature sensitivity of the pre-mRNA splicing defects, RNA was prepared from these strains grown at the permissive temperature and compared to RNA from cells that had been shifted to 37°C for 1 and 2 hours. Use of both the exon and intron probes revealed that normal levels of mRNA were produced at 23°C and no pre-mRNA was detected, whereas the loss of the mRNA signal was essentially complete by 1 hour at 37°C and was accompanied by an accumulation of pre-mRNA. The kinetics of pre-mRNA accumulation differed slightly among the three splicing mutants. The results of the Northern analysis reflect directly on the splicing of only intron 4 of β-tubulin. However, the size of the pre-mRNA that is present at 37°C appears to be identical in all three splicing mutants, and the difference between this size and that of the mRNA seen at 23°C suggests that the splicing of all five introns of β-tubulin is blocked in *ts32*, *ts46*, and *ts50*.

To further characterize the molecular phenotype of the splicing mutants, we showed that the pre-mRNA produced by all three mutants at 37°C could be selected on an oligo(dT)-cellulose column. This result indicates that the block in splicing in the mutants is not associated with or due to a lack of polyadenylation. Thus, in *S. pombe*, as in mammals, polyadenylation can occur on an unspliced pre-mRNA. We do not know from this experiment if polyadenylation has occurred at the correct site. A similar analysis with a probe for the alcohol dehydrogenase mRNA showed that neither the size of the mRNA nor its ability to be selected on oligo(dT)-cellulose was affected by a growth shift from 23°C to 37°C in *ts32*, *ts46*, and *ts50*. To test whether the splicing defect was specific for pre-mRNA, we probed RNA from *ts32*, *ts46*, and *ts50* grown at 37°C with an oligo specific for the intron of a *S. pombe* pre-tRNA^{Ser} (a gift from I. Willis, Yale). An accumulation of tRNA precursors was not evident in the pre-mRNA splicing mutants. Finally, since U1 and U2 RNAs are required for pre-mRNA splicing, we checked the mutants for decreased levels of these snRNAs using probes derived from human se-

quences but which hybridize to the *S. pombe* U1 and U2 RNA homologs. The U1 and U2 RNA concentrations appeared to be normal in all three mutants at the nonpermissive temperature.

In all of our molecular analyses, we have studied the block in pre-mRNA splicing by following one intron in the β-tubulin pre-mRNA. An important question that remained unresolved concerned the generality of the splicing defect. Although it is highly unlikely that all three splicing mutants are specific for β-tubulin, we had not eliminated the possibility that one was due to a mutation that affects the splicing of only the β-tubulin pre-mRNA or only intron 4 of β-tubulin. To address this point, we have assayed the splicing of another pre-mRNA, that for α1-tubulin, by an RNase protection procedure. These experiments confirmed the Northern blot results. The α1-tubulin pre-mRNA accumulated at the nonpermissive temperature in all three splicing mutants, and the patterns of inhibition were identical to those observed for β-tubulin.

To summarize the molecular analyses, *ts32*, *ts46*, and *ts50* possess temperature-sensitive lesions that lead to a block in splicing at 37°C. The defects are specific for the splicing of mRNA precursors and are not caused by a loss of polyadenylation or reduced levels of U1 or U2 RNA.

The three pre-mRNA splicing mutants have also been characterized genetically. Each mutant was backcrossed to a wild-type strain at least two times. The resultant diploids were sporulated, and tetrad analysis showed that all three mutants exhibited 2:2 segregation of both the *ts* growth and the splicing-defect phenotypes. Therefore, for each mutant, the *ts* phenotype almost certainly results from a lesion in a single gene, and this gene is required for pre-mRNA splicing. Diploid progeny from crosses with the wild-type strain were not temperature-sensitive for growth at 37°C and did not exhibit accumulation of pre-mRNA, demonstrating that the mutations are recessive. The mutants were also crossed with each other. The result of these crosses was that each of the mutants could complement the others for the *ts* growth phenotype. Thus, *ts32*, *ts46*, and *ts50* fall into three separate complementation groups. We have designated the three pre-mRNA splicing mutants *rna 1*, *rna 2*, and *rna 3*.

Further characterization of the pre-mRNA splicing mutants is in progress. The ultimate genetic analysis of the mutants is to clone and sequence the affected genes. We have already crossed the mutants into a genetic background that will allow us to transform them with an *S. pombe* genomic library in an attempt to clone the *rna* genes by functional complementation of the *ts* phenotype.

A Possible U2 RNA Mutant

J. Potashkin, A. Serrano, R. Li, D. Frendewey

As mentioned in the previous section, part of the analysis of the pre-mRNA splicing mutants consisted of determining if they were defective in the synthesis of U2 RNA. We did this by probing the blots containing RNAs from the first 70 temperature-sensitive mutants with an oligo complementary to U2 RNA. Nearly all of the RNAs, including the three splicing mutants *ts32*, *ts46*, and *ts50*, appeared to have normal levels of U2 RNA. The U2 signal for one of the mutants, *ts45*, was quantitatively and qualitatively different. The level of U2 RNA in *ts45* appeared to be reduced compared to the other samples, and a new RNA of approximately 400 nucleotides in length was detected. U2 RNA is 180 nucleotides in *S. pombe*. To confirm that the U2 RNA pattern exhibited by *ts45* was abnormal, we fractionated equal amounts of the same *ts45* RNA and RNA from wild-type cells grown at 30°C (the normal growth temperature) on a polyacrylamide gel. These RNAs were then blotted and probed with the U2-specific oligo. Again, the amount of U2 RNA in *ts45* was reduced compared to that of the wild type, and the 400-nucleotide signal was seen only in the *ts45* sample.

The RNA used for the initial analyses was prepared from the *ts45* after a 2-hour shift to 37°C. To investigate the temperature dependence of the molecular phenotype in *ts45*, we prepared RNA from the mutant grown to mid-log stage at 23°C and after a shift to 37°C for various times out to 7 hours. After polyacrylamide gel electrophoresis and Northern blotting, the RNAs were probed with the U2 RNA oligo. This analysis showed that the U2 RNA levels at 23°C and 37°C were identical and remained unchanged out to 7 hours after shift-up to the nonpermissive temperature. On the other hand, the 400-nucleotide RNA was present only at 37°C, increasing up to 3 hours and then leveling off. In addition, a new RNA of approximately 110 nucleotides was seen whose signal was faint at 23°C and increased steadily during the 37°C incubation. A parallel experiment was performed with wild-type cells. In this

case, neither the 400- nor the 110-nucleotide RNA was observed regardless of the temperature. Comparison of the two experiments indicated a two- to fivefold lower level of mature-size U2 RNA in *ts45* versus the wild type. Subsequent analysis of the same RNAs showed that U1 RNA and mRNA levels were normal in *ts45* even after extended incubation at 37°C, and there was no accumulation of pre-mRNA.

At this point, the molecular phenotype of *ts45* can be described as follows. The mutant exhibits a slight decrease in the steady-state levels of U2 RNA that is independent of a shift in growth temperature from 23°C to 37°C. This decrease in U2 RNA is insufficient to affect pre-mRNA splicing. At 37°C, two new RNAs appear that are related to U2 RNA in that they hybridize to a U2-specific probe. These RNAs are approximately 400 and 110 nucleotides in length. We are currently trying to determine the nature of these RNAs. We have also initiated a genetic analysis of *ts45* to determine whether a single mutation is responsible for the molecular phenotype and whether the U2 RNA defect segregates with the weak *ts* growth phenotype exhibited by *ts45*.

Biochemical Characterization of *S. Pombe* Pre-mRNA Splicing Components

C. Leptac, A. Serrano, D. Frendewey [in collaboration with A. Krainer, Cold Spring Harbor Laboratory]

Another aspect of our work has been an attempt to characterize pre-mRNA splicing in *S. pombe* biochemically. The direct approach to this problem is, of course, to study splicing in vitro. However, after trying several different types of extracts and a variety of pre-mRNA substrates and conditions, we have not been successful in obtaining in vitro splicing in extracts derived from *S. pombe*. We will continue to devote considerable efforts along these lines because of the significant value of an in vitro system. In the meantime, we have taken an alternative indirect approach by investigating the relatedness between the *S. pombe* and the well-characterized HeLa cell pre-mRNA splicing systems. We tested the ability of *S. pombe* introns to be spliced by a HeLa cell in-vitro-splicing extract (a gift from A. Krainer). Eight introns from four different *S. pombe* genes were tested. None of the introns spliced correctly in the HeLa extract. Only intron 5 of the β-tubulin gene spliced at all, but aberrantly. Its 5′ splice site was used, but a cryptic 3′ splice site and branchpoint downstream from the *S. pombe* sites were chosen.

The splicing of the *S. pombe* β-tubulin substrate in the HeLa extract suggested an experiment. Could we force the HeLa system to choose the *S. pombe* 3′ splice site by supplying it with *S. pombe* components and thereby identify an *S. pombe* factor involved in 3′ splice-site selection? The β-tubulin pre-mRNA is inefficiently spliced in the HeLa extract. When reduced amounts of extract are used for the splicing reaction, no splicing of the β-tubulin substrate is detected. Under these conditions, if the HeLa extract is reconstituted with a *S. pombe* whole-cell extract, splicing of the β-tubulin pre-mRNA is restored. The *S. pombe* extract does not splice the β-tubulin pre-mRNA on its own. The restored splicing in the mixing experiment appears to be the same as that in the complete HeLa reaction; i.e., the aberrant 3′ splice site is chosen. Although this experiment failed to identify an *S. pombe* 3′ splice-site selection factor, it suggests that such a reconstitution assay may prove useful for investigating components involved in pre-mRNA splicing in *S. pombe*.

We have also compared the *S. pombe* snRNPs with their human counterparts. In collaboration with A. Krainer, we have enriched the *S. pombe* snRNPs from a whole-cell extract by immunoaffinity chromatography with an anti-M_3G monoclonal antibody. This antibody recognizes the trimethylated guanosine "caps" on the 5′ ends of the snRNAs. The purified *S. pombe* snRNPs contain four or five small RNAs that are similar in size to the human snRNAs. Analysis of the proteins in the purified snRNPs by SDS-PAGE and silver staining revealed a simple pattern of 12–15 polypeptides. Only one of these was identical in size to a human snRNP protein, that being the D protein found in all human snRNPs. The anti-M_3G antibody should be very helpful in the further characterization of the *S. pombe* snRNAs and snRNPs.

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PLANT GENETICS

V. Sundaresan B.A. Lowe R. Morfeld P.S. Chomet
T. Peterson B. Elliott M. Papazain J. Sorrentino
S. Briggs S. Ismail S. Allan

Molecular Genetics of Robertson's Mutator

R. Morfeld, M. Papazian, J. Sorrentino, V. Sundaresan

Robertson's mutator is a transposable element system in maize identified by D. Robertson in 1978 (*Mutat. Res., 51:* 21 [1978]). *Mutator* (*Mu*) lines of maize generate new recessive mutations at high frequencies; these mutations are caused by insertional inactivation of genes by transposable elements known as *Mu* transposons. The *Mu* transposons consist of three types of elements, *Mu1*, *Mu1.7*, and *Mu3*; they are characterized by common 200-bp-terminal inverted repeats flanking a variable internal sequence. We have been studying the mechanism and regulation of transposition of the *Mu* transposable elements.

MECHANISM OF *MU* TRANSPOSITION

To understand the mechanism of *Mu* transposition, we have been studying DNA molecules that might correspond to intermediates or products of transposition. An extrachromosomal form of the *Mu* transposons has been identified (Sundaresan and Freeling, *Proc. Natl. Acad. Sci. 84:* 4924 [1987]). This is a circular supercoiled DNA molecule that can be isolated from *Mu* plants and that corresponds to a circularized form of the transposon that is free from the host DNA. The extrachromosomal *Mu* is not abundant; it is present at less than 1 copy/cell and its presence correlates with the timing of *Mu* activity in the plant. We have cloned some of the extrachromosomal circular *Mu* elements in order to characterize them in detail. In the process, we have found that some of the clones (called *MuC4*, *MuC6*, *MuC7*, and *MuC8*) are new additions to the *Mu* transposon family. They have the same terminal inverted repeats as *Mu1*, but with internal sequences unrelated to *Mu1* and to each other. *MuC4*, *MuC7*, and *MuC8* appear to be homologous to elements identified in the genome by their *Mu* termini, but not previously shown to be transposable (V. Chandler, University of Oregon). It is likely that all elements of the *Mu* transposon family generate extrachromosomal circles (Fig. 1). The role of circles in *Mu* transposition (e.g., whether they are real or abortive transposition/replication intermediates) is yet to be elucidated.

THE PHENOMENON OF *MU* INACTIVATION

Spontaneous inactivation of *Mu* lines can occur during propagation through either outcrossing or selfing. These inactive derivatives of *Mu* lines carry *Mu* elements, but the elements no longer transpose, and furthermore, the circular *Mu* disappears. This inactive state of the *Mu* elements has been found to be correlated with methylation of these elements at sites within the inverted repeats (Chandler and Walbot, *Proc. Natl. Acad. Sci. 83:* 1767 [1986]; Bennetzen, *Mol. Gen. Genet. 208:* 45 [1987]). We have asked the question, Can a "dead" line be revived? We derived inactive lines by following the instability of a *Mu* insertion in the *Bronze1* (*Bz1*) gene. In an active line, this insertion produces spotted bronze kernels,

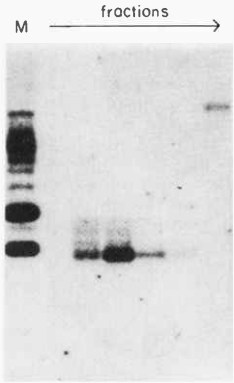

FIGURE 1 Autoradiogram of uncut DNA fractions from a Robertson *Mu* line fractionated on a CsCl/EthBr gradient showing at least four circular DNA molecules including *Mu1* hybridizing to a complete *Mu1* probe. Arrow indicates concentrations of CsCl from high to low, with linear fractions on the right. (M) Closed circular DNA size markers.

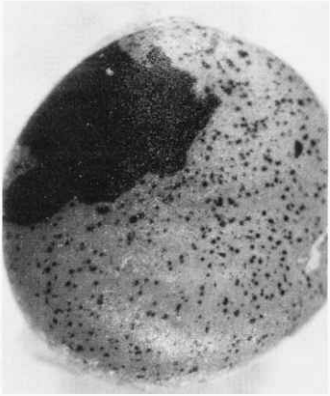

FIGURE 2 A kernel from an ear of *bz1-Mum9-inactive* crossed to *bz1*; *Mu*-active pollen showing a large purple sector reflecting an early reactivation event.

whereas in an inactive derivative, the kernels are stable bronze and the *Mu* elements are methylated. Inactive lines were obtained by outcrossing active lines to normal maize and identifying stable bronze kernels (ON × NORMAL→ON + few OFF). When these inactive lines are crossed to normal maize lines, the progeny are stable bronze, showing that, in general, a dead line remains dead, i.e., spontaneous reactivation does not occur (OFF × NORMAL→ OFF). However, if crossed to an active *Mu* line (with a standard recessive *bronze* allele), we find complete restoration of the spotted bronze phenotype (Fig. 2); the restoration is the same regardless of which parent is the female (OFF × ON→ON). Furthermore, the methylation of the *Mu* elements is reversed, i.e., all of the *Mu* elements in the progeny are unmethylated (Fig. 3). This shows that the changes that occur during inactivation are reversible and that the *Mu* elements in an OFF line retain their ability to transpose. B. McClintock has suggested that the genomes of higher eukaryotes carry many elements (including transposons) capable of reorganizing the genome that are normally silent or unexpressed, but that these can be activated or triggered by stress or shock to the organism (McClintock, *Stadler Symp. 10:* 25 [1978]). Because the inactive *Mu* elements retain their ability to transpose, they may be capable of spontaneous reactivation in response to stress. We have begun experiments to test this possibility.

USE OF *MU* IN GENE TAGGING

Another area of interest is the use of *Mu* to study genes important in plant growth and development by gene tagging. The rate of new mutants generated by *Mu* lines is exceptionally high, and 5–15% of the progeny in a *Mu* outcross carry new recessive mutations. We have therefore been using *Mu* to generate recessive mutations in different genes, with the idea of studying them at the molecular level by cloning the gene using a *Mu* transposon probe. In theory, this can be done by identifying a *Mu*-hybridizing band on a Southern blot that cosegregates with the mutant phenotype. In practice, because of the large number (20 or more copies) and the high transposition rates of the *Mu* elements, this is a difficult proposition. To get around this problem, we are utilizing a system in which the *Mu* insertion at *Bz1* is used to obtain inactive progeny from the mutant plants by identifying stable bronze kernels. The inactive progeny have a stable number of *Mu* elements; these are outcrossed to follow the segregation of *Mu*

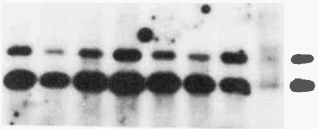

FIGURE 3 Autoradiogram of a Southern blot of *Hin*fI digests of DNA isolated from progeny and parents of a cross of an inactive *Mu* female to an active *Mu* male. *Hin*fI cuts *Mu1* and *Mu1.7* at the termini to generate 1.4-kb and 1.7-kb fragments, respectively; if these sites are modified, larger fragments are seen. (Lanes *1–7*) DNA from the progeny; (lane F) DNA from the female parent (inactive *Mu* with modified *Mu* elements); (lane M) DNA from the male parent (active *Mu* with no modified *Mu* elements). Lane F was deliberately underloaded to contain 25% as much DNA as the other lanes to show that higher-molecular-weight bands can be detected under our conditions with one-fourth the DNA than in lanes *1–7*.

elements with the mutant phenotype while reducing the *Mu* copy number.

One set of genes that we are applying this technique to are the *dwarf* genes. These genes are involved in the synthesis and response to gibberellin (GA_1), a plant growth regulator. The *dwarf* mutants are characterized by greatly shortened internodes, broad dark-green wrinkled leaves, and anthers in the ear; the mature *dwarf* plant is one-fourth to one-eighth the height of a normal plant. We have a *Mu* insertion in the *d1* gene; this gene regulates the amount of GA_1 in the plant by encoding the enzyme that synthesizes GA_1 from GA_{20}, the biologically inactive precursor. We have obtained stable derivatives of this mutant using the *Bz1* marker, and we are now in a position to begin the segregation analysis. Other unmapped *dwarf* mutants have been generated in our *Mu* stocks, and we have begun their characterization. There is evidence from restriction-fragment-length polymorphism analysis that the variation in height in maize lines might be due to differences in the alleles at one of the *dwarf* loci (Helentjaris, *Trends Genet. 3:* 217 [1987]). Thus, study of the *dwarf* genes might also help us to understand the basis of quantitative trait variation in field crops.

A Change in the State of the *Ac*-induced *P-VV* Allele Is Associated with Inversion of *Ac*

T. Peterson, S. Allan [in collaboration with W.J. Peacock and E.S. Dennis, CSIRO-Division of Plant Industry, Canberra, Australia]

The maize *P* locus controls pigmentation of certain floral tissues, including the pericarp and glumes of the cob. Since pericarp and cob pigmentation can differ, *P* alleles are commonly given a two-letter designation indicating the expression in each tissue. Thus, the *P-RR*, *P-RW*, *P-WR*, and *P-WW* alleles specify red pericarp/red cob, red pericarp/white cob, white pericarp/red cob, and white pericarp/white cob, respectively. The *P-VV* allele, which gives variegated pericarp and cob, comprises the transposable element *Ac* situated at the *P* locus. We have previously isolated a DNA clone from *P-VV*, and we are now using *P* DNA sequences to study the structure and expression of *P*. The long-term objective is to determine how the *P* gene is expressed in specific floral tissues during development of the maize plant.

I have isolated an allele termed *P-OVOV* (orange variegated pericarp and cob) derived from *P-VV*. The *P-OVOV* allele was recovered from a sector of 40 kernels, with orange variegated pericarp on an ear with otherwise ordinary variegated pericarp, from a plant of *P-VV/P-WR* genotype. *P-VV* specifies colorless pericarp with red sectors, whereas *P-OVOV* specifies orange pericarp with many dark-red sectors and some colorless sectors (Fig. 4). Thus, *P-OVOV* represents a change in state of the *P-VV* allele.

Two hypotheses have been proposed to explain the molecular basis for changes in state. The composition hypothesis attributes changes in state to changes within the transposable element, such as internal deletions or DNA modification. The position hypothesis proposes that the element is unchanged but that it has moved to a new position within the locus. The *P-OVOV* allele was studied to determine the basis of this change in state.

The genetic properties of the *P-OVOV* allele were investigated in progeny of the cross *P-OVOV/P-WR* × *P-WW*. For each progeny plant, ear phenotype was scored by inspection, and the presence or absence of *Ac* was determined in tests crosses. The results are shown in Table 1. Three points can be drawn from these data. First, the number of *P-WR* ears (126) is approximately equal to the combined number of *P-OVOV*, *P-RR*, and variant ears (122).

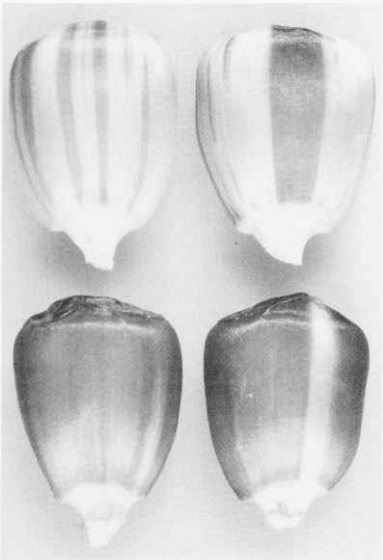

FIGURE 4 Pericarp phenotypes specified by the progenitor *P-VV* allele (*top*) and the new *P-OVOV* allele (*bottom*).

This shows that *P-OVOV* segregates as an allele of *P-WR*. The *P-OVOV* phenotype does not depend on unlinked modifying factors. Second, *Ac* activity is tightly linked to *P-OVOV*; 109 plants producing *P-OVOV* ears carried *Ac*. There were no examples of plants producing *P-OVOV* ears that did not carry *Ac*. Third, the *P-OVOV* allele is itself unstable, as evidenced by the *P-RR* and "other" ears among the progeny. These variants are presumed to arise from mutation of *P-OVOV* because the *P-WR* allele used in this cross is stable.

Has the *Ac* element at the *P* locus undergone a compositional change in the mutation of *P-VV* to *P-OVOV*? This possibility was tested by comparing the abilities of the *Ac* elements associated with the *P-VV* and *P-OVOV* alleles to *trans*-activate a *Ds* element. No differences in the *Ds* response were seen, suggesting that the *trans*-acting functions of *Ac* are unchanged.

Southern analysis of DNA from the progenitor

TABLE 1 Segregation of *Ac* Activity with the *P-OVOV* Allele

Cross: *P-OVOV/P-WR* × *P-WW*

	Ear phenotype			
	WR	*OVOV*	*RR*	other
Ac +	2	109	5	2
Ac −	124	0	5	1
Total	126		122	

P-VV allele and the *P-OVOV* allele shows that the *Ac* element associated with *P-OVOV* has remained within a 850-bp genomic DNA fragment at the *P* locus. Interestingly, the *Ac* element is in an inverted orientation in *P-OVOV* compared to *P-VV*. These results suggest that the change in state of *P-VV* to *P-OVOV* is due to inversion of *Ac*.

I propose the following explanation for the origin and behavior of the *P-OVOV* allele (Fig. 5). The progenitor *P-VV* allele comprises the *Ac* element inserted in a particular orientation at the *P* locus. The *P-OVOV* allele may have arisen by an event in which *Ac* has inverted, or flipped, from its orientation in *P-VV* to the opposite orientation. Inversion might have occurred by (1) short-range transposition and reinsertion in an inverted orientation, (2) a specialized inversion mechanism of *Ac*, or (3) inversion mediated by sequences just outside *Ac*. In the inverted orientation, the *Ac* insertion may partially suppress *P* expression, resulting in a lowered level of *P* function, less pigment formation, and thereby an orange pericarp color. *Ac* can excise from *P-OVOV* to restore a *P-RR* gene, producing the red sectors on *P-OVOV* kernels and *P-RR* germinal revertants. The colorless sectors may result from other mutagenic *Ac* activities, such as imprecise excision, formation of deletions, and transposition of *Ac* to a new site within the *P* locus, where it may partially or completely suppress *P* expression. I have already isolated a number of new alleles derived from *P-OVOV* that specify novel patterns of *P* gene expression. Preliminary results show that some of these new alleles have structural alterations at the *P* locus consistent with local transpositions of *Ac*. We are currently using the *P-OVOV* allele to mutagenize the *P* locus with *Ac* insertions efficiently, and we expect to correlate the sites of *Ac* insertion with the mutant phenotypes to produce a fine-structure map of the *P* gene.

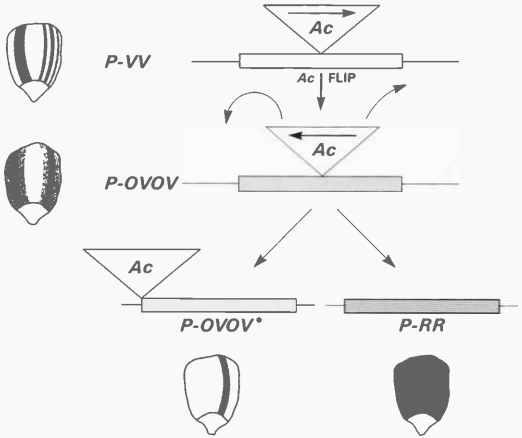

FIGURE 5 Model for the origin and behavior of the *P-OVOV* allele (see text for details).

Function of the *Hm1* Gene in Disease Resistance

S. Briggs

Resistance to the maize pathogen *Cochliobolus carbonum* race 1 maps to two nuclear genes, *Hm1* and *Hm2*. *Hm1* conditions immunity, even in the heterozygote, but *Hm2* can provide only intermediate resistance. Certain alleles at *Hm1* can also confer intermediate resistance, in which case the action of *Hm1* and *Hm2* is additive. A correspondence between disease resistance and 100-fold decreased sensitivity to a *C. carbonum* race 1 metabolite (HC toxin; Fig. 6) provides circumstantial evidence that *Hm1* is pleiomorphic, controlling both resistance to the fungus and lack of sensitivity to the fungal metabolite. Alternatively, two linked genes could be determining these traits. Direct evidence for *Hm1* control of both traits was obtained by testing susceptible mutants from a transposon-mutagenesis screen for sensitivity to HC toxin. In all cases examined, the loss of resistance to the fungus was accompanied by an acquired sensitivity to HC toxin. These results suggest that the function of the *Hm1* gene is to block the action of HC toxin.

Suppression of Disease Resistance by HC Toxin

S. Briggs, B. Elliott [in collaboration with J. Duvick, Pioneer Hi-Bred International]

C. (*Helminthosporium*) *carbonum*, a filamentous ascomycete, exists in nature as three races defined by virulence and host range. Race 1 is the most virulent and only infects genotypes of maize that are recessive at *Hm1*, race 2 is avirulent on maize, and race 3 is moderately virulent, and the interaction with maize is under polygenic control. *C. carbonum* is interfertile with *Cochliobolus victoriae*, a pathogen of oats. Progeny from a cross between race 1 and *C. victoriae* show segregation for two unlinked genes, of which one permits pathogenicity on maize and the other permits pathogenicity on oats. Strictly correlated with pathogenicity are the production of a toxin that only affects susceptible maize (HC toxin) and the production of a toxin that only affects susceptible oats (HV toxin). These results indicate that both host range and pathogenicity of *C. carbonum* race 1 (and of *C. victoriae*) are a consequence of host-selective toxin production.

The mechanism by which HC toxin permits in-

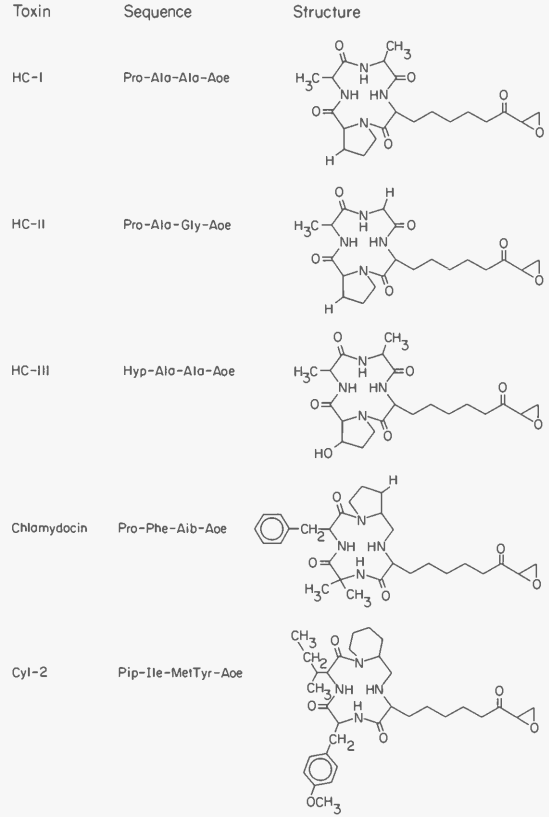

FIGURE 6 HC toxin and related toxins.

gress of *C. carbonum* is unknown. Studies of toxin responses show that, in contrast to most fungal toxins, HC toxin action is slow to manifest and is first seen as a stimulated uptake of selected solutes 2–4 hours after exposure, followed several hours later by leakage of electrolytes and cell death. Histological studies show that penetration of susceptible cells precedes cell death, suggesting that the toxin is not simply killing cells in advance of saprophytic invasion. At low doses, HC toxin actually stimulates growth up to 25%. This effect is seen in other species as well; both sorghum and tomato grow faster at low doses of HC toxin but are killed at high doses. Cultured mammalian cancer cells are even more sensitive to HC toxin than is susceptible maize; the ED_{50} for growth is 0.01 μM and 1.0 μM for mastocytoma cells and maize roots, respectively. The epoxide moiety of HC toxin is required for biological activity, but certain amino acids can be substituted at other positions in the molecule.

To describe further the action of HC toxin, we wish to compare two-dimensional gel profiles of [^{35}S]methionine-labeled proteins from tissue cultures of resistant and susceptible plants. Conditions for labeling, extracting, and electrophoresing maize proteins have been determined. The acidic nature of the proteins required the use of a pH 4–8 isoelectric focusing range for optimal separation (Fig. 7). The effects of HC toxin are now being studied.

Molecular Cloning of *Hm1*

S. Briggs, S. Ismail

Thirty recessive mutations at *Hm1* were recovered from a cross between Mutator *Hm1 Hm1* × *hm1 hm1*. The susceptible mutants were crossed with testers. Progeny from these crosses were screened with restriction-fragment-length polymorphism (RFLP) probes that are linked to *Hm1* to distinguish those that carry the mutant allele from those that received the standard recessive allele. Progeny that carry the mutant allele were examined to determine the copy number of *Mu1*-homologous fragments. One plant was selected that appears to have 17 separate inserts. A genomic library was constructed in Stratagene's λ-DASH vector. Approximately 1.3 × 10^6 plaques were screened, and 63 *Mu*-homologous clones were recovered. The clones are being screened by using the T7 and T3 promoters in the arms of λ-DASH to generate hybridization probes. Two screening strategies are being used. The probes are hybridized to genomic DNA of isogenic lines that differ only near the *Hm1* locus (they are derived from backcross siblings); each DNA is cut with several en-

FIGURE 7 [^{35}S]Methionine-labeled proteins from maize cell cultures (pH 4–8).

zymes to identify RFLPs. Polymorphisms should only be detected when using probes from clones that come from the *Hm1* region. The second strategy is to hybridize the probes to genomic blots of DNA from recombinant inbred families. This provides the chromosomal map location for each clone. The expected pattern for the *Hm1* gene has not been detected in the clones that have been screened.

Transposon Mutagenesis of the *Rp1* Gene

S. Briggs [in collaboration with D. Wilkinson and R. Christiansen, Pioneer Hi-Bred International, and S. Dellaporta, Yale University]

Resistance to the obligate maize rust pathogen, *Puccinia sorghi*, maps to several nuclear loci. The *Rp1* locus is known to have at least 14 different alleles, each encoding resistance to a different subset of rust races. Interest in the *Rp* genes stems from their diversity, genetic instability, and agronomic value. There is no information regarding the nature of the *Rp* gene products nor the means by which they act.

The *Rp1-d* allele was recombined under homozygous conditions with the heterozygous *r-nj:m1* allele. The *r-nj:m1* allele contains an active *Ac* element at the *R* locus, on the same chromosome as *Rp1*. This line was crossed with a homozygous *rp* tester that carried resistance to pathogens other than rust. Kernals in which a germinal transposition of *Ac* had occurred were screened for mutations at *Rp1-d*. Seventy-eight susceptible mutants were recovered. Tests are under way to determine whether any of the mutations were caused by insertion of *Ac* at *Rp1*.

Cloning Genes by Random Transposon-Mutagenesis

S. Briggs, P.S. Chomet, B.A. Lowe [in collaboration with S. Dellaporta and J. Chen, Yale University]

Maize kernal genetics were used to select progeny in which the *Ac* element had transposed and remained active (hence, clonable) at a new site. Ears from the cross *P-VV/P-WR r-g* × *P-WR r-m3* were examined for revertant kernels or ear sectors in which *Ac* had excised from the *P* locus. These kernels or sectors were recognized by a uniform red pigmentation in the pericarp caused by restoration of the wild-type *P-RR* allele from the *Ac*-containing *P-VV* allele. Revertant kernals were also examined for spots in the aleurone to detect the presence of a transposed *Ac*. Since reversion occurs in only one homolog, segregation at meiosis will cause the transposed *Ac* to be transmitted to only half of the red kernals. Furthermore, in approximately one third of all cases, the revertant kernals or sectors receive no transposed *Ac* due to an alternate pattern of transposition. Therefore, selection based solely upon reversion of *P* will give *Ac*-containing kernals only one sixth of the time. In our case, transposed *Ac* was detected by the somatic reversion of *r-m3* (a *Ds*-containing allele) to *R-sc*, causing spots (revertant sectors) in the aleurone. *Ac*-containing mutant alleles are relatively easy to clone because they are hypomethylated in comparison to the many other *Ac*-homologous genomic sequences. Methylation-sensitive enzymes cut only the desired restriction fragment, vastly enriching genomic libraries for the clone of interest. Families from selfs of the selected progeny were screened for segregating mutations. Several new mutations were observed, with the *Ac* element linked to the mutant locus. Cloning of the active *Ac* element from these mutants has provided probes for genes involved in floral determination and chloroplast biogenesis. This appears to be a general and efficient method for cloning maize genes that are associated with a mutant phenotype.

Map Positions of Cryptic *Ac* Elements

S. Briggs [in collaboration with S. Dellaporta and J. Chen, Yale University]

Southern blot analysis of all maize lines tested revealed the presence of several *Ac*-homologous sequences, whether or not an active *Ac* element was present. These cryptic elements have no known function but may be inactive or mutant elements. To gain further insight into their distribution in the genome, the cryptic elements from two different lines were mapped by segregation analysis in recombinant inbreds. Although the study is not yet complete, it is clear that some of the cryptic elements in one line are polymorphic alleles of cryptic elements in the other line, suggesting an extensive period of quiescence. Elements appear to be clustered at certain chromosome locations. There was no evidence that the cryptic elements transpose in the presence of an active *Ac*.

Functional Analysis of Inhibitor-Diffuse

B.A. Lowe [in collaboration with Irwin Greenblatt, University of Connecticut, Storrs]

Inhibitor-diffuse (*Idf*) is a dominant inhibitor of pigment biosynthesis in maize. Genetic analysis has mapped *Idf* to the long arm of chromosome 4. Allelism tests with the *C2* gene have shown no recombinants between *C2* and *Idf* in a population of 20,000 kernels, indicating that *Idf* is allelic or closely linked to *C2*. *C2* is one of many genes required in the dominant form for anthocyanin production in the plant body as well as in the seed pericarp and aleurone. *C2* encodes chalcone synthase, the first flavonoid-specific enzyme in the pathway. When homozygous, *Idf* inhibits pigment formation even though all dominant color genes are present (Fig. 8A). *Idf/C2* heterozygotes have pale color in the aleurone but have no detectable pigment in the plant or pericarp (Fig. 8B). In contrast, a *C2* homozygote has full color (Fig. 8C). The dominant inhibitory effect of *Idf* is most pronounced in the diploid pericarp and plant tissues, where no pigment is detectable with a single dose of *Idf*. In contrast, a single dose of *Idf* in the aleurone allows some pigment production. Because the aleurone is a triploid tissue, two *C2* alleles may be able to overcome the inhibitory effect of *Idf*. Only one other dominant inhibitor of anthocyanin synthesis has been reported. *I* or *C-I* is an allele of the *C* locus. Inhibition is thought to result from the overproduction of a competing homologous RNA product rather than by suppression of mRNA levels. The mechanism by which *Idf* inhibits anthocyanin biosynthesis is unknown.

To test whether the inhibitory effect of *Idf* is similarly caused by an overproduction of a competing homologous product, poly(A)$^+$ RNAs from immature kernels (23 days after pollination) of *Idf* homozygotes, *C2* homozygotes, and *C2/Idf* heterozygotes were hybridized with gene probes of *C2* (Fig. 9A) and *P* (Fig. 9B). *P* is required for pigmentation in the pericarp but not the aleurone. The waxy gene probe was used to confirm that similar amounts of RNA were loaded in each lane (Fig. 9C). Two RNA species hybridizing to the *C2* probe were detected in the *C2* homozygotes. The 1.5-kb band corresponds to the spliced *C2* transcript and the larger 2.8-kb

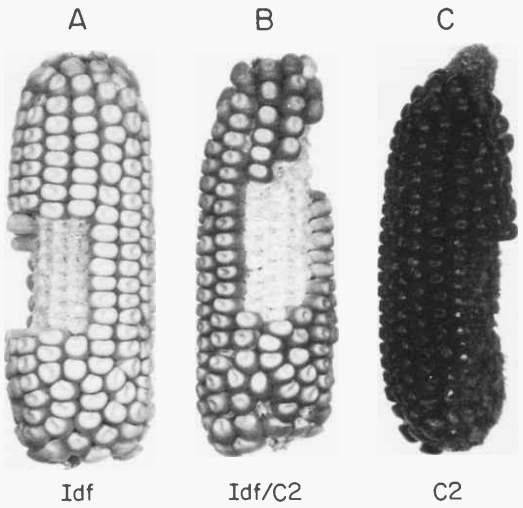

FIGURE 8 Phenotypes of *Idf* (*A*), *Idf/C2* heterozygote (*B*), and *C2* (*C*) alleles.

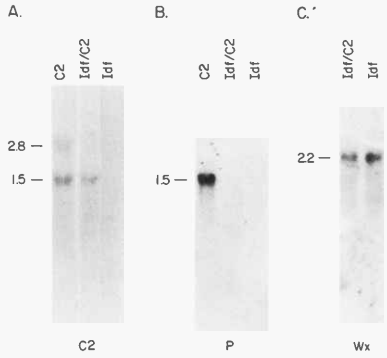

FIGURE 9 Poly(A)$^+$ RNA in kernel tissue homologous to the *C2*, *P*, and *Wx* genes. Poly(A)$^+$ (10 μg) RNA isolated from kernels homozygous for *C2* (lane *1* in *A* and *B*), heterozygous for *Idf/C2* (lane *2* in *A* and *B*, lane *1* in *C*), and homozygous for *Idf* (lane *3* in *A* and *B*, lane *2* in *C*) was fractionated on a 1% formaldehyde-agarose gel, transferred to nitrocellulose filters, and hybridized with labeled probes for the *C2* (*A*), *P* (*B*), and *Wx* (*C*) genes.

band is thought to be the unspliced form representing the 1.5-kb coding region plus the 1.3-kb intron. No RNA species were detected with the *C2* probe in the *Idf* homozygote. A faint 1.3-kb RNA was detected in the *C2/Idf* heterozygote, indicating that the *C2* allele is functioning in the heterozygote. The absence of the larger 2.8-kb RNA species may indicate that a splicing enzyme is limiting in the *C2* homozygote. The *P* probe detects a 1.5- and a 1.8-kb doublet in the *C2* homozygote, but no RNA hybridizes in the *C2/Idf* heterozygote or *Idf* homozygote. These results show that the *C2* and *P* gene probes detect the transcriptional activity that is reflected visually when the ears are examined for pigment. No pigment in the pericarp is visible in the *C2/Idf* heterozygote and no *P* transcript is detected. In contrast, some pigment is visible in the aleurone of the *C2/Idf* heterozygote and *C2* transcript is detected. These results indicate that *Idf* is somehow suppressing mRNA for both *C2* and *P*, concomitant with a suppression of the phenotypes that are determined by each gene. This is in marked contrast to the action of the *C-I* inhibitor. Experiments are in progress to determine the effect of *Idf* on the mRNA levels of other genes in the pathway, such as *A1*, *Cl*, *Bz1*, and *R*.

Attempts are being made to clone the *Idf* allele. A *C2*-specific probe hybridizes to three fragments of a *Hin*dIII digest in *Idf* and a single fragment in *C2* material (Fig. 10). The initial step is to identify which of the three fragments in *Idf* plants are associated with the *Idf* phenotype. When a *C2/Idf* plant is self-pollinated, the resulting ear shows kernels that segregate 1 full color (*C2/C2*):2 pale (*C2/Idf*):1 colorless (*Idf/Idf*). DNA was isolated from kernels of the three phenotypes and compared with the *Idf* progenitor by genomic blot analysis. Only two of the three *Hin*dIII fragments in the *Idf* progenitor are detected in the *Idf/Idf* derived by selecting for the *Idf* phenotype. Presumably, these two fragments are the minimum required to have a functional inhibitor. Genomic libraries are being screened to obtain clones of *Idf*. The molecular structure of *Idf* may explain its inhibitory nature.

Idf was first observed as a mutable allele (*Idf-m*) visible in the pericarp as colorless stripes on a colored background (Fig. 11A). The *Idf-m* phenotype may be caused by a transposon insertion into the *Idf* allele. Transposition away from *Idf* could restore *Idf* function, resulting in colorless stripes. Early mitotic sectoring on the ear is common and the meiotic transmissability of *Idf* derived from *Idf-m* depends on whether the mutation occurred in the L2 or L1 cell lineages. If the mutation occurs in the L1, it is not

FIGURE 10 DNA in kernels homologous to the *C2* gene. DNA (3 μg) isolated from kernels homozygous for *C2* (lane *1*), heterozygous for *Idf/C2* (lane *2*), homozygous for *Idf* derived from selfing *Idf/C2* (lane *3*), and homozygous for the *Idf* progenitor (lane *4*) was restricted with *Hin*dIII, fractionated on a 0.7% agarose gel, transferred to nitrocellulose, and hybridized with labeled probe for the *C2* gene.

FIGURE 11 Phenotypes of the *Idf*-mutable (*A*) and *Idf*-stable (*B*) alleles.

heritable. If it occurs in the L2, from which the germ line is derived, the mutation is heritable (Fig. 11B). Attempts are being made to determine if the mutation is mediated by a transposon. Comparison of *C2*-hybridizing DNA fragments from mutable plants and stable revertants may reveal a fragment associated with an insertion. If so, the fragment will be cloned and characterized.

The Transposable Element *Activator*

P.S. Chomet, S.L. Dellaporta

Our research efforts continue to focus on the biology and behavior of the transposable element *Activator* (*Ac*). *Ac*, the autonomous element, encodes a mutator function that can generate a *trans*-acting product (a transposase) capable of promoting transposition of itself and of *Ds* elements. The transposition of these elements is affected by the dosage of active *Ac* elements in the genome and possibly by other signals in the cell (McClintock, *Cold Spring Harbor Symp. Quant. Biol. 15:* 13 [1951]). We have investigated three distinct changes that affect *Ac*: (1) a metastable event that shifts *Ac* between active and inactive phases, (2) an aberrant *Ac* element with an altered pattern of transposition, and (3) a phenotypic change to a variegation pattern at the *waxy* locus under the control of an *Ac* element.

WX-M7: CHANGES IN-PHASE

The *Ac* element, like the *Spm* and *Dt* elements of maize, has the ability to undergo cyclical changes in its activity (McClintock, *Carnegie Inst. Wash. Yearbook 56:* 393 [1957], *Carnegie Inst. Wash. Yearbook 62:* 486 [1963]; Doerschug, *Theor. Appl. Genet. 43:* 182 [1973]). The initial studies of *Ac* "phase changes" involved the *Ac*-induced *waxy* mutation, *wx-m7* (McClintock, *Carnegie Inst. Wash. Yearbook 62:* 486 [1963]). The *waxy* locus codes for a UDPG:glucosyl transferase that is involved in the synthesis of amylose in endosperm and pollen cells (Nelson and Rines, *Biochem. Biophys. Res. Commun. 9:* 297 [1962]). When *Ac* at *wx-m7* is inactive, the *Ac* element can no longer transpose autonomously, *trans*-activate a *Ds* element, or contribute to overall *Ac* dosage effects (McClintock, *Carnegie Inst. Wash. Yearbook 63:* 592 [1964]). When *Ac* activity is regained, each of these functions is restored. The active *Ac* at the *wx-m7* allele has been cloned and sequenced (Behrens et al., *Mol. Gen. Genet. 194:* 346 [1984]; Muller-Neumann et al., *Mol. Gen. Genet. 198:* 19 [1985]) and shown to be structurally indistinguishable from other *Ac* elements at *wx-m9* and *bz-m2* (Fedoroff et al., *Cell 35:* 235 [1983]; *Proc. Natl. Acad. Sci. 81:* 3825 [1984]).

In past reports, we have shown that when the element is inactive, *Ac* DNA is methylated at all of the *Pvu*II, *Sst*II, and *Eco*RII restriction endonuclease sites (Chomet et al., *EMBO J. 6:* 295 [1987]). The *Sst*II, *Eco*RII, and one of the *Pvu*II sites are situated near the 5′ end of the transcribed region of *Ac* (Kunze et al., *EMBO J. 6:* 1555 [1987]). These sites are hypomethylated when *Ac* is active.

We have cloned the inactive *Ac* element and shown it to be structurally indistinguishable from the active *Ac7* element by detailed restriction analysis; it is also in the same orientation and position in the *waxy* gene. The methylation is specific for the *Ac* DNA, since the flanking *waxy* DNA is hypomethylated whether *Ac* is active or inactive.

Inheritance studies of *Ac* at *wx-m7* show that *Ac*

can exist in at least three heritable states: active (*Ac:a*), cycling (*Ac:cyc*), and inactive *(Ac:i*). In the active line, *Ac* at *wx-m7* is maintained as an active element through meiotic generations. In the inactive line, *Ac* is maintained as an inactive element, although *Ac:a* and *Ac:i* can change to a new state at a low frequency. The stock derived from the original *waxy* mutable kernel was an active line. From this apparently normal *Ac* allele, a stock was derived in which *Ac* underwent changes to its activity in subsequent mitotic and meiotic generations (McClintock, *Carnegie Inst. Wash. Yearbook 62:* 486 [1963]). We have termed this the *Ac:cyc* allele.

Inheritance of Ac:cyc. The *Ac:cyc* allele is defined by the ability of *Ac* to undergo changes in its activity readily during endosperm development. Inheritance of these endosperm phenotypes was tested in the next plant generation by planting kernels that phenotypically did or did not show *Ac* activity in the endosperm and reciprocally crossing to *wx/wx al-m3(Ds)* plants.

Kernels in which the Endosperm Showed *Ac* Activity. Of 23 plants grown from these kernels and crossed as a female, 22 bore ears that showed the *Ac:cyc* phenotype; i.e., the endosperm of kernels on these ears showed *Ac* active, cycling, and inactive phenotypes.

Kernels in which Endosperm Showed No *Ac* Activity. Of 57 plants grown from these kernels, 32 bore ears that exhibited the *Ac:cyc* phenotype when crossed as a female; 25 of 57 plants grown from these kernels bore ears, when crossed as a female, showing the *Ac* inactive phenotype in all the kernels (the *Ac:i* line). This phenotype is inherited through the germ line as *Ac:i*. We have tested a number of independently derived *Ac:i* lines for at least one more generation, and they continue to show the inactive phenotype on the entire ear. A second generation has been tested this summer. Three independently derived *Ac:i* lines (derived by B. McClintock and B. Burr) have been propagated in this laboratory for three generations with no evidence of *Ac* activity when backcrossed in similar genetic backgrounds.

These *Ac:i* alleles do have the ability to revert to the *Ac:cyc* allele at a low frequency. This occurred when the *Ac:i* alleles were backcrossed into a new genetic background for two generations, although it is unclear whether the new genetic background was the cause of this reversion event. Experiments are under way to answer this question.

Male Transmission of the *Ac:cyc* Allele. In every reciprocal cross of the above-mentioned crosses, there was a greater than 60% reduction in the activity of *Ac* when the *Ac:cyc* allele was transmitted through the pollen rather than the embryo sac. Most often, the *Ac:cyc* allele showed no *Ac* activity in the endosperm of kernels on these test-crossed ears.

Further tests of plants grown from these phenotypically inactive kernels showed that *Ac* can regain activity through the germ line (upon subsequent female transmission of the allele). Some ears of plants grown from kernels showing no *Ac* activity (the male-transmitted *Ac:cyc* allele) showed the *Ac:cyc* phenotype, whereas 9 out of 24 ears showed the *Ac:i* allele phenotype. These data are comparable to germ-line expression of *Ac:cyc* when transmitted through the female; i.e., male transmission of *Ac:cyc* has a drastic (reduction) effect on *Ac* activity in the immediate generation only. The mode of transmission has little or no effect on the subsequent germ-line activity of the *Ac:cyc* allele. We are currently testing whether this effect is actually due to the mode of transmission or simply the dosage of the *Ac:cyc* allele in the endosperm. If it is the mode of transmission that causes this phenomenon, then parallels can be drawn between this type of behavior and the imprinting phenomenon in maize.

Ac:i *in the Presence of an Active* Ac (bz-m2 *Allele*). To understand whether inactivity of the *Ac* element at *wx-m7* was a consequence of its position in the genome, we performed crosses to isolate germinal transpositions of the *Ac:i* and to study its behavior at new positions in the chromosome. It has been reported that the *Ac:i* can respond to the transposase of an active *Ac* (McClintock, *Carnegie Inst. Wash. Yearbook 63:* 592 [1964]). We introduced the active *Ac* at the *bz-m2* allele with the *wx-m7:i* allele.

Many kernels on these ears showed a very late (developmentally) pattern of *wx-m7:i* response to the active *Ac*, as would be expected with the two doses of the active *Ac* in the endosperm. However, in kernels showing the *bz-m2* phenotype (2 dose) and carrying the *wx-m7:i* allele, the inactive *Ac* did not always respond to the active *Ac*. This conclusion was interpreted from kernels showing the *bz-m2* spotting pattern in the aleurone and the *wx-m7* inactive pattern in underlying endosperm cells.

A reciprocal cross of the above (and the cross *sh bz wx-m7:i/sh bz wx* × *Sh bz-m2 wx/sh bz wx*) produced progeny in which the inactive *Ac* responded to one dose of the active *Ac* (*bz-m2*). Many kernels

were noted in which *wx-m7:i* responded (by expressing a clonal "excision" pattern of *Ac* at *waxy*) to *bz-m2* early in kernel development, as would be expected with one dose of an active *Ac* in the endosperm. Furthermore, the *bz-m2* spotting pattern was unaltered by changes in the *waxy* phenotype. These data suggest that inactive *Ac* at *wx-m7* is responding to the transposase of the active *Ac* at *bz-m2*, but the inactive *Ac* has not been activated, since no increase in *Ac* dosage is reflected in the *bz-m2* pattern.

Methylation of the Inactive Ac *in the Presence of an Active* Ac. Since the inactive *Ac* could respond to an active *Ac* but apparently was not activated, we asked if the modification of the *Ac:i* was still maintained in the presence of the active *Ac*'s transposase. DNA from plants of the genotype *Sh bz-m2 wx-m7:i/sh bz wx* (+ *Ac* activity) and *Sh Bz wx-m7:i/sh bz wx* (−*Ac* activity) was isolated and cleaved with *Sst*I and *Sst*II. This blot was probed with pWx0.4 (*Sal*I-*Sma*I fragment of *Wx*). No changes in the modification of the *Sst*II sites within *Ac:i* were seen whether the active *Ac* at *bz-m2* was present or absent. These data imply that the active *Ac* element does not regulate the methylation at the 5′ end of the transposase gene in the *Ac:i* element. Furthermore, it is the expression of the transposase of *Ac*, not the ability of *Ac* to transpose, that is correlated with the methylation of *Ac* DNA (at *Sst*II sites).

Ac-cyc *in the Presence of an Active* Ac (bz-m2). We have also crossed an active *Ac* into the *wx-m7:cyc* stock to isolate transpositions of the *Ac:cyc* element. In the progeny of the cross, *Sh b3-m2 wx/Sh Bx wx-m7:cyc* × *sh bz wx*, some kernels showed changes in the *bz-m2* spotting pattern, from the two-dose to a three- or four-dose *Ac* pattern. A few kernels of this type also heterozygous for the *wx-m7:cyc* allele revealed that the *Ac* at *wx-m7:cyc* could undergo cycling even in the presence of an active *Ac*. Underneath the two-dose *Ac* spotting pattern of *bz-m2* in the aleurone, the *Ac:cyc* element appeared inactive, as assayed by the "nonclonal" expression of *wx-m7* in the starchy endosperm. Underneath the higher-dose *Ac bz-m2* pattern was the excision pattern of *wx-m7*. This suggested that *Ac* at *wx-m7* cycled between the inactive and active state, as revealed by its own excision pattern at *waxy* and by its contribution to *Ac* dosage in only a sector of the endosperm.

Conclusions

1. *Ac* at *wx-m7* can exist in three heritable states: active, cycling, and inactive.
2. *Ac:a* and *Ac:i* are relatively stable alleles, whereas *Ac:cyc* readily changes to *Ac:i*.
3. The *Ac:i* allele was associated with specific methylation of the *Ac* DNA, whereas the *Ac:a* allele was hypomethylated.
4. Male transmission of the *Ac:cyc* allele drastically reduced *Ac* activity in the endosperm, whereas *Ac* activity through the germ line (in subsequent generations) was not affected. This imprinting effect is associated with the *Ac:cyc* allele only.
5. The inactive *Ac* does not always respond to the transposase of an active *Ac*, unlike *Ds* elements. This may be due to a local chromatin structure change associated with the methylation.
6. *Ac* at *wx-m7* can undergo a change in its transposase expression (change in-phase) even in the presence of an active *Ac*. This suggests that control of the shift in *Ac* activity is not a general control over the *Ac-Ds* system, but rather a local control over *Ac* at *waxy*.
7. The inactive *Ac* DNA remains methylated in the presence of an active *Ac*, again suggesting that the control of activity and methylation of *Ac* is specific for *Ac* at *waxy*.

AC-STABILIZED

An unstable allele caused by a controlling element insertion can undergo heritable changes that affect the pattern of somatic variegation. These changes then give rise to new states of the locus that differ from the parental state in the pattern of somatic mutation (McClintock, *Carnegie Inst. Wash. Yearbook 54:* 245 [1955]). New states of the *dSpm* controlling element have been characterized molecularly and shown to be rearrangements within the responding element (Schiefelbein et al., *Proc. Natl. Acad. Sci. 82:* 4783 [1985]).

A new state of the *Ac* element, termed *Ac*-stabilized (*Ac-st*), is characterized by the late and infrequent action of its transposase (B. McClintock, unpubl.). Unlike other *Ac* elements, this late response occurs in the presence of one dose of the *Ac-st* element in the genome. This element is not located within a scorable gene; therefore, its effect is monitored on *Ds*-suppressed alleles. Other heritable changes that affect the activity of the autonomous element have not been well characterized. Some in-

ternal deletions of *Ac* have been shown to destroy the transposase function of the element (Fedoroff et al., *Cell 35:* 235 [1983a]; Pohlman et al., *Cell 37:* 635 [1984]). *Ac-st* is unique in that a mutation has altered the action of the transposase without the complete loss of *Ac* activity.

The Late-acting Action of Ac-st *Is Independent of the* Ds *Allele on which It Acts.* A stock carrying I *Ds* (standard position) was crossed as a male onto stock heterozygous for *Ac-st*. Similarly, a stock carrying *bz-m2* (derivative 1) was crossed as a male to the *Ac-st* stock. Both chromosome breaks mediated by *Ds*, and transpositions of *Ds*, occurred late in endosperm development as compared to the action of a normal *Ac* (B. McClintock, unpubl.). The *r-m3* and *a1-m3 Ds* alleles also showed a developmentally late and infrequent response to one *Ac-st*, as compared to other *Ac* elements (*wx-m7:a*, *P-VV*).

Genetic Background Has No Effect on the Late Action of Ac-st. Inbred W22 *Ac-st/+*, *r/r-m3* was crossed into inbred W22, W23, Mo17, A632, and OH 43 lines for three generations. In the F_1 progeny, no change in the behavior of *Ac-st* (as assayed by *r-m3* response) was observed. This suggests that a *trans*-acting factor is not necessary for the late action of *Ac-st*.

Ac-st *Has Little or No Effect of Dosage upon Itself.* A plant of the genotype *r-m3/r, +/Ac-st* was crossed reciprocally to a homozygous *r-m3* plant. Among the progeny of the reciprocal crosses, the timing of the *r-m3* response was indistinguishable when *Ac-st* was present in one or two doses. This test of dosage contribution has been repeated this summer with plants of the genotype *Ac-st/+*, *a1-m3*. These plants were reciprocally crossed to *a1-m3* plants as well as selfed. This construction allows for the monitoring of the effect of *Ac-st* dosage, whereas the dosage of *a1-m3* does not vary.

Ac-st *Makes Little or No Contribution to the Dosage of a Normal* Ac *in the Cell.* Plants of the genotype *wx-m7:active/wx*, *al-m3* were crossed by a plant of the genotype *Ac-st/+*, *A1/al-m3*. Half of the progeny kernels carrying the *wx-m7* and *al-m3* alleles should carry the *Ac-st* allele. No variation in the variegation pattern at *waxy* and *a1* was evident among these kernels. This experiment was repeated this summer using *P-VV* as the donor of an active *Ac*.

Ac-st *Cloning.* We have cloned a *Bam*HI fragment containing most of *Ac-st* and 3 kb of flanking DNA. *Bam*HI, *Sst*I, and *Bgl*II digests of genomic DNA show that one of the nine *Ac* homologous bands is present in *Ac-st* stocks [W22] but is absent in W22 lines lacking *Ac-st*. When genomic DNA is cleaved with *Pvu*II, this fragment is replaced by a 2.5-kb fragment that represents the internal portion of an active *Ac*. Restriction analysis of the *Bam*HI clone shows that the 4.3 kb of *Ac-st* is indistinguishable from *Ac7*. The remaining 0.2 kb of *Ac-st* is yet to be characterized. A flanking unique probe of *Ac-st* has been isolated and will be used to clone a *Bgl*II fragment containing the entire *Ac-st* element. Sequence analysis will be performed on this clone to uncover any change to the *Ac-st* element.

Ac-st *Maps to Position 26 on Chromosome 10L.* We have mapped the *Ac-st* flanking probe to its chromosomal position using recombinant inbred lines of maize (Burr et al., *Genetics* [1988] in press). This method has replaced the element at approximately position 26 on the long arm of chromosome 10. Moreover, genetic linkage data of *Ac-st* with the *R* locus have placed the element at approximately the same position. The confirmation of the data with these two independent methods assures the map position of *Ac-st* and shows that the *Ac-st Bam*HI fragment is the correct clone for *Ac-st*.

Conclusions

These data suggest that *Ac-st* is associated with a reduction in transposase activity, since *Ac-st* action is recessive to a standard *Ac*. An alternative explanation of the phenotype of *Ac-st* would be an increase in the suppression of transposase activity associated with the *Ac* dosage effect. Our studies rule this out, since *Ac-st* action should display a dominant effect over a standard *Ac* element if the dosage contribution is increased. It is interesting to note that timing and *Ac* dosage contribution associated with this *Ac* are simultaneously affected, suggesting that these functions may be both associated with the transposase.

CHANGE IN STATE OF *WX-M7:A*

A new state of the *wx-m7* allele has been derived that shows a heritable change in the expression of waxy (B. McClintock, unpubl.). The pattern of this allele (designated *wx-m404*) no longer shows frequent wild-

type revertant sectors of starchy endosperm as seen with the *wx-m7* progenitor. Most revertant sectors contain low levels of amylose (as detected by IKI staining), with wild-type sectors appearing infrequently in the endosperm. The *wx-m404* allele is still under the control of the *Ac-Ds* system. This allele will be used to define the alteration associated with the change in state of *wx-m7*.

One Ac *Element Is Associated with the* Waxy *Locus in* wx-m404. A plant with the genotype *wx-m404/wx, a2-m4* was reciprocally crossed to a plant carrying *a2-m4, −Ac*. All of the A2 mutable kernels (50%) were also mutable for *waxy*, indicating that one *Ac* was segregating and that it was associated with the *waxy* locus. *Ac* also showed the expected dosage effect when crossed reciprocally.

Ac *Is no Longer Present at the Original Location of* Ac *in* wx-m7. A 3.2-kb *Sal* C fragment of *Wx* was present, indicating the loss of the entire *Ac* element from this fragment as is seen in *wx-m7*. An increase of 4.5 kb is seen for the *Sal* D fragment, from 2.0 kb to 6.5 kb. This indicates that *Ac* is now present in the *Sal* D fragment of the *wx-m404* allele. This insertion has been mapped to within 200 bp of the 3′ end of the *Sal* D fragment.

Ac *Is in the Opposite Orientation in Relation to* Wx *When Compared to the Original* wx-m7 *Position.* We have cloned the putative donor (location of *Ac7*) and the receptor site of this transposition event. Both sites have been recovered on an *Eco*RI clone spanning half of the *Ac* element into the 5′ end of the *wx* gene. This clone has been restriction mapped and is consistent with results from genomic Southern blot data.

We have sequenced across the 5′ junction region of *Ac* and *Wx*. This sequence shows that the insertion site of *Ac* in *wx-m404* is in exon 9 of the *waxy* gene. This location is 32 bp 5′ of the insertion site of *Ds1* in the *wx-m1* allele (Wessler et al., *EMBO J. 5:* 2427 [1986]).

Conclusions

The *wx-m404* allele is associated with a change in position and orientation of the *Ac* element relative to *Wx*. This change in location is from the 5′ promoter region of the *waxy* gene to a site in the coding region of the gene. The phenotypic change associated with the *wx-m404* allele may be due to the new position of *Ac* in exon sequence or a rearrangement in the promoter of *Wx* (donor site) as a consequence of transposition. These two possibilities may be distinguished when the donor site is sequenced.

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CELL BIOLOGY

The Cell Biology Section includes six laboratories using diverse approaches to analyze various aspects of the cellular biology of eukaryotes. The control of cell growth is the focus of the laboratory of J. Feramisco. Cellular oncogenes (e.g., the *ras* and the *fos* oncogenes) that influence the proliferative capacity of cells are being studied with respect to their biological effects and biochemical functions. The analysis of cellular *trans*-activators of gene expression is the focus of the laboratory of R. Franza. Cellular proteins that interact with specific, genetically defined control elements for gene expression are being identified and characterized. The laboratory of J. Garrels (The QUEST 2D Gel Facility) is continuing to build and to analyze the REF52 rat cell database for the study of protein synthesis modification and turnover in normal, transformed, and serum-stimulated cells. Similar database studies (in collaboration with C. McLaughlin and J. Warner) are now under way in yeast. The control of expression of the tropomyosins is the focus of the laboratory of D. Helfman. The tissue-specific tropomyosin isoforms are expressed from a single gene by alternative RNA splicing and polyadenylation. The molecular basis for this tissue-specific expression is being studied. The laboratory of D. Spector is investigating the structural and functional organization of the cell nucleus. Nuclear components that interact with the *myc* oncoprotein are being analyzed. The laboratory of W. Welch continues to focus on the biology and biochemistry of the stress proteins. Using monoclonal and polyclonal antibodies, the intracellular localization and the biochemical properties of the stress proteins are being determined.

MOLECULAR MECHANISMS OF GROWTH CONTROL

J.R. Feramisco C. Arrigo D. Bar-Sagi J.C. Lamb K. Riabowol S. Kaplan J. Suhan

Phospholipase A_2 and Cell Transformation

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

Phospholipase A_2 (PLA_2) is a calcium-requiring esterase catalyzing the hydrolysis of fatty acid ester bonds specifically at the sn-2 position of glycerophospholipids. The activity of PLA_2 has been postulated to play a regulatory role in several metabolic pathways, including the biosynthesis of prostaglandins, the turnover of membrane phospholipids, and the generation of receptor-mediated mitogenic signals. Following our earlier observation that microinjection of the *ras* oncogene protein into fibroblasts stimulates PLA_2 activity, we have initiated studies aimed at the characterization of the molecular and biological properties of this enzyme. Our goal is to understand the role of PLA_2 in the regulation of normal cell growth and in the transformation process. We have been pursuing our research in three main directions as described below.

GENERATION OF MONOCLONAL AND POLYCLONAL ANTI-PLA_2 ANTIBODIES

We have been using homogeneous preparations of porcine pancreatic PLA_2 as immunogen for raising antibodies in rabbits and mice. Within a 2–3-month period following the first injection, we have obtained high-titer serum as determined by the enzyme-linked immunosorbent assay. The rabbit antisera were purified on PLA_2 affinity columns, and the resulting affinity-purified anti-PLA_2 antibodies have been

extensively utilized during the past year for the analysis of the intracellular localization of PLA_2 in normal and *ras*-transformed cells. Immunofluorescence microscopy indicated that PLA_2 was diffusely distributed throughout the cell. Increased concentration of PLA_2 was detected under membrane ruffles in normal and *ras*-transformed cells (Fig. 1). Specific immunofluorescence staining was also detected on the outer surface of the cells. Immunoelectron microscopy demonstrated the increased accumulation of PLA_2 in membrane ruffles and also revealed the presence of the enzyme in microvilli and its association with intracellular vesicles. Ultrastructural localization of PLA_2 and the *ras* oncogene protein, using a double immunogold-labeling technique, indicated a spatial proximity between PLA_2 and *ras* proteins in the ruffles of *ras*-transformed cells. We plan to utilize further these antibodies for microinjection studies as well as for the biochemical characterization of PLA_2. In particular, we will attempt to immunoprecipitate PLA_2 polypeptide from metabolically labeled cells in order to analyze the biosynthesis and posttranslational modifications of this enzyme.

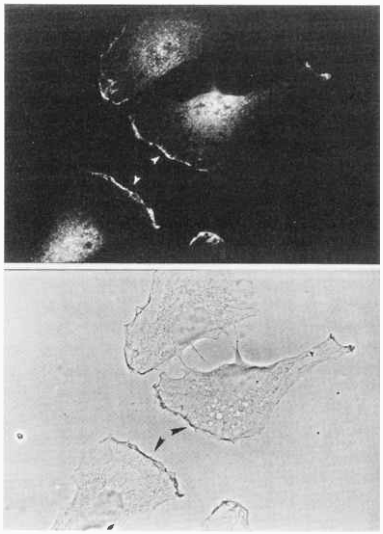

FIGURE 1 Localization of cellular PLA_2 by immunofluorescence. NRK cells grown on glass coverslips were fixed in 3.7% formaldehyde and permeabilized by brief extraction with 0.2% Triton X-100. The cells were stained with monoclonal anti-PLA_2 antibody (PLA29) followed by fluorescein-conjugated goat anti-mouse IgG. Note the intense staining in membrane ruffles (arrowheads).

CLONING OF cDNAs ENCODING CELLULAR PLA_2

The calcium-dependent phospholipase A_2 enzymes occurring in mammalian tissues are classified into two groups in terms of their localization. Extracellular PLA_2 is abundant in pancreatic tissue and has been studied extensively with respect to its physical and chemical characteristics. The intracellular PLA_2 has been characterized to a much lesser extent than the pancreatic enzyme because of its low cellular content and low specific activity. To circumvent these limitations, we have been working toward the isolation and characterization of PLA_2 cDNAs. In our studies to date, we have used a rat skeletal muscle cDNA expression library prepared in plasmid (pUC8 or pUC9) vectors. This cDNA library was screened with an oligonucleotide derived from the published cDNA sequence of the rat pancreatic PLA_2. We chose the sequence Tyr-Gly-Cys-Tyr-Cys-Gly-Leu-Gly for the synthesis of a 25-mer probe. This sequence encodes a highly conserved region of secretory phospholipase A_2 enzymes from various species. Thus far, we have obtained 12 independent recombinants that hybridize to the oligonucleotide probe. The plasmid DNAs from these clones were isolated, and cDNA inserts were found to range from 500 to 3000 bp. We are currently sequencing and characterizing some of these clones. In addition, we have recently obtained a rat brain and a rat pancreatic λgt11 cDNA library. We are using these libraries for immunological screening with our specific anti-PLA_2 antibodies.

BIOCHEMICAL MECHANISMS OF PLA_2 ACTIVATION

Activation of PLA_2 has been implicated in mitogenic transmembrane signaling. The proliferative response of Swiss-3T3 cells to platelet-derived growth factor (PDGF) appears to be accompanied by PLA_2 stimulation and prostaglandin production. In addition, PLA_2 seems to be an important component of the mechanism by which mitogens activate Na^+/H^+ exchange. This activation leads to cytoplasmic alkalinization, which has been shown to be required for the initiation of the mitogenic response. We have found that addition of serum to quiescent

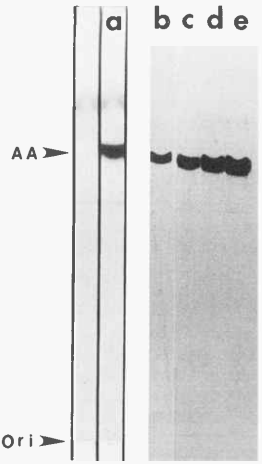

FIGURE 2 Activation of PLA_2 by serum and Ca^{++} ionophore. Confluent serum-deprived NRK cells were labeled with [^{3}H]arachidonic acid. Label was removed after 24 hr, and cells were treated with DME alone (*b*), DME + Ca^{++} ionophore (A23187, 2 μM) (*c*), DME + 5% serum (*d*), DME + serum (5%) + Ca^{++} ionophore (2 μM) (*e*). [^{3}H]Arachidonic acid released to the medium was extracted and analyzed by thin-layer chromatography (TLC). The position of standard arachidonic acid (stained with iodine) is shown in lane a. (Ori) Origin of TLC plate.

serum-deprived NRK cells results in a three- to five-fold enhancement of PLA_2 activity within 15 minutes of exposure to the serum (see Fig. 2b,d). The release of [^{3}H]arachidonic acid was used in these experiments as a measure of PLA_2 activity. We have begun to characterize the mechanisms underlying the stimulatory effects of serum on PLA_2 activity by investigating the Ca^{++} requirement for the activation of arachidonic acid release by serum. All phospholipase A_2 enzymes that have been adequately characterized are activated by Ca^{++} ions. Since the action of several serum growth factors is accompanied by enhancement of Ca^{++} influx, the resulting increase in intracellular calcium level constitutes a plausible mechanism for the activation of Ca^{++}-dependent PLA_2. We have found that the Ca^{++} ionophore A23187 (2 μM), when added to NRK cells in the absence of serum, stimulates [^{3}H]arachidonic acid release (Fig. 2c). The extent of stimulation is lower than that obtained by serum treatment (Fig. 2d). Furthermore, we have found that the effects of serum and Ca^{++} ionophore on [^{3}H]arachidonic acid release are additive (Fig. 2e), an observation consistent with two independently activatable release mechanisms. We will utilize similar approaches to analyze further the identity of the serum growth factors that are responsible for the stimulation of PLA_2 activity and to investigate the mechanistic aspects of this activation process.

Microinjection of *fos*-specific Antibodies Blocks DNA Synthesis in Fibroblast Cells

K. Riabowol, J.C. Lamb, J.R. Feramisco
[in collaboration with R. Vosatka and E. Ziff,
New York University Medical Center]

Transcription of the proto-oncogene c-*fos* is increased more than tenfold within minutes of treating fibroblasts with serum or purified growth factors. Due to this rapid transcriptional induction and accumulation of c-*fos* protein seen following mitogen stimulation of quiescent fibroblasts, the DNA-binding properties of the protein, and the reported ability of v-*fos* to *trans*-activate transcription from heterologous promoter sequences, it has been suggested that c-*fos* serves a role in coupling external stimuli, such as mitogens, to long-term transcriptional responses, leading to cell proliferation. Recent experiments using mouse 3T3 cell lines containing inducible *fos* antisense RNA constructs have shown that induced *fos* antisense RNA transcripts cause either a marked inhibition of growth in continuously proliferating cells or a minimal effect, except during the transition from a quiescent (G_0) state into the cell cycle. We have also isolated lines of rat fibroblast cells transfected with a glucocorticoid-inducible *fos* antisense RNA construct, and we have found that high levels of *fos* antisense RNA reduced the level of c-*fos* protein seen upon addition of serum to quiescent cells and that fewer cells were able to incorporate [^{3}H]thymidine in the presence of dexamethasone and serum compared to untransfected cells. In some of our cell lines, however, production of *fos* antisense RNA did not correlate well with reduced levels of *fos* protein, nor with a decreased rate of logarithmic growth. Since intracellular production of large amounts of antisense RNA does not completely block gene expression, we used the technique of needle microinjection to introduce polyclonal, affinity-purified antibodies

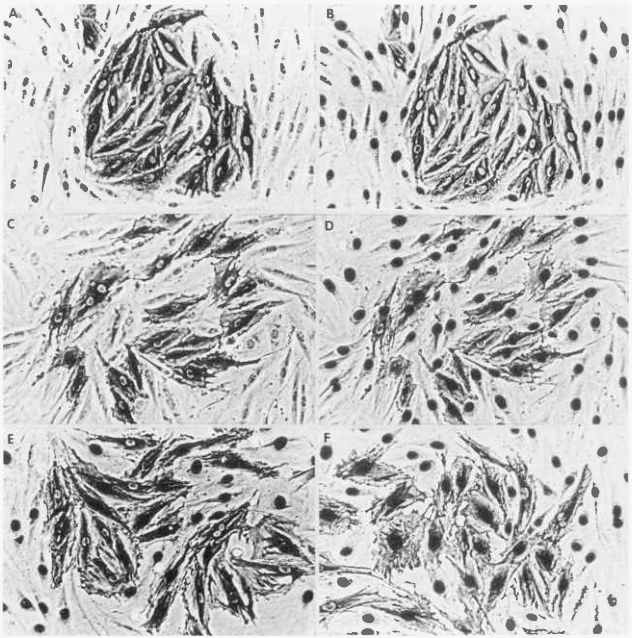

FIGURE 3 Microinjection of antibodies. At various times following injection of antibodies, cells were fixed, incubated with biotinylated goat anti-rabbit IgG, washed with PBS, incubated with Strep-avidin-conjugated HRP, and stained for the presence of antibodies. Stained fields of cells were photographed and processed for emulsion autoradiography. (*A–D*) Two fields of cells fixed after injection with *fos* (*A,B*) or control (*C,D*) antibodies 2 hr prior to addition of 15% FCS and 2 μCi/ml of [^{3}H]thymidine for 24 hr. (*A,C*) HRP staining; (*B,D*) the corresponding fields of cells following emulsion autoradiography. (*E,F*) Fields of cells microinjected with *fos* antibodies 8 and 12 hr, respectively, after addition of FCS and [^{3}H]thymidine. These results were obtained by injecting control and *fos*-specific antibodies at a concentration of 5 mg/ml. Injection of *fos* antibodies 2 hr prior to addition of medium containing 15% FCS at concentrations of 2.5 mg/ml, 0.5 mg/ml, and 0.1 mg/ml inhibited synthesis 92%, 26%, and 2% as well, respectively, as when used at 5 mg/ml.

directed against the *fos* protein into both quiescent (serum-deprived) cells and logarithmically growing cells to determine if, and when during the cell cycle, functional c-*fos* protein is required for cell proliferation. As shown in Figure 3, we find that microinjected *fos* antibodies efficiently block serum-stimulated DNA synthesis when injected up to 6–8 hours following serum stimulation of quiescent REF52 fibroblasts. To define further when the function of c-*fos* was required for cell growth, injection of antibodies was carried out at various times following serum stimulation. Figure 4 shows that by 12 hours following addition of serum, injection of *fos* antibodies had no effect on the ability of cells to synthesize DNA 6–8 hours later. Furthermore, when *fos* antibodies were injected into asynchronously growing cells, a consistently greater number of cells were prevented from synthesizing DNA than when cells were injected with nonspecific immunoglobulins. Thus, although the activity of c-*fos*

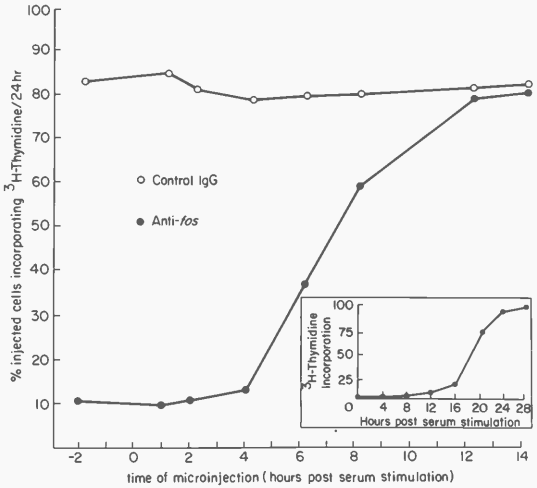

FIGURE 4 Time-course injections of anti-*fos* and control antibodies. Fibroblasts grown for 24 hr in medium containing 0.1% FCS were injected before or after the addition of 15% FCS and 2 μCi/ml [^{3}H]thymidine as indicated on the abscissa. Fields of 25–40 cells were injected for each time point in three separate experiments. Thus, each time point represents data from 75–120 injected cells. Cells that received antibody were identified by staining for Igs with HRP, and the percentage incorporating [^{3}H]thymidine was determined following emulsion autoradiography. (*Inset*) A time course of [^{3}H]thymidine incorporation following addition of serum to quiescent REF52 cells under the conditions used in these studies.

may be necessary for transition of fibroblasts from G_0 to G_1 of the cell cycle, its function is also required during the early G_1 portion of the cell cycle to allow subsequent DNA synthesis.

Although this approach has determined that functional c-*fos* is required for transit of fibroblast cells through the cell cycle, it does not address the question of the role of *fos* protein in this process. It has recently been reported that the c-*fos* protein binds to an upstream regulatory element of the AP2 gene, whose product, a fat-binding protein, is expressed in 3T3-F44A cells only upon their differentiation into adipocytes. We have obtained these cells, as well as antisera to the AP2 protein, from B. Spigelman and are presently investigating whether manipulation of the intracellular levels of functional c-*fos* protein by microinjection of *fos* antibodies has any effect on the expression of AP2.

The Catalytic Subunit of cAMP-dependent Protein Kinase Is Sufficient to Induce Expression of the c-*fos* Gene and a Synthetic Gene That also Contains a cAMP-responsive DNA Sequence Element

K. Riabowol, J.R. Feramisco [in collaboration with M. Gilman, Cold Spring Harbor Laboratory, R. Goodman and S. Fink, Tufts University, and D. Walsh, University of California at Davis]

Changes in the phosphorylation state of proteins by protein kinases and phosphatases are a major process for regulating cell function. The cAMP-dependent protein kinase exists as an inactive tetramer consisting of two molecules of catalytic (C) subunit complexed with two molecules of regulatory

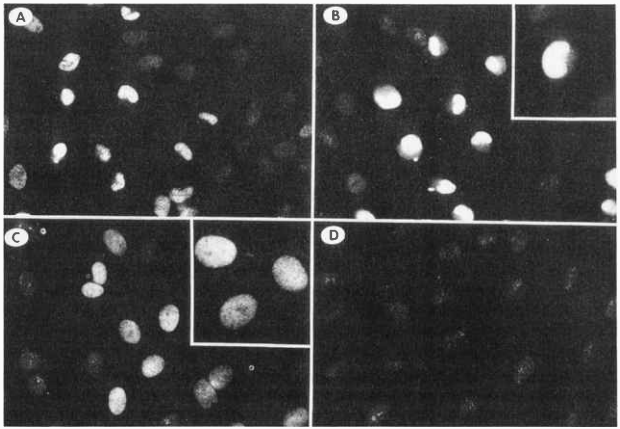

FIGURE 5 Induction of c-*fos* expression by microinjection of *ras* and cAMP-dependent protein kinase. Rat 208F cells were made quiescent by incubation in serum-poor medium (0.5% FCS) for 24 hr prior to injection of the catalytic subunit of cAMP-dependent protein kinase (*A,B,D*) or bacterially produced *ras* (T-24) protein. Cells were then fixed 1 hr (*A*) or 4 hr (*B–D*) after injection and incubated with affinity-purified *fos* antibodies, followed by fluorescein-conjugated goat anti-rabbit IgG. Nuclear fluorescence indicating the production of c-*fos* was evident within 1 hr of microinjection of *C*, and reached a maximum by 4 hr following injection. Injection of *ras* protein also resulted in expression of c-*fos*, but was not evident until 2–3 hr following injection. (*D*) Cells injected with R_{II} subunit and stained with *fos* antibodies.

(R) subunit. It is believed that cAMP affects cellular processes by binding to the R subunit, which results in the liberation of enzymatically active C subunits. Since the expression of several genes has been shown to be induced by the addition of cAMP analogs to cells, we tested the idea of whether introduction of purified C subunit (provided by D. Walsh) could alter the expression of two different test genes. Initially, a cell line containing a cAMP-responsive DNA sequence linked to β-galactosidase (provided by R. Goodman) was injected with C subunit alone and in the presence of the two forms of R subunits. We found that microinjection of the C subunit, in the absence of cyclic nucleotides, was sufficient to induce production of β-galactosidase, whereas injection of regulatory subunits R_I and R_II had no discernible effect on expression of this gene construct. We next tested the effect of C-subunit injection on the expression of c-*fos*. Using affinity-purified *fos* antibodies to assess levels of c-*fos* expression, we found that the C subunit dramatically induced the expression of c-*fos* when injected into cells made quiescent by incubation in serum-poor medium (0.5% serum, 24 hr) or by contact inhibition in complete medium (10% serum). Figure 5 shows that injected cells display high levels of *fos* staining both I hour (A) and 4 hours (B) after injection of the C subunit. For comparison, Figure 5C shows the effect of *ras* (T-24, previously reported to induce c-*fos* expression) on the level of c-*fos* staining 4 hours after injection, and Figure 5D shows a field of cells injected with the R_II subunit and stained with *fos* antibodies. These results demonstrate that increasing the activity of cAMP-dependent protein kinase within individual cells rapidly induces the expression of genes containing DNA sequences, termed cAMP-response elements (CREs), and that this induction can occur in the absence of cyclic nucleotides and the R subunits of cAMP-dependent protein kinase. We are now attempting to determine how the C subunit and *ras* affect expression of c-*fos* and the CRE-β-galactosidase construct.

Expression of c-*fos* and Entry into DNA Synthesis Is Prevented by Microinjection of Serum Response Element DNA Oligonucleotides

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

The 5′ region of the c-*fos* gene contains several promoter regions, including an element with a region of dyad symmetry termed the serum response element (SRE), previously shown to be necessary for transcriptional induction of gene constructs by factors present in serum. The induction of gene expression seen upon serum stimulation of quiescent cells is thought to occur by the activation and binding of positively acting (or inactivation of negatively acting) transcription factors within this DNA sequence. To address this question, we designed experiments

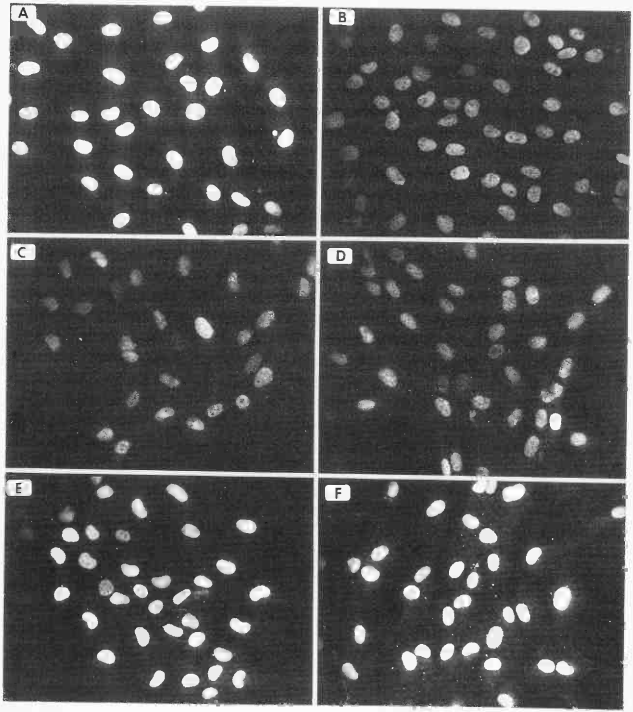

FIGURE 6 Expression of c-*fos* in cells injected with oligonucleotides. Serum-deprived (0.5% serum, 24 hr) rat 208F fibroblasts were fixed and processed for indirect immunofluorescence analysis before (*B*) or after (*A,C–F*) stimulation with medium containing 10% serum. Oligonucleotides injected into cells were as follows: (*A,B*) not injected; (*C*) 0.5 mg/ml SRE; (*D*) 0.1 mg/ml SRE; (*E*) 0.5 mg/ml SRE point mutant; (*F*) 0.5 mg/ml SV40 control oligonucleotide. Staining was done by incubation of fixed cells with affinity-purified rabbit anti-*fos* antibodies, followed by incubation with fluorescein-conjugated goat anti-rabbit IgG antibodies.

to determine if expression of the endogenous c-*fos* gene could be altered by affecting the availability of active transcription factors within serum-stimulated fibroblast cells. Double-stranded DNA oligonucleotides corresponding to (1) the wild-type SRE; (2) a point mutant of the SRE known to bind protein factors much less avidly, as assayed by gel-shift assay; and (3) two mutant sequences of the SV40 promoter that display no enhancer activity (kindly supplied by B. Ondek and W. Herr, Tumor Viruses Section) were microinjected into quiescent, serum-deprived cells. Cells were refed with medium containing 10% serum, incubated 90 minutes, and fixed and processed for indirect immunofluorescence analysis by incubation with affinity-purified *fos* antibodies, followed by fluorescein-conjugated goat anti-rabbit IgG antibodies. We find that cells injected with buffer or with mutant SV40 promoter oligonucleotides show levels of nuclear fluorescence similar to those in uninjected cells. In contrast, cells injected with oligonucleotides corresponding to the wild-type SRE show levels of nuclear fluorescence similar to those in quiescent cells, whereas cells injected with the SRE point mutant show intermediate levels of nuclear fluorescence. Figure 6 shows the nuclear-staining pattern obtained when quiescent, serum-deprived (Fig. 6B) or serum-stimulated 208F cells were injected with SRE oligonucleotides (0.5 mg/ml, panel C; 0.1 mg/ml, panel D), SRE point mutant (0.5 mg/ml, panel E), and SV40 control oligonucleotides (0.5 mg/ml, panel F) or were left uninjected (panel A). Pictures were automatically exposed for the same period of time to allow comparison of fluorescence intensity between fields of cells. In addition to affecting c-*fos* expression, microinjection of the SRE oligonucleotides into serum-deprived 208F fibroblasts inhibited the incorporation of [^{3}H]thymidine when cells were subsequently stimulated by the addition of medium containing 10% serum and incubated for 24 hours prior to emulsion autoradiography. This inhibition was not as complete as seen when cells were injected with antibodies directed against the *fos* protein, which may reflect the $t_{1/2}$ of the oligonucleotides, compared to antibody molecules, when injected into fibroblast cells.

We are now in the process of determining if this experimental approach can be used in conjunction with other methods, to determine which cell-signal pathways operate through DNA sequences known to be important in the control of gene expression.

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MAMMALIAN HEAT-SHOCK (STRESS) RESPONSE

W.J. Welch A.-P. Arrigo K.T. Riabowol M. Mulcahy
L.A. Mizzen J.P. Suhan L. Cipp

We have continued our efforts to understand how mammalian cells recognize and confront adverse changes in their local growth environment. Our system of study, the so-called heat-shock or stress response, is best characterized by the rapid and preferential transcription and translation of a set of genes that lead to the synthesis and accumulation of a group of proteins, the stress proteins. Collectively, these proteins appear to serve a function in either protecting the cell during the particular stress event at hand or facilitating the return of normal cellular functions when the cell is placed back into its normal environment. Consequently, most of our efforts have centered around purifying and characterizing the individual stress proteins with the ultimate objective, of course, of defining their exact biochemical function. Toward these ends, we have succeeded in purifying most of the major stress proteins and have prepared antibodies to each, and, as discussed further below, we are quite involved in experiments aimed at determining the function of these proteins both in the normal cell and in cells under stress.

Within the past year or two, we have witnessed a considerable increase in the number of laboratories investigating the structure/function of the heat-shock proteins. This new interest stems from recent observations implicating a role for many of the stress proteins in diverse areas of cell biology. These include a role for the 70-kD stress proteins in the ATP-dependent uncoating of clathrin-coated vesicles and recent reports of rather stable associations between members of the 70-kD stress-protein family and various oncogene proteins, including the SV40 large T antigen, the adenovirus E1A protein, polyoma middle T antigen, and the cellular p53 protein. With respect to the very abundant and highly phosphorylated 90-kD stress protein, we and other investigators have shown that the protein is a component of (and perhaps regulator of) various steroid receptor complexes and is involved in the intracellular transport of various protein tyrosine kinases such as $pp60^{v\text{-}src}$. In the case of the low-molecular-mass heat-shock protein, 28 kD, recent studies are beginning to implicate a role for the protein in proliferation and/or differentiation. Finally, in the last year or so, it has been shown by a number of laboratories that members of the 70-kD stress-protein family are involved in the translocation of proteins across the intracellular membranes and trafficking through the endoplasmic reticulum and Golgi complex. These studies, in sum, both underlie the importance of the stress proteins in normal cellular processes and provide us with a solid foundation by which to examine their biochemical function further. Moreover, with this influx of laboratories beginning to focus on the stress proteins from so many different directions, I am confident that the field will realize considerable acceleration with respect to determining the structure/function of the stress proteins.

Defining the Thermotolerant Cell

L.A. Mizzen, W.J. Welch

Perhaps the most important clue we have concerning the role of the heat-shock response and the possible function of the heat-shock proteins comes from studies examining the ability of cells to survive multiple stress events. For example, if cells are presented a mild heat-shock treatment and then allowed to recover, they acquire a transient resistance to a subsequent and otherwise lethal heat-shock challenge. This phenomenon, referred to as acquired thermotolerance, appears to be dependent on the prior production of functional stress proteins during the conditioning heat-shock treatment. To understand more fully how cells become thermoresistant and to determine the possible role of the stress proteins in the acquisition of the thermotolerant state, we undertook a study to define the major differences exhibited by the tolerant cell, as compared to the nontolerant cell in response to a severe heat-shock challenge.

As discussed in last year's Annual Report, one major difference exhibited by the tolerant cell is the protection of translational activity following the second and more severe heat-shock event. For example, if cells are brought immediately to a rather high temperature (45°C for 30 min), overall protein synthe-

sis activity is severely impaired and does not recover for as long as 5 hours following the return of the cells back to 37°C. In contrast, cells first made thermotolerant (e.g., a 43°C, 1-hr heat shock and recovery at 37°C for 8 hr) and then presented the 45°C, 30-minute shock exhibit a return of translational activity by as soon as 1 hour. In a number of different studies, we have observed a strict correlation between the levels of the 70-kD stress proteins and the ability of the cells to acquire resistance to hyperthermic treatment, as assayed by their ability to recover translational activity. Moreover, using this new assay, we are trying to determine directly, via microinjection studies of either the purified 70-kD stress proteins or alternatively antibodies specific to 70-kD, whether the 70-kD stress proteins are directly involved in protecting the translational machinery against inactivation by high-temperature exposure of the cell (Mizzen and Welch, *J. Cell Biol. 106:* 1105 [1988]).

Acquisition of the thermotolerant state is manifested in a number of other events that are perturbed in the cell following heat shock. For example, although processing of heterogeneous nuclear RNA (hnRNA) (i.e., splicing) is severely curtailed in cells given a severe heat-shock treatment, such RNA processing is significantly less inhibited if the cells are first made thermotolerant. In this respect, we are intrigued by our preliminary observations showing that a portion of the 70-kD stress proteins colocalize within the nucleus with the so-called small nuclear ribonucleoproteins (snRNPs), which are involved in RNA-processing activities (W. Welch et al., unpubl.). Finally, we have found that a number of other lesions that occur in cells as a consequence of heat-shock treatment, including the collapse of the intermediate filaments and the disruption of the nucleolar integrity and function, either do not occur or are repaired faster if the cells are first rendered thermotolerant (Welch and Mizzen, *J. Cell Biol. 106:* 1117 [1988]).

We think it likely that the stress proteins, particularly the 70-kD proteins, serve a critical role in protecting various cellular machineries from heat-induced damage and as such facilitate the acquisition of the thermotolerant state. Understanding the mechanism of thermotolerance acquisition will prove to be quite important with respect to a number of areas in biology and medicine. For example, it has been shown that developmental abnormalities arising from exposure to various teratogenic agents can be prevented if the embryo is first made thermotolerant. In addition, because new enthusiasm is resurfacing concerning the use of site-directed hyperthermia as a cancer treatment modality, understanding how cells (and tumors) defend themselves (and become thermotolerant) will prove critical to the successful development of this new approach to cancer treatment.

A Family of ATP-binding Stress Proteins

L.A. Mizzen, W.J. Welch

If the 70-kD stress proteins are indeed involved in protecting macromolecular proteinaceous complexes from protein denaturation and/or repairing such denaturation events, the obvious question arises as to how this might occur mechanistically. A number of recent observations are beginning to shed new light on the possible biochemical functions of hsp70. First, the simple fact that the cell contains such high levels of hsp70 both before or after heat-shock treatment is indicative of the proteins having some general, mass-acting function in the cell, as opposed to a very defined catalytic role. Second, the hsp70 proteins appear to be capable of binding and perhaps hydrolyzing ATP. Finally, it has been shown that a protein involved in the proper assembly of immunoglobulins within the endoplasmic reticulum is in fact related to the hsp70 proteins. This protein, referred to as BiP, was first observed to form transient interactions with immunoglobulin heavy chains in pre-B cells, with the interaction dependent on ATP. A number of experiments indicate that BiP functions in the ordered assembly of the immunoglobulin heavy chains (H) with the immunoglobulin light chains (L), thereby allowing for the mature form of the immunoglobulin, H_2L_2. Both cDNA sequence analysis and immunological studies have shown that BiP is identical for the glucose-regulated 80-kD stress protein and exhibits approximately 50% homology with the hsp70 proteins.

Using this model in which BiP transiently interacts in an ATP-dependent manner with proteins trafficking through the endoplasmic reticulum, we think it possible that the 70-kD stress proteins similarly might form transient interactions with denatured proteins in the cell following stress. Using the energy provided by ATP hydrolysis, the 70-kD proteins might function to either promote refolding of these partially denatured proteins or prevent such a denaturation event from ever occurring. In this way,

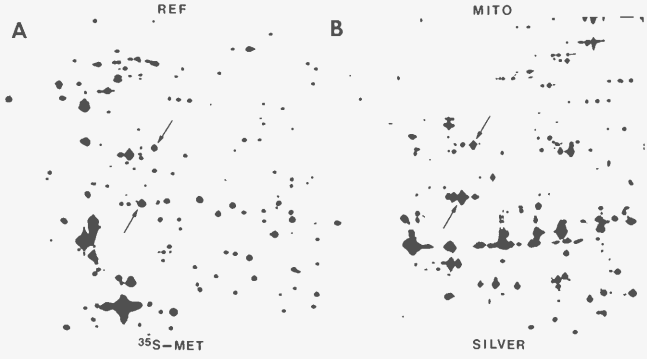

FIGURE 1 Identification of stress proteins present in the mitochondria. Mitochondria were purified from rat liver, and the mitochondrial proteins were analyzed by two-dimensional gel electrophoresis and visualized by silver staining of the gel (in collaboration with J. Garrels). In parallel, rat embryo fibroblasts, growing at 37°C, were labeled with [^{35}S]methionine, the radiolabeled proteins were analyzed by two-dimensional gel electrophoresis, and the proteins were visualized by fluorography. (*A*) [^{35}S]methionine-labeled proteins of rat fibroblasts; (*B*) protein constituents of isolated rat liver mitochondria. The positions of the 75-kD and 56-kD mitochondrial stress proteins are indicated by the downward and upward pointing arrows, respectively.

one could imagine then how the 70-kD stress proteins might function in providing a protective effect of macromolecular complexes (e.g., translational machinery and RNA-processing machinery) known to be affected by heat shock or other stress-inducing agents (discussed above). Hence, using a number of different approaches, we are examining the possibility that the 70-kD protein interacts with partially denatured proteins and that such an interaction is dependent on ATP.

We should also mention that we have found what appears to be yet another member of the hsp70 protein family, what we refer to as the 75-kD protein. We have found that the 75-kD protein appears to be structurally related to hsp72, hsp73, and the glucose-regulated 80-kD protein (BiP) as determined by both comparative peptide mapping and immunological cross-reactivity. This protein, like the other proteins of the family, binds avidly to ATP covalently linked to Sepharose, and this property of the protein has facilitated our purification of the protein to apparent homogeneity. In addition, in a collaborative effort with J. Garrels' laboratory, we have found that the 75-kD protein is compartmentalized within the mitochondria and, as such, is synthesized as a precursor with an apparent mitochondrial signal leader sequence (Fig. 1). Presently, we are attempting to determine the exact location of the 75-kD protein within the mitochondria as well as other proteins with which it might interact. Finally, we find it intriguing that the 75-kD protein, like the 80-kD (BiP) protein, is both a compartmentalized form of hsp70 and is increased in cells as a result of either glucose or calcium deprivation (L.A. Mizzen et al., in prep.). Thus, we think it likely that the 75-kD protein may function, like its related counterpart, the 80-kD protein (BiP), in regulating protein-trafficking events within the mitochondria in an ATP-dependent manner.

Characterization of the Low-molecular-mass 28-kD Mammalian Stress Protein

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

As discussed in last year's Annual Report, we have finally succeeded in purifying, to apparent homogeneity, the smaller 28,000-kD stress protein from HeLa cells. This was a difficult endeavor owing to the fact that this protein, unlike the other stress proteins, does not accumulate to high levels in cells following heat-shock treatment. Nevertheless, using a combination of ion-exchange and gel-filtration chromatography, the protein has been isolated in

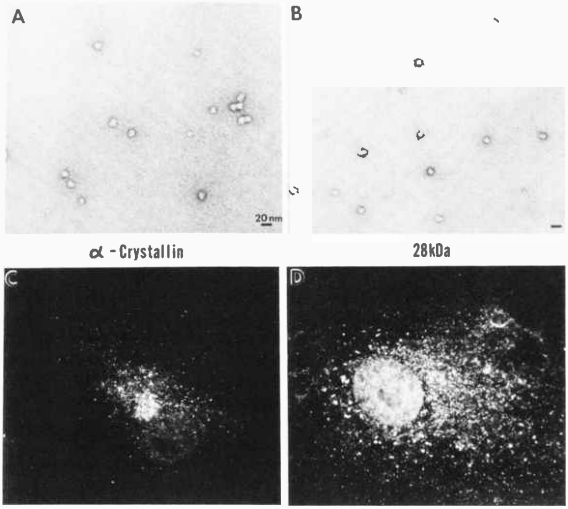

FIGURE 2 The 28-kD mammalian stress protein exists as a defined structure and is localized near the Golgi complex in monkey cells. The 28-kD stress protein was purified to homogeneity, and the isolated protein was analyzed by high-voltage electron microscopy (*B*). Note the well-defined 15–20-nm spherical structure and its structural homology with that of α-crystallin proteins (*A*), which exhibit approximately 50% sequence homology with the low-molecular-weight 28-kD stress protein. Using the purified protein, a rabbit polyclonal antibody specific to 28-kD was obtained and used to determine the intracellular locale of 28-kD in monkey cells (CV-1) before and after heat-shock treatment via indirect immunofluorescence. Panel C shows the distribution of the protein in cells grown at 37°C and panel D shows the location of 28-kD following heat shock. The perinuclear distribution of the protein in the 37°C cells corresponds to the distribution of the Golgi complex as determined by double-label staining using anti-28kD and a lectin specific for Golgi-associated components.

pure form (Arrigo and Welch, *J. Biol. Chem. 262:* 15359 [1987]). Interestingly, the purified protein appears to be an oligomer with an approximate native mass of 300–500 kD. In the electron microscope, the protein appears as a circular particle with an average diameter of ≈18 nm (Fig. 2).

Using the purified protein as antigen, we have succeeded in raising polyclonal antibodies specific for the 28-kD protein. Using these antibodies, we have examined the intracellular locale of the protein before and after heat-shock treatment via indirect immunofluorescence (Fig. 2). In cells grown at 37°C, the 28-kD protein is observed to localize primarily within the perinuclear region. Because the staining patterns appeared to be similar to that observed for the Golgi compartment, we obtained a fluorescently labeled lectin that recognizes a number of proteins known to reside within the Golgi. Using double-label fluorescence techniques, we have found that the 28-kD protein is closely aligned in and around the Golgi stacks. After heat-shock treatment, this staining pattern appears to "break up," and there is a corresponding increased staining within the peripheral regions of the nucleus. We have previously shown that the Golgi complex itself similarly becomes fragmented and somewhat dispersed after heat-shock treatment of the cells. We are currently investigating whether the 28-kD protein might actually be an

integral component of the Golgi or a more peripheral protein situated on the external side of the individual Golgi stacks/vesicles.

We have observed a number of interesting properties of the 28-kD protein that appear to differ depending on the physiological state of the cell. First, as mentioned above, the 28-kD protein is an oligomeric protein with an apparent mass of at least 300 kD in cells grown at their normal temperature. After heat-shock treatment, however, the protein appears to form large oligomers and exhibits a native size between 500 kD and approximately 2×10^6 daltons. With recovery from heat-shock treatment, the protein slowly returns to its smaller size. A second property of the protein is its different intracellular distribution and solubility in the cell as a function of stress. Although much of the protein is found in the low-speed supernatant of detergent-extracted cells incubated under normal conditions, most of the protein redistributes within the detergent-insoluble, low-speed pellet following heat-shock treatment. However, rendering the cells thermotolerant (by exposure to a prior mild heat-shock treatment and subsequent recovery period at 37°C) results in the protein to now remain in the detergent-soluble phase following a second and more severe heat-shock treatment. As the cells slowly lose their thermotolerant status, more of the protein is observed to fractionate within the low-speed pellet following the heat-shock treatment. Using this simple observation, i.e., the distribution within the low-speed supernatant versus the pellet, we can now fairly well predict the thermotolerant "status" of the cell (A.-P. Arrigo et al., in prep.).

One direction we plan to pursue with respect to the 28-kD protein involves the response of cells to steroids and/or growth factors. Recently, we came across reports from workers in the steroid field showing that the addition of steroids to various target cells results in an increased synthesis of a protein of approximately 27 kD and that has now been shown to be the 28-kD heat-shock protein. This result, coupled with our previous observation showing that the phosphorylation of the 28-kD protein increases in cells following addition of mitogens or tumor promoters, leads us to believe that the 28-kD heat-shock protein may play an essential role in proliferation, development, and/or differentiation. Further support for this idea follows from other observations that the expression of the *Drosophila melanogaster* equivalent of hsp28 appears to be regulated at different times during embryonic development. Consequently, we are beginning to examine the properties of the 28-kD protein in both normal and transformed cells and are setting up the appropriate tissue-culture cell lines that will respond in a known fashion to added steroids to facilitate our search for 28-kD function.

Identification of the Prosome Particle as a Large Multifunctional Protease

A.-P. Arrigo, J.P. Suhan, W.J. Welch [in collaboration with A.L. Goldberg, Harvard Medical School]

For a number of years, there have been numerous reports of a ring-shaped 19S-20S particle in both the cytoplasm and nucleus of eukaryotic cells. Because these particles were often found to copurify with inactive mRNAs, they were assumed to function in the regulation of translation of certain classes of mRNAs and hence were named prosomes (for program-osome). Prosomes isolated from many different sources exhibit the same polypeptide composition, with approximately six to ten proteins ranging in size between 25 kD and 35 kD. In fact, because of their subunit composition and appearance as a defined particle in the electron microscope, the prosome was once thought to be composed of the low-molecular-weight heat-shock proteins. However, this is not the case; instead, the low-molecular-weight heat-shock proteins, as detailed above, form their own 19S-20S particle and as such, often copurify with the 19S-20S prosome particle. In the course of examining the prosome from HeLa cells, we noted that it was similar in many ways to a high-molecular-weight protease complex purified in A.L. Goldberg's laboratory. This 70-kD enzyme complex, designated LAMP (for large alkaline-sensitive multifunctional protease) contains three endoproteolytic sites that function at neutral or alkaline pH and are specific for the hydrolysis of proteins, hydrophobic peptides, or basic peptides. Using a number of criteria including electron microscopy, immunological cross-reactivity, and peptide mapping, we have shown that the prosome is in fact identical to this large protease, LAMP (Arrigo et al., *Nature 331:* 192 [1988]). Currently, in conjunction with A.L. Goldberg's laboratory, we are investigating whether LAMP may serve a role in intracellular protein breakdown in cells subjected to physiologic stress.

Association of hsp70 with Different Oncogene Proteins

W.J. Welch [in collaboration with J. Jenkins, Marie Curie Cancer Research Foundation; G. Walter, University of California at San Diego; E. White and D. Spector, Cold Spring Harbor Laboratory]

During the past few years, it has become apparent that the expression of at least one form of hsp70 appears to be cell-cycle-regulated and in some cases, its levels are increased in highly proliferating cells. Moreover, it has been demonstrated that hsp70 levels increase in cells transfected with a number of so-called cooperating oncogene products, including SV40 large T antigen, polyomavirus T, c-*myc*, adenovirus E1A, and the cellular p53 protein. In addition to promoting hsp70 expression, a number of these oncogene proteins have been observed to form an association with hsp70, and in some cases, these associations appear to be ATP-dependent. In a collaborative effort with other investigators, we have shown that mutated forms of both polyomavirus medium T antigen and the cellular p53 protein form a stable complex with hsp70 (Walter et al., *J. Virol. 61:* 405 [1987]; Sturzbecher et al., *Oncogene 1:* 201 [1987]). Very recently, in a collaborative effort with E. White and D. Spector, we observed a similar coassociation of hsp70 with the adenovirus E1A (12S and 13S) protein (E. White et al., submitted). At the present time, the biological relevance of these associations of various oncogene proteins with hsp70 is not clear. One interpretation is that the high-level expression of these oncogene proteins is recognized as deleterious to the cell, and therefore the association with hsp70 reflects a housekeeping function of the protein perhaps similar to its role in the cell after stress. Alternatively, these various associations of hsp70 may be important to the stability, regulation, and/or function of these oncogene proteins and the maintenance of the transformed phenotype.

Heat Shock Is Lethal to Fibroblasts Microinjected with Antibodies Directed against hsp70

K.T. Riabowol, L.A. Mizzen, W.J. Welch

One approach we are using to dissect the function of the individual stress proteins has employed the technique of microinjection. Through finely drawn glass needles, we are able to introduce into the cell either purified proteins, their corresponding antibodies, mRNAs, or antisense RNA and then assess the effects by morphological or biochemical criterion. Using this approach, we have introduced a mixture of affinity-purified monoclonal antibodies directed against hsp70 and have examined the cells following a subsequent heat-shock treatment. We have found that the introduction of hsp70 antibodies into the cytoplasm of the cell completely inhibits the heat-dependent redistribution of hsp70 from the cytoplasm into the nucleus and nucleolus. Moreover, the injected cells are unable to survive the heat-shock treatment (K.T. Riabowol et al., submitted). These results then demonstrate that hsp70 is required for the survival of cells exposed to thermal stress. This result nicely complements previous genetic studies demonstrating a similar requirement for hsp70 in the ability of either yeast or *E. coli* to grow and acclimate to high temperatures.

Using this same approach, we are beginning to assess the effects either of introducing antibodies specific for the other stress proteins or of modulating the intracellular levels of the stress proteins directly by injection of the purified stress proteins. In the latter case, we plan to examine whether the absolute levels of the individual stress proteins serve in regulating their own synthesis and whether such regulation is in fact dependent on the severity of the stress treatment. Finally, because one or more of the stress proteins may be involved in the acquisition of thermotolerance, we plan to determine whether introduction of specific antibodies against the individual stress proteins might result in an inability of the cell to acquire the thermotolerant state.

COLLABORATIVE EFFORTS

Numerous laboratories working in many different areas of cell biology are beginning to converge in the areas of the mammalian stress response and the function of the stress proteins. Owing to our having purified all but one of the stress proteins, as well as raising the appropriate antibodies to each, we have entered into a number of exciting collaborative efforts including (1) the possible role of the heat-shock proteins, and in particular hsp70, in the regulation and/or function of intracellular protein breakdown in cells following heat-shock treatment (with N. Spector, S. Fraser, and A.L. Goldberg, Harvard Medical School); (2) the induction and accumulation of hsp70 in rodent brains following transient

ischemia (with T. Nowak, National Institutes of Health); (3) the possible participation of hsp70 in hnRNA processing (splicing) (with R. Morimoto, Northwestern University, and D. Spector and A. Krainer, Cold Spring Harbor Laboratory); (4) the role of the 90-kD heat-shock protein in regulating the activity of the translation factor eIF-2α protein kinase (with B. Hardesty, University of Texas, Austin); (5) the stimulation of heat-shock protein synthesis in T lymphocytes treated with various T-cell mitogens (with W. Farrar, National Cancer Institute); and (6) the participation of both hsp70 and hsp90 in the regulation and activity of steroid receptors (with D. Edwards, University of Colorado, and D. Toft, Mayo Clinic).

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MOLECULAR BIOLOGY OF THE CYTOSKELETON

D.M. Helfman S. Cheley S. Erster L. Goodwin J.P. Lees-Miller L.A. Finn W.M. Ricci

The emphasis of our research is directed toward understanding the molecular and cellular biology of tropomyosins in normal and transformed cells. Tropomyosins comprise a family of related actin-binding proteins present in muscle (skeletal, cardiac, and smooth) and nonmuscle cells. Although they are present in all cells, different isoforms of the protein are characteristic of specific cell types. During the past year, we have concentrated our efforts on studying the genes that encode tropomyosins and on developing experimental assays to study their expression in muscle and nonmuscle cells. Work from our

laboratory and others has demonstrated that the generation of tropomyosin isoform diversity involves the expression of multiple genes, most of which code for more than one isoform by the use of alternatively spliced exons. Some of our efforts are aimed at studying the molecular basis for the tissue-specific RNA processing that is involved in the generation of tropomyosin isoform diversity. In addition, we have continued to investigate the mechanism and functional significance of the alterations in tropomyosin expression in transformed cells. The following is a brief description of these studies.

Studies of Alternative Splicing Pathways of Tropomyosin Pre-mRNAs In Vitro

D.M. Helfman, W.M. Ricci

Work from our laboratory has previously demonstrated that a single gene encodes both rat fibroblast tropomyosin 1 (TM-1) and skeletal-muscle β-tropomyosin by alternative RNA splicing (Helfman et al., *Mol. Cell. Biol. 6:* 3582 [1986]). The gene contains 11 exons and spans approximately 10 kb (Fig. 1). Exons 1 through 5 and exons 8 and 9 are constitutive exons common to all mRNAs expressed from this gene. Exons 6 and 11 are used in fibroblasts as well as smooth-muscle cells, whereas exons 7 and 10 are used exclusively in skeletal muscle. The structural features of this gene raises a number of questions concerning the mechanisms involved in tissue-specific RNA processing. The existence of a mechanism(s) whereby the same gene can generate unique isoforms in cells as different as skeletal muscle and fibroblasts underlies the importance of regulatory factors (both *cis* and *trans*) that must be implicated in alternative RNA processing. At present, the nature of these *cis*- and *trans*-acting factors are not known and constitutes a major focus of our research.

We have been studying alternative RNA splicing of the rat TM-1 gene in vitro using nuclear extracts derived from HeLa cells. We have used nuclear extracts derived from HeLa cells because human cells contain the same isogene as the rat and HeLa cells express the same isoform as rat fibroblasts. We find that in vitro splicing of ^{32}P-labeled pre-mRNAs composed of the internal alternatively spliced region of the gene (exons 5 through 8) results in exon skipping, wherein exon 5 is spliced efficiently to exon 8. The lack of splicing of exon 5 to exon 6 or 7 was not due to a simple *cis*-competition for 3′ splice sites because truncated pre-mRNAs containing only exons 5 and 6 or exons 5 through 7 did not splice exon 5 to 6 or 7. Interestingly, we were able to detect efficient splicing of exon 5 to 6 (fibroblast-type splice) and exon 5 to 7 (skeletal-muscle-type splice), using precursors in which exon 6 or 7 was first joined to exon 8. The ability of HeLa cell nuclear extracts to splice exon 5 to exon 7 was unexpected, since this splice occurs exclusively in skeletal muscle in vivo. Since HeLa cell nuclear extracts were able to utilize the 3′ splice sites of both exon 6 (fibroblast-type splice) and exon 7 (skeletal-muscle-type splice), it seems likely that 3′ splice-site recognition of these exons does not require cell-type-specific factors. However, it is possible that 3′ splice-site discrimination is lost using the in vitro system. This could be due to the loss of a regulatory factor during the extract preparation. On the other hand, splicing of exon 5 to either exon 6 or exon 7 may not strictly be a regulated event, but dependent solely on either of the alternative exons being spliced to exon 8 (see below).

The observation that both of the internal alternatively spliced exons (exon 6 and exon 7) must first

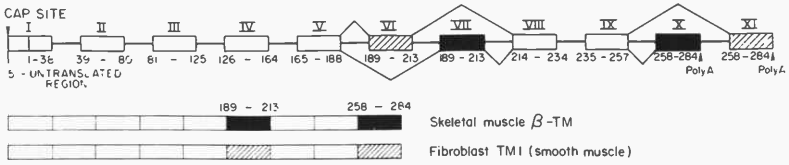

FIGURE 1 Schematic diagram of the tropomyosin gene and model for the generation of rat fibroblast TM-1 and skeletal-muscle β-tropomyosin mRNAs by alternative RNA processing. Open boxes represent common exons, hatched boxes represent fibroblast or smooth-muscle exons, closed boxes represent skeletal-muscle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

be joined to the downstream constitutive exon before they can be spliced to the upstream constitutive exon has important implications in understanding the regulation of splice-site selection. The data are consistent with a model in which the critical event in alternative splicing occurs during the joining of exon 6 to exon 8 (fibroblast-type splice) or exon 7 to exon 8 (skeletal-muscle-type splice). However, even if this model is correct, we cannot conclude that splicing exon 6 to exon 8 and exon 7 to exon 8 are both regulated processes. For example, splicing of exon 6 to exon 8 may simply reflect the constitutive or default pathway, whereas splicing of exon 7 to exon 8 requires tissue-specific factors expressed in skeletal muscle. Experiments are in progress to determine if splicing of exon 6 to exon 8 or exon 7 to exon 8 requires tissue-specific splicing factors.

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

D.M. Helfman, L.A. Finn

We are also studying the tissue-specific splicing of the TM-1 gene by introducing into tissue-culture cells DNA constructions (minigenes) containing defined regions of DNA to determine which sequences are required for alternative RNA processing. To study the internal alternatively spliced region of the gene, we have cloned genomic sequences from exons 5 through 9 into a eukaryotic expression vector containing the SV40 early promoter and a functional poly(A) site. Transient and stable expression of this minigene in rat fibroblasts, HeLa cells, L6 myoblasts, and C2 myoblasts resulted in splice products that contain exon 5+6+8+9. No other products were detected, and no RNAs were detected that contain exon 7. These results are consistent with the splice products expected if this minigene is being processed in a tissue-specific mannner in these cells types. In addition, we have established a number of stable transformants containing this minigene in mouse C2 myoblasts, a cell line that mimics myogenesis. Thus, when the myoblasts fuse to form myotubes, there is cessation of cell division, together with repression of fibroblast TM-1 synthesis, followed by fusion of the myoblasts with induction of skeletal-muscle β-tropomyosin. As described above, expression of this minigene in the myoblasts results in the generation of RNAs that contain only the fibroblast exon (exon 6). We are currently in the process of determining if expression of this minigene in the differentiated myotubes exhibits utilization of the skeletal-muscle-specific exon (exon 7).

Studies of Alternative 3′-end Processing of Tropomyosin Pre-mRNA In Vivo

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

The 3′-end processing events of the TM-1 gene requires both alternative exon selection and utilization of the appropriate poly(A) site (Fig. 1). To study the alternative 3′-end processing events, we have generated a series of wild-type and mutant minigenes containing genomic sequences from exon 9 to exon 11. These minigenes have been introduced into cultured cells by transfection, and the RNA processing products generated after transient expression have been characterized by S1-nuclease analysis and by a modified primer-extension assay (see below). Transient expression in COS cells results in splicing of exon 9 to both exon 10 (skeletal-muscle-type splice) and exon 11 (fibroblast-type splice) and utilization of both poly(A) sites, with a preference for the fibroblast-type pathway. In contrast, expression of wild-type constructions in 3T3 fibroblasts results exclusively in splicing of exon 9 to exon 11 (fibroblast-type splice). We have generated several deletions within intron 9 that result in an increase in the efficiency of splicing of exon 9 to exon 10 in COS cells, as well as utilization of exon 10 in 3T3 cells. We are currently determining whether the deletions in intron 9 that result in utilization of the skeletal-muscle exon (exon 10) in 3T3 cells are simply the result of the decrease in intron length or the removal of specific sequences. In addition, we are characterizing the processing products of the minigenes in the mouse myogenic cell C2.

Development of a New Method to Analyze Splice-site Selection

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

To facilitate our studies of RNA splicing, we have developed a procedure using a modification of stan-

dard techniques to study RNA splice-site selection. First, an oligodeoxynucleotide complementary to exon sequences upstream of a 5′ splice donor site of interest is annealed to the RNA. The DNA-RNA hybrid is then digested with RNase H, resulting in RNA molecules that are cleaved precisely at the same site. The cleaved RNAs are then subjected to primer extension using a ^{32}P-labeled primer complementary to sequences downstream from an appropriate 3′ splice site. Since the primer-extension products all terminate at the site of RNase H cleavage, their lengths are indicative of the splice sites utilized. The method simplifies the study of splicing of complex pre-mRNAs by allowing individual introns to be analyzed separately. We have used this method to characterize successfully rat tropomyosin mRNAs expressed in different tissues and RNAs generated in vivo by transient expression of tropomyosin minigene constructions in cultured cells. In addition, we have used this approach to study the differential splicing at both the 3′ end and at the internal region of the TM-1 gene. One of the advantages of the technique is the ability to detect exon skipping, which was undetected using available cDNA probes. Furthermore, 3′ exon splicing can be studied with this procedure, since there is no need to primer-extend a long RNA from the 3′ end all the way to the 5′ end.

Identification of Novel Tropomyosin Isoforms Expressed in Rat Brain via Tissue-specific RNA Processing

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

Using ^{32}P-labeled probes prepared from a cDNA clone that encode skeletal muscle α-tropomyosin (α-TM), we have detected three mRNAs of approximately 3.0, 1.8, and 1.3 kb in rat brain. It has already been shown that a single gene encodes skeletal-muscle α-TM, smooth-muscle α-TM, and fibroblast TM-2 by an alternative RNA-splicing mechanism. To determine the structure of the brain mRNAs, and to investigate a possible structural relationship with other mRNAs, we have isolated and characterized a number of cDNA clones from a rat brain library. At present, we have isolated 20 cDNA clones that contain sequences related to the α-TM. The longest cDNA is approximately 2.8 kb; however, sequence analysis indicates that it is not full length. This cDNA starts at amino acid 83 and contains sequences through the poly(A) tract. Comparison of the brain cDNA indicates that it is identical to skeletal-muscle α-TM, smooth-muscle α-TM, and fibroblast TM-2 from the region encoding amino acids 83–257. These data indicate that it is the product of the same gene that encodes these three other isoforms. However, the brain cDNA was found to contain a novel carboxy-terminal region that would encode a tropomyosin containing 281 amino acids. This is assuming that this message has the same 5′-coding sequences characteristic of other tropomyosin isoforms expressed from this gene. At present, the structure of the 5′ end of this mRNA is unknown, and experiments are under way to determine its structure. Further sequence analysis of the 2.8-kb cDNA indicates that it contains a long 3′-untranslated region, terminating with the same sequences also found to encode the carboxy-terminal end and the 3′-untranslated sequences through the poly(A) site used in fibroblasts and smooth muscle. Analysis of genomic clones has revealed that the 3-kb mRNA expressed in the brain results from use of a brain-specific splice located upstream of the exon used in fibroblasts and smooth-muscle cells. In addition, we have determined that there are two additional poly(A) sites utilized in the brain that are located downstream from this brain-specific splice site that give rise to 1.3- and 1.8-kb mRNAs. We have also isolated a full-length clone of approximately 1.3 kb from the brain cDNA library that contains a portion of the 5′-untranslated sequences through the poly(A) tract. This clone contains coding sequences that are identical to those of skeletal-muscle α-TM from amino acids 1 to 257 and the novel brain-type carboxy-terminal end corresponding to amino acids 258–281. The significance of the three different poly(A) sites associated with the same carboxy-terminal coding sequences is unclear at present. We are now determining if the differences in the 3′-untranslated regions of these mRNAs correlate with distinct 5′-coding sequences. In addition, studies are under way to determine the expression of the brain tropomyosin isoforms with respect to region, cell type, and developmental-stage specificity.

Structural Analysis of the Gene Encoding Rat Fibroblast Tropomyosin 4

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

cDNA clones for TM-4 that we previously identified were used to isolate the gene that encodes this

isoform. Four overlapping λ clones that contain the entire transcription unit were isolated from a genomic library. Restriction enzyme analysis indicated that all sequences represented in the rat genome are continuous at a single locus. The gene spans approximately 15 kb. For detailed mapping, genomic DNA fragments were subcloned into plasmid vectors pUC18/pUC19, and the intron/exon organization of the gene was determined. The gene was found to contain eight exons that encode the TM-4 mRNA. At present, we do not know whether this gene contains alternatively spliced exons used in different tissues and cell types. Work from our laboratory has already suggested that this gene may encode at least two mRNAs: a 2.2-kb mRNA expressed in fibroblasts and smooth muscle and a 1.3-kb mRNA expressed in skeletal muscle (Yamawaki-Kataoka and Helfman, *J. Biol. Chem. 262:* 10791 [1987]). Although we detected two different mRNAs with cDNAs encoding TM-4, it is not known if this gene encodes two different RNAs via tissue-specific RNA processing or if the two RNAs simply represent cross-hybridization with the products of two different genes. Work is currently under way to answer this question.

Expression and Function of Tropomyosin in Normal and Transformed Cells

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

One of the major differences between normal and transformed cells in tissue culture is their morphological appearance. Transformed cells are usually more rounded or spindle-shaped, and their stress fibers (microfilament bundles) are reduced or even absent. The molecular and biochemical bases for these changes in the cytoskeleton of transformed cells are poorly understood. We have focused our attention on tropomyosin because recent studies have demonstrated profound alterations in the pattern of tropomyosin expression in cells transformed by oncogenic tumor viruses and carcinogens. This may, in part, be reponsible for the reduction of microfilament bundles (stress fibers) and the accompanying alterations of cell shape characteristic of many transformed cells. Neither the mechanism(s) responsible for these altered patterns of tropomyosin synthesis in transformed cells nor the functional significance of the five different isoforms of tropomyosin in fibroblasts is known.

To study the function of tropomyosin in normal and transformed cells, we want to introduce specific tropomyosin isoforms into living cells by direct microinjection of purified RNA or proteins. To synthesize biologically active mRNAs encoding specific tropomyosin isoforms, the full-length cDNAs encoding TM-1, TM-2, and TM-4 were subcloned into SP6 plasmids. Direct purification of individual tropomyosin isoforms from rat fibroblasts has been difficult using conventional purification methods, which is likely due to the fact that the different protein isoforms have such similar physicochemical properties. To prepare large quantities of each of the major fibroblast tropomyosin isoforms (TM-1, TM-2, and TM-4), we have utilized a plasmid cloning system that allows for the production of the protein in *Escherichia coli*. Using this system, we plan to prepare homogeneous preparations of each isoform. In the future, we plan to label each of the different isoforms with fluorescent probes in order to determine the subcellular distribution of each protein species following microinjection into living cells. In addition, we plan to express particular tropomyosin isoforms in transformed cells where the cellular gene product is repressed. Such studies may determine if expression of tropomyosin results in changes in microfilament organization and cell shape. We have already prepared large quantities of purified TM-2 from *E. coli* for these studies.

Normal rat fibroblasts contain five tropomyosin isoforms. To date, we have obtained full-length cDNA clones of the three major fibroblast tropomyosin isoforms (TM-1, TM-2, and TM-4). To obtain cDNA clones to the minor isoforms (TM-3 and TM-5), we are preparing cDNA libraries from two transformed cells lines: an adenovirus-transformed cell line (Ad5D.4A) and a Kirsten-virus-transformed cell line (NRK 1569). These cell lines exhibit increased levels of TM-3 and TM-5, respectively. In addition, previous work from our laboratory has demonstrated that although the Kirsten virus NRK 1569 cell line and the Rous sarcoma virus NRK 4/435 cell line do not express TM-2, they still express a 1.8-kb mRNA associated with this isoform (Yamawaki-Kataoka and Helfman, *J. Biol. Chem. 262:* 10891 [1987]). Preliminary experiments using RNA protection analysis indicate that the mRNA expressed in transformed cells is structurally different from the mRNA expressed in normal cells. We are in the process of isolating the corresponding cDNA clone from a cDNA library prepared from the Kirsten-virus-transformed cells to establish fully the structure of the RNA in these cells.

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CELL BIOLOGY OF THE NUCLEUS

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

Our laboratory is divided into two sections, a research group studying the structural and functional organization of the cell nucleus and a core Electron Microscopy Facility. Our core facility, through the expert technical achievements of Mike Delannoy and Joe Suhan, has provided a diverse range of services to numerous scientists at Cold Spring Harbor Laboratory, including standard transmission and scanning electron microscopy, immunoperoxidase and immunocolloidal gold electron microscopy, DNA spreading, negative staining, and metal shadowing. Descriptions of many of these projects can be found in the individual research sections in this Annual Report.

Our research program continues to proceed in two related areas: (1) the elucidation of specific functional regions within the cell nucleus and (2) the biochemical identification of the nuclear components that interact with the *myc* oncoprotein.

Structural and Functional Organization of the Cell Nucleus

D.L. Spector, M.R. Delannoy

In a previous series of immunocytochemical studies, we examined the spatial organization of a group of nuclear proteins, some of which are associated with small nuclear ribonucleoprotein particles (snRNP). The proteins investigated colocalized within a discrete nuclear domain that excludes the nucleolus and parts of the nucleoplasm. We are now at the stage of being able to examine the functions associated with this nuclear region and to relate these findings to the general organization of the interphase cell nucleus. Our first series of experiments were designed to localize the sites of DNA replication in situ by using high-resolution autoradiography and to compare these nuclear sites with the immunolocalization of the *myc* oncoprotein. Quail cells (Q8) nonproductively transformed with the avian myelocytomatosis virus MC29 were pulsed for 5 minutes with [^{3}H]thymidine (sp. act. 74 Ci/mM) at 50 μCi/ml. Short pulses were used in order to be certain that we were looking at the initial sites of label incorporation, rather than label that has moved around the cell nucleus subsequent to incorporation. After [^{3}H]thymidine labeling, cells were fixed and immunolabeled with an antibody raised against the human recombinant c-*myc* protein followed by peroxidase-conjugated secondary antibodies. Sections of cells (0.5 μm) embedded in resin were affixed to slides and coated with autoradiographic emulsion. After 20 days of exposure, the sections were chemically developed and examined microscopically. Panels A and B in Fig-

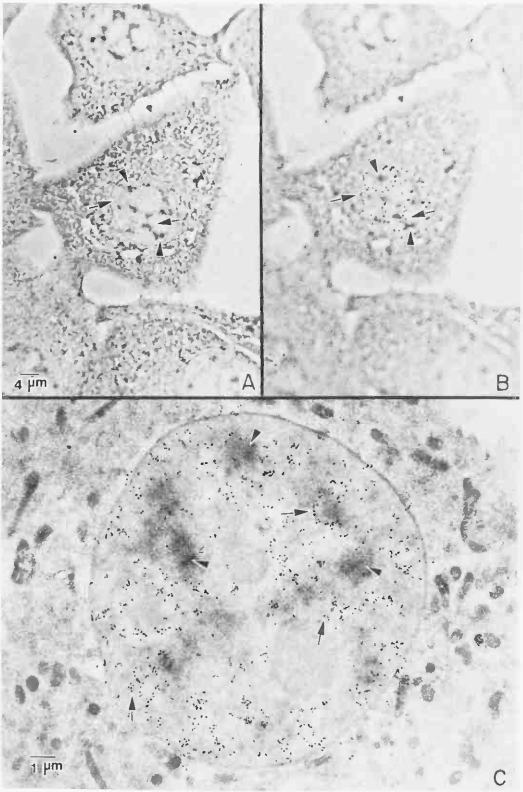

FIGURE 1 Quail Q8 cells immunolabeled with antibodies against the *myc* oncoprotein and radiolabeled with [^{3}H]thymidine. Panels *A* and *B* are the same cell. Panel *A* is focused on the immunoperoxidase staining of v-*myc*, and panel *B* is focused on the autoradiographic grains. As shown, the localization of v-*myc* (arrowheads) is not coincident with the sites of incorporation of [^{3}H]thymidine (arrows). Panel *C*: Immunoelectron microscopic localization of v-*myc* showing that the bulk of autoradiographic grains due to [^{3}H]thymidine labeling (arrows) are not coincident with v-*myc* immunostaining (arrowheads).

ure 1 are the same 100-nm cell section; however, panel A is focused on the immunolabel and panel B is focused on the autoradiographic grains that occur on the section surface. Immunostaining of the *myc* oncoprotein was restricted to a reticular network within the cell nucleus (panels A and B, arrowheads). Autoradiographic grains resulting from [^{3}H]thymidine incorporation were present in regions of the nucleoplasm, which were not coincident with the *myc* immunostaining (compare arrows in panel B, pointing

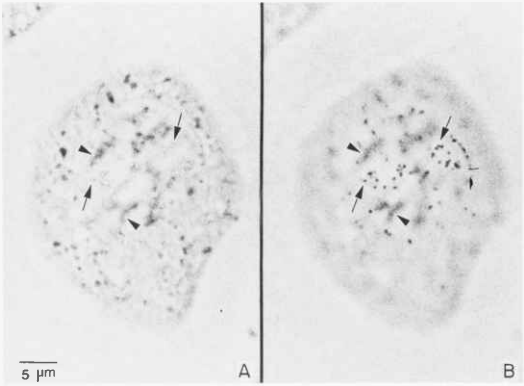

FIGURE 2 Quail Q8 cells immunolabeled with antibodies against the *myc* oncoprotein and radiolabeled with [^{3}H]uridine. *v-myc* immunostaining (arrowheads) is not coincident with [^{3}H]uridine incorporation (arrows).

to autoradiographic grains, and arrows in panel A, showing no immunolabeling in these regions). Two other cells in panels A and B show immunolabeling, but no autoradiographic grains, since they were not in S phase during the 5-minute incorporation of [^{3}H]thymidine. Although our light microscopic data are quite convincing, we decided to evaluate samples at the electron microscopic level so that we could attain higher resolution. Improved resolution was attained at three levels. Most obviously is the inherent increase in resolution that the electron microscope has over the light microscope (2.04 Å vs. 0.2 μm). In addition, the use of an electron microscopic autoradiographic emulsion (L4) that is applied to the section in a monolayer provides a more precise grain distribution. Finally, the use of physical development, rather than chemical development, increases the sensitivity as well as the resolution by virtue of the small and consistently round grain size, so that underlying structures remain easily visible. Combining all of these methods, we evaluated the distribution of [^{3}H]thymidine, and we found that, consistent with our light microscopic data, the sites of *myc* immunostaining were distinct from the sites of [^{3}H]thymidine incorporation (Fig. 1C). In fact, in some cases, the autoradiographic grains appeared to surround (rather than overlap with) the immunostained regions. In all cases, little to no background labeling was observed in the cytoplasm.

A second series of experiments evaluated the sites of transcription using [^{3}H]uridine incorporation. Cells were labeled with [^{3}H]uridine (sp. act. 18 Ci/mM) at 50 μCi/ml for 5 minutes and processed as above. Light microscopic autoradiography showed the sites of incorporation of [^{3}H]uridine to be distinct from the sites of immunoreactivity (Fig. 2A, B). Similar results have been obtained in CHOC 400 cells using the anti-Sm antibody that recognizes U1, U2, U4/U6, and U5 snRNPs (data not shown). Taken together, these data suggest that the nuclear network identified with *myc* and anti-Sm antibodies is distinct from those nuclear regions involved in DNA replication and transcription. Our current hypothesis is that the network-like staining observed with these antibodies represents the sites of heterogeneous nuclear RNA (hnRNA) processing. To address this hypothesis, we are employing pulse-chase experiments to determine whether the [^{3}H]uridine label can be chased into the speckled-staining region. In addition, we are preparing biotinylated SP6 probes to specific cellular mRNAs and oligonucleotide probes to splicing intermediates to determine if, in

fact, we can localize splicing intermediates to the regions of the cell nucleus that are concentrated in snRNPs.

Nuclear Associations of the *myc* Oncoprotein

W.-K. Chan, M.R. Delannoy, D.L. Spector

Our laboratory has previously observed that the *myc* oncoprotein is localized within distinct nuclear clusters (speckles). Our current investigations are aimed at identifying the polypeptides and/or nucleic acids that are directly associated with the *myc* protein in the cell nucleus. Several approaches will be used to achieve this goal. First, coimmunoprecipitation experiments will be performed to examine whether particular protein species will be coimmunoprecipitated using *myc*-specific antibodies. To facilitate the coimmunoprecipitation experiments, we have recently gel-purified the human recombinant c-*myc* oncoprotein expressed in *E. coli* (from M. Rosenberg, Smith, Kline and Beckman Laboratories) and have injected the purified protein into rabbits to generate polyclonal antibodies against *myc*. Cross-linking studies using bifunctional cross-linking reagents will be used to confirm the coimmunoprecipitation experiments. As *myc* may interact with particular species of nuclear RNA, such as snRNA, which resides in the speckled region of the nucleus in the form of snRNPs, we will perform UV-cross-linking experiments that can covalently link the protein bound to the nucleic acid and allow us to investigate the possibility of direct interactions between *myc* and RNA molecules.

Although the *myc* protein is readily available from *E. coli*, the expressed protein aggregates to form granular structures within the bacterium (Fig. 3A),

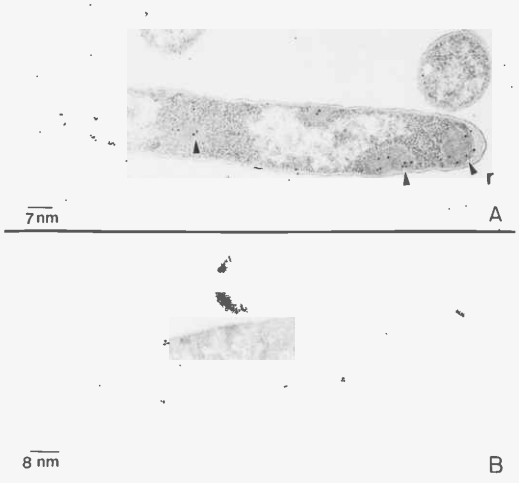

FIGURE 3 *E. coli* cells overexpressing the *myc* oncoprotein exhibit large granules within the cells, which can be immunolabeled with *myc* antibodies coupled to colloidal-gold-conjugated secondary antibodies (*A*). Control cells not expressing the *myc* oncoprotein do not exhibit such granules (*B*).

making it difficult to solubilize the protein. We have used immunocolloidal gold-labeling techniques to prove that the granule is composed of *myc* protein (Fig. 3A). We are now attempting to optimize the expression of *myc* in soluble form and subsequently to purify this soluble form of *myc* protein. The purified protein will be used in in-vitro-binding studies to investigate its nucleic-acid-binding capability and specificity. The purified protein will also be used for crystalization studies in collaboration with J. Anderson (Structure Section) to determine its three-dimensional structure. In addition, in collaboration with D. Carroll in D. Marshak's laboratory (Tumor Viruses Section), we will investigate the effect of phosphorylation on the properties and function(s) of the protein.

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QUEST LABORATORY/TWO-DIMENSIONAL GEL BIOTECHNOLOGY RESOURCE

J.I. Garrels	C. Chang	M. Hannaford	G. Mak
B.R. Franza	H. Sacco	P. Myers	A. Rudman
M.E. Lambert	J. Kos	J. Ross	Salah-Ud-Din

The QUEST Laboratory has concentrated its efforts on the analysis of the REF52 database and of the yeast database, which is now growing rapidly through collaborative work with Calvin McLaughlin and Jonathan Warner.

Software tools for the construction and analysis of databases have matured so that a thorough analysis of the REF52 data, including construction of permanent spot sets and numerous spot annotations, has been possible. Detailed reports on the methods of database construction and on the REF52 database itself have been accepted for publication. This completed work now sets an example that can be followed in the development of the yeast database and in human databases yet to be constructed.

A new member of the laboratory, Salah-Ud-Din, has begun to explore better ways to identify protein spots in the two-dimensional gels. Having set up the blotting techniques for antibody detection and microsequencing of proteins from the gels, and for enriching minor spots through prefractionation, he will attempt to identify and characterize one or more of the nuclear proteins with proliferating cell nuclear antigen (PCNA)-like regulation. This work is being done in collaboration with Bruce Stillman's laboratory (see Tumor Viruses Section).

Michael Lambert has moved on to the Scripps Clinic in La Jolla, where he has received a staff position. He will continue to work on cellular responses to DNA damage and plans to set up a facility for two-dimensional gel analysis at the Scripps Clinic.

The REF52 Database

J.I. Garrels, B.R. Franza

As described in previous Annual Reports, the REF52 database is built on a family of ten normal and virus-transformed rat fibroblast cell lines of the REF52 family. Cells from each line have been radiolabeled and analyzed at four stages, during growth from low-plating density to confluence and again after stimulation of confluent cells with fresh serum. Three lines (normal, SV40-transformed, and adenovirus-transformed) have also been radiolabeled early (1–4 hr) and late (21–24 hr) after addition of fresh serum to serum-deprived cells.

The REF52 database also contains experiments to identify cytoskeletal, nuclear, mitochondrial, and mannose-labeled proteins. Permanent protein sets have been created and stored in the database to identify the members of each group. Other sets have been generated to indicate those proteins that are coregulated during transformation, during growth to confluence, and at early or late times after response to serum stimulation. At the workstation, any set can be recalled from the database, and its members can be highlighted on the gel image being displayed. The QUEST software allows us to characterize the behavior of each set quantitatively, as a whole and as individual proteins. Names and other annotations for each member of a set can be listed, based on the annotations previously entered into the database.

CYTOSKELETAL PROTEINS

In transformed cells, the fraction of protein synthesis represented by the cytoskeletal proteins is reduced by as much as threefold. More highly transformed REF52 derivatives have lower total cytoskeletal protein synthesis. Many individual cytoskeletal proteins have marked virus-specific responses to transformation. Tropomyosin 3 is the most interesting of these because it is highly elevated in adenovirus-transformed cells (at low density, but not at high density), yet it is suppressed in retrovirus-transformed cells and in most SV40-transformed cells. Members of the cytoskeletal group appear in other sets selected for response to serum stimulation, progression to anchorage-independence, and responses to viral mutations (see below).

NUCLEAR PROTEINS

A set of 255 nuclear proteins was identified by comparing gels of purified nuclei to gels representing total cellular proteins. Nuclear proteins were defined to be those that were enriched by twofold or more during nuclear purification. The quantitative analysis of enrichment is necessary to screen out major cytoplasmic and cytoskeletal proteins present in nuclear preparations as contaminants. About 20% of the transformation-sensitive proteins are nuclear. Only three nuclear proteins were detected among the proteins induced early after serum stimulation, and none of these have been identified. Ten nuclear proteins are among the 40 major late responses to serum stimulation, and these include PCNA and lamin-B. The set of late-responding nuclear proteins is regulated quite coordinately during growth, refeeding, and serum stimulation. Furthermore, most are elevated substantially in transformed cells. A set of 11 nuclear proteins has been selected for having regulation similar to that of PCNA, and some of these, like PCNA, might be involved in DNA replication.

MANNOSE-LABELED PROTEINS

A set of 50 glycoproteins was identified by labeling with [3H]mannose and confirmed by tunicamycin treatment (which blocks glycosylation of these proteins). This set includes many secreted proteins: the heat-shock protein (hsp100), glucose-stimulated protein, and the p45 group characterized by J.F. Santaren and R. Bravo (EMBL, Heidelberg). The latter family of proteins is prominent at early times after serum stimulation but barely detectable at other times. In transformed cells, mannose-labeled proteins represent a two- to threefold lower fraction of total protein synthesis, and the responses of mannose-labeled proteins to serum stimulation are absent.

SERUM-STIMULATED PROTEINS

The proteins induced early after serum stimulation of normal cells include three families of glycoproteins (including the p45 family), one cytoskeletal protein (tropomyosin 3), and three unknown nuclear proteins. The members of this set may overlap with the products of genes identified in other laboratories by differential screening of cDNA libraries made from serum-stimulated cells. Most early-response genes found by other investigators are transiently induced. We find that nearly all members of the set of early responses have returned to normal levels by 24 hours after stimulation. Proteins induced late after serum stimulation include ten nuclear proteins, but no members of the mannose-labeled or cytoskeletal sets. The induced proteins include PCNA, lamin-B, and possibly other proteins involved in DNA synthesis or cell-cycle-related events. In transformed cells, most of the early responses to serum do not occur at all, and most members of the late-responding set are expressed at high levels regardless of the presence or absence of serum.

TRANSFORMATION-SENSITIVE PROTEINS

Proteins induced by SV40 transformation are generally also induced by adenovirus but are much less

likely to be induced by Kirsten murine sarcoma virus (Ki-MSV), a retrovirus. Proteins repressed by adenovirus transformation are usually repressed in all other transformants. Of the induced proteins, about 20% are nuclear and only 1% or fewer are cytoskeletal. The repressed proteins contain nuclear, mannose-labeled, and cytoskeletal proteins in about equal proportions.

PROTEINS REPRESSED DURING PROGRESSION TO ANCHORAGE INDEPENDENCE

SV40 transformants in different stages of progression to anchorage independence and tumorigenicity were analyzed for proteins that might correlate with progression. A set of nine proteins was selected that are synthesized at lower rates in cells of higher tumorigenicity. None of these proteins are nuclear. One was identified as tropomyosin 4, and another is a protein that responds early to serum stimulation. Most of these nine proteins are synthesized at very low rates in adenovirus-transformed cells, which is significant because no adenovirus-transformed cells were used in the selection of this set.

PROTEINS DIFFERENTIALLY REGULATED IN AN ADENOVIRUS E1B MUTANT

Of the proteins normally repressed by adenovirus transformation, 19 were much less repressed in cells transformed by an adenovirus E1B mutant. Five of these are known cytoskeletal proteins (α-sm-actin, tropomyosin 2, an isoform of vinculin, nonmuscle caldesmon, and calpactin-I). Adenovirus transformation usually represses these cytoskeletal proteins much more than do the other transforming viruses, but the mutant adenovirus seems to suppress them only to the same level as the other viruses.

AN UNUSUAL SET OF PROTEINS IN NU2 CELLS

One tumorigenic SV40 transformant (NU2) was found to contain more than 60 proteins never seen in other rat cell lines. Most of these proteins were found to comigrate with proteins in mouse 3T3 cells. In particular, both rat and mouse forms of PCNA, β-F1-ATPase, calpactin-I, and calpactin-II, seem to be present. Detailed chromosome analysis by W. Nash (H&W Cytogenetic Services Inc.) has confirmed that NU2 contains both rat and mouse chromosomes, apparently derived from a fusion event that occurred when NU2 was grown as a tumor in nude mice. Until database analysis was done, this important property of NU2 cells had never been recognized.

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

M.E. Lambert, J.I. Garrels

Studies of REF52 and human cells exposed to DNA-damaging agents have continued with the analysis of many more two-dimensional gel patterns of cells exposed to chemical carcinogens and irradiation. DNA damage is a form of cellular stress that, in normal cells, induces growth arrest, DNA-repair pathways, and yet undefined cellular responses mediated by diffusible factors. The complex pattern of changes induced by each agent in normal and transformed rat cells requires the full utilization of the REF52 database to understand which responses are related to growth arrest; which responses have to do with DNA-replication; which responses are induced by other forms of stress, such as heat shock; which responses are nuclear versus cytoplasmic or membrane-associated; and which responses are specific to particular types of damage. With the REF52 database fully assembled and analyzed with respect to our studies of growth regulation and viral transformation, the analysis of our extensive data on DNA damage can be completed. Already, it is clear that some aspects of the DNA-damage response, in particular the ability to arrest growth, are different in transformed cells and that not all of the induced responses are nuclear. Most of the induced proteins are yet unknown, but one that is known, PCNA, is both a part of the growth-arrest response and the DNA-repair mechanism.

A particular aspect of these studies that has been pursued in the last year is the induction of the major histocompatibility complex (MHC) class I proteins in human fibroblasts and keratinocytes by DNA damage. The members of this family of cell-surface proteins are induced severalfold in synthesis after treatment of cells with UV light or BPDE, a metabolically activated form of benzo[a]pyrene. Cycloheximide or the DNA-synthesis inhibitor aphidicolin induces some members of the family to much higher levels, but UV light of a nondamaging wavelength and another DNA-synthesis inhibitor, hydroxyurea, had no effect. The induction of MHC class

I was not a secondary result of growth arrest because it could be shown to occur in quiescent, nondividing cells. Cells defective in DNA repair (from patients with xeroderma pigmentosum) show even higher levels of MHC class I induction. These findings indicate that cells respond to some types of DNA damage and to the abnormal arrest of DNA synthesis in part through the altered expression of cell-surface molecules.

The Yeast Database

J.I. Garrels [in collaboration with Calvin McLaughlin, University of California, Irvine, and Jonathan Warner, Albert Einstein College of Medicine]

The goal of the yeast database is to describe the protein metabolism of *Saccharomyces cerevisiae* under conditions that are of major interest to the yeast genetics and molecular biology communities. We are constructing the database so that it will be a major resource together with the genetic map and DNA and protein sequence databases.

Our first problem was to determine how closely related the common laboratory yeast strains are to each other at the protein level. If the common strains exhibit a large number of protein-level polymorphisms, the matching of protein patterns from one strain to another strain could be very difficult. We examined the protein patterns in strains S288c, W303, A364A, D273, 10B, SKQ2N, and X2180. Although a substantial number of polymorphisms exist, we have been able to match these patterns, providing the "linker" experiment that allows subsequent data from different stains to be traced through the database. Interestingly, on the basis of the number of polymorphisms observed, we will be able to determine how related the common laboratory strains are to each other.

The identification of individual proteins in the pattern is being pursued by several different approaches. One of the most useful involves transformation by high-copy-number plasmids based on 2-μm vectors. The coefficient of variation for most spots in replicate gels is less than 25%, and thus a three- or four-fold overproduction of a protein stands out very clearly. We have used this technique to identify the proteins encoded by *URA3*, *LEU2*, and *TRP1*. Purified proteins and immunoprecipitates are being used for protein identification. We are labeling cells with each of the 20 amino acids in turn to obtain the amino acid composition of many of the proteins. These data are being standardized to ten well-resolved known spots to calibrate for pool and interconversion problems. We also plan to pursue protein identification from the two-dimensional gels for protein identification.

The synthesis, processing, and turnover of proteins have been examined by pulse-labeling and pulse-chase techniques. Although the majority of the yeast proteins are stable and made at normal rates in our shortest pulse of 30 seconds, a surprising number do show more complex kinetic behavior, indicating that they are unstable or are involved in precursor-product relationships. We are using the *rna1* mutation to determine the effective mRNA half-life for the proteins. We have initiated a systematic study of protein modification. Our initial focus is on phosphorylation, acetylation, and carbohydrate modification of proteins. To determine the subcellular localization of proteins, we have started a series of cell fractionations using labeled cells for nuclei, mitochondria, ribosomes, and cytoplasmic and membrane-bound proteins. We also anticipate a general study of nucleic-acid-binding proteins.

To interface the database with the yeast field, we are focusing our experiments on several traditional areas of interest. To identify sex-specific proteins, we have examined a, α, and diploid isogenic strains of W303 growing in glucose. Under these conditions, very few proteins are sex-specific. In contrast, we have used *rna2* mutants to determine the number of proteins with introns or whose synthesis depends on a protein with an intron, and we find that 5–10% of the proteins in yeast have introns as defined by *rna2*. In another experiment, over 50 proteins involved in the heat-shock response have been detected. This is the most complete description of the heat-shock response yet carried out in yeast.

In the coming year, we propose to examine carbon, nitrogen, and sulfur starvation; growth on fermentable and nonfermentable carbon sources to interface with gene regulation studies, and entry into and exit from G_0. We will also examine α-factor arrest, sporulation, and the meiotic and mitotic cell cycles.

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CELLULAR *TRANS*-ACTIVATORS OF GENE EXPRESSION

B.R. Franza J.M. Ross
S. Losse

For the past 3 years, our effort has been focused on developing a strategy for both qualitative and quantitative analyses of cellular proteins that interact with genetically defined nucleic acid sequence elements. The long terminal repeat (LTR) region of the human immunodeficiency virus type 1 (HIV-1) was chosen for initial investigation. This region has been shown to mediate the inducible expression of either the viral genome itself or genetic recombinants consisting of the LTR linked to different "reporter" genes. From the outset, it was anticipated that more than one protein would associate with specific sequence elements. It was considered likely that proteins would be found that interact with such elements by complexing with the proteins that directly contact the nucleic acid. Therefore, the strategy had to permit evaluation of both direct and indirect association of proteins with specific control elements. The strategy was designed not only to identify such proteins, but also to allow the subsequent study of the regulation of each protein throughout experimental simulations of normal cellular growth and/or development. Database analysis is the system that will be used to accomplish this task. Simply described, database analysis provides the means to quantify and link experimental observations of any cellular protein resolved by two-dimensional gel electrophoresis. The following is a simple description of the strategy and a discussion of the results of its application to the study of two control regions in the HIV-1 LTR.

MICROSCALE DNA-AFFINITY PRECIPITATION ASSAY

The basic principle of the strategy is to analyze directly protein–nucleic acid interactions yielding quantifiable information on the proteins involved. To accomplish this task, an assay was developed that utilizes fragments of nucleic acid that can be recovered from a solution of proteins by virtue of the high affinity that avidin has for any molecule containing a biotin group. A biotin molecule is covalently attached to the nucleic acid fragment. The fragment is mixed with the proteins and recovered from the solution by addition of avidin that is linked to an insoluble matrix. The proteins are then eluted from the matrix and separated on high-resolution, two-

dimensional (HR2D) gels. The two-dimensional array of spots, each spot representing distinct proteins, can be detected, quantified, and entered into the database analysis environment for study. The assay structure is highly flexible in that it permits controlled alterations of the numerous cell-growth conditions, extraction conditions, and reaction parameters required to dissect thoroughly the potential each control element has for interacting with different cellular proteins. Such flexibility is also required to characterize the differential rates of synthesis, posttranslational modification, and turnover of the proteins associating with a specific sequence element. The assay is accomplished on a small scale (1 ml or less reaction volumes) and is rapid (no more than 2 hr from time of extracting the cellular proteins to having them ready for gel separation). Either synthetic oligonucleotides or restriction fragments containing the control element(s) to be investigated have been used successfully in the assay. The results discussed below involved the study of control elements present on double-stranded DNA; however, assays of protein interactions with single-stranded DNA and RNA have also been studied.

HIVEN86A IS AN INDUCIBLE CELLULAR PROTEIN THAT INTERACTS SPECIFICALLY WITH THE HIV ENHANCER

HIV, like other retroviruses, integrates into the genome of the cell it infects. It actually becomes part of the cellular DNA, and it is capable of responding to extracellular stimuli much like many cellular genes. Elements, coded within the DNA in the LTR, mediate this responsiveness. One of these elements (see Fig. 1, −105 to −80) is referred to as an enhancer and confers inducible expression to the genes linked to it. Enhancer activity of a sequence element means that it contributes in a positive way to the promotion of transcription of functionally linked genes. Inducible expression means that the linked gene is either wholly or partly dependent on certain signals for transcription of new mRNA. We have identified several cellular proteins, produced in human T-lymphoblast cells, that interact specifically with the HIV enhancer. We have used synthetic oligonucleotides representing either the "wild-type" enhancer or mutants thereof to detect these proteins. *Some* of these proteins, expressed in the H9 cell line ($CD4^+$, supports replication of HIV), are shown in

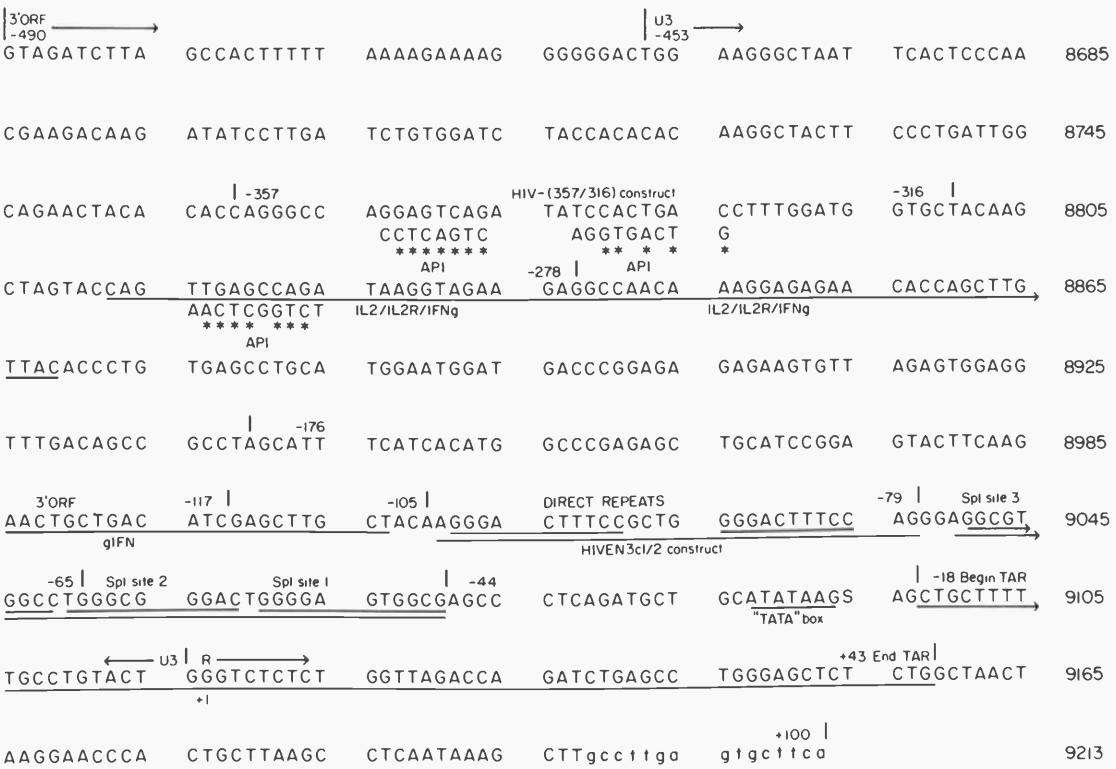

FIGURE 1 A schematic of the HIV-1 LTR with various elements and sequence similarities noted.

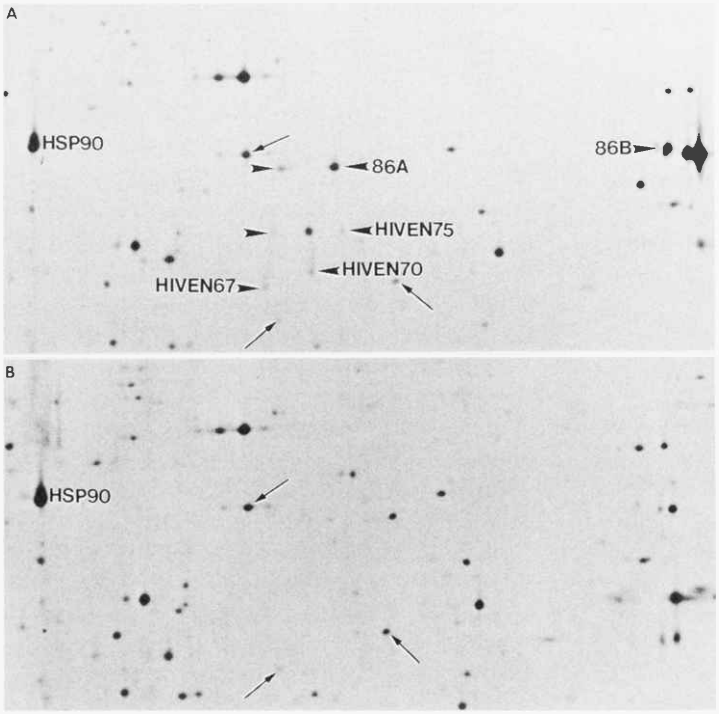

FIGURE 2 HIV-1 enhancer-associated proteins isolated from human T-lymphoblast cells. (*A*) Proteins isolated on the HIV enhancer (HIVEN) wild-type probe; (*B*) proteins isolated on the HIV enhancer mutant probe (for details, see Franza et al. *Nature 330:* 391 [1987]).

Figure 2A. We now know that the protein designated 86A in the figure (referred to as HIVEN86A for HIV enhancer-binding protein of 86-kD apparent molecular size) is an inducible protein. In certain lymphoblast lines, grown under normal conditions, HIVEN86A is virtually nondetectable. However, when these cells are stimulated by agents that induce maturation of human T lymphoblasts, HIVEN86A is expressed early-on, and associates with the HIV enhancer probe. The expression of HIVEN86A is rapid enough to be a transducer of activating signals to responsive, normal cellular genes, such as the interleukin-2 receptor (IL2R) gene, as well as to control elements of the integrated HIV provirus. Work is in progress to determine if HIVEN86A functions to activate growth and/or differentiation control genes. We do know that its expression is positively correlated with early production of both HIV mRNA and IL2R mRNA (in collaboration with W. Greene, Duke University Medical School, Durham, North Carolina).

More than one protein is found to interact specifically with the HIV enhancer as shown in Figure 2A. One of these proteins, 86B (HIVEN86B), is found only in H9 cells. It is not inducible in the other human lymphoblasts studied thus far. The other proteins shown in Figure 2A are variably detected in several different human lymphoblast cell lines and appear to be responsive to certain extracellular stimuli. The specificity of the interaction of each of these HIV enhancer proteins is indicated by the fact that they do not associate with the mutant construct of the enhancer element (compare panels A and B in Fig. 2). One exception is that small amounts of HIVEN86B still associate with the mutant construct. Given the fact that only three of the ten bases in the HIV enhancer core element were altered in the mutant construct, it may be that HIVEN86B is less dependent than the others on those bases for binding. The surrounding proteins, seen in both panels, nonspecifically associate with the matrix and are therefore seen independent of whatever biotinylated nucleic acid is added to the reaction. They are useful in two respects: (1) They provide a means for normalizing any set of DNAP assays and (2) they assist in alignment of one HR2D gel image with any other HR2D gel image.

ANOTHER SET OF INDUCIBLE CELLULAR PROTEINS ASSOCIATES WITH HIV-1 LTR CONTROL ELEMENTS DISTINCT FROM THE ENHANCER

A region in the HIV-1 LTR (−360 to −278, see Fig. 1) was noted to contain three sequence elements simi-

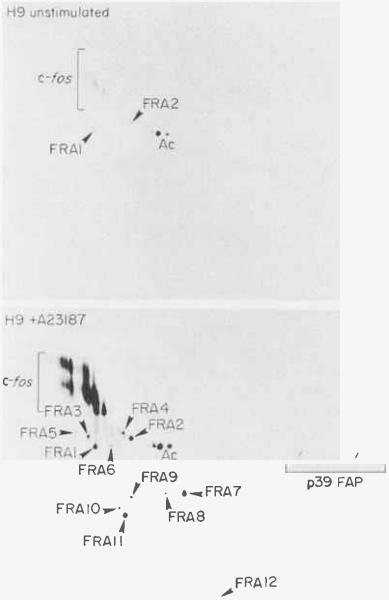

FIGURE 3 Human T-lymphoblast proteins immunoprecipitated by an anti-Fos peptide antibody. (*A*) Proteins immunoprecipitated from unstimulated H9 cells; (*B*) proteins immunoprecipitated from calcium ionophore (A23187)-stimulated cells (for details, see Franza et al., *Science 239:* 1150 [1988]).

lar to the recently described HeLa cell transcriptional activator protein 1 (AP-1) site. This region had been partly characterized as a negative regulatory unit by other investigators. It is not clear how many different types of activities might be mediated by this region, since only one experimental setting has been explored thus far. Specifically, these investigators studied the expression of a linked reporter gene several hours after its transfection into tissue-culture cells. Our interest in this region resulted from the observation that the product of the cellular proto-oncogene, c-*fos*, was reported to associate with a control element from a differentiation-sensitive adipocyte gene. I noticed that this element contained a site similar to the AP-1 site in the human metallothionein II gene, the SV40 enhancer, the gibbon ape leukemia virus enhancer, the c-*fos* promoter, and the HIV-1 LTR. T. Curran (Roche Institute, Nutley, New Jersey) and I had successfully collaborated on the HR2D gel analysis of the complex modifications of the c-*fos* product (Fos), the numerous Fos-related antigens, and the Fos-associated proteins, including the different isoforms of p39. Along with F. Rauscher III, a postdoctoral fellow in T. Curran's laboratory, and S.F. Josephs at the National Cancer Institute, we began a detailed DNAP analysis of those cellular proteins that interacted with AP-1 sites in several different viral and cellular control regions. It quickly became clear that Fos, several Fos-related antigens, and the Fos-associated protein (FAP) p39 were recovered on probes containing intact AP-1 sites. None of these inducible cellular proteins associated with the HIV enhancer probes, confirming not only the specificity of the assay, but also the fact that the same agent could induce entirely different cellular proteins that could be distinguished by their association with distinct control elements.

An example of the proteins immunoprecipitated with an antisera raised against a region of the Fos protein is shown in Figure 3. In uninduced H9 cells, some Fos, certain Fos-related antigens (FRA), and barely detectable amounts of p39 were recovered and

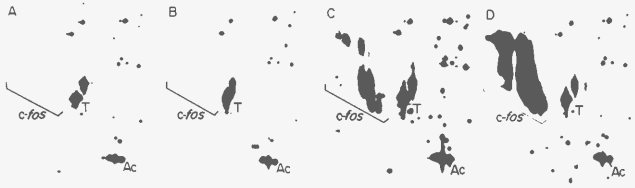

FIGURE 4 Human T-lymphoblast proteins that interact with AP-1 sites. (*A*) Proteins recovered from control reaction containing no biotinylated (b-) probe; (*B*) proteins recovered from a control reaction containing b-HIV enhancer probe; (*C*) proteins recovered from a reaction containing b-HIV AP-1 probe; (*D*) proteins recovered from a reaction containing a b-probe containing the AP-1 site in the fat-specific element from the adipocyte aP2 gene. All extracts were obtained from H9 cells stimulated with calcium ionophore, A23187, using conditions identical to those used in preparing extracts for immunoprecipitations as seen in Fig. 3. For details, see Franza et al. (*Science 239:* 1150 [1988]).

resolved on HR2D gels (Fig. 3A). On induction with the calcium ionophore, A23187, considerable increases in Fos, FRAs, and FAP p39, or the tumor promoter, PMA, are easily detected (Fig. 3B). FRA refers to the fact that the antibody can still immunoprecipitate these proteins even after the extracts containing them have been denatured by boiling them in the presence of the detergent, SDS. FAP indicates that p39 is immunoprecipitated only because it is complexed with Fos or one of the FRAs. Figure 4 presents the results of four different DNAP assays on similarly treated H9 cells. In Figure 4A, no biotinylated probe was added, and in Figure 4B, biotinylated HIV enhancer probe was added to the reaction. In both cases, although nonspecific proteins are detected on the HR2D gels, no Fos is detected. In Figure 4, C and D, different probes containing AP-1 sites were added to respective reactions, and it is clear that the heterogeneous population of modified forms of Fos are recovered in each case. Figure 5, A and B, shows that not only was Fos recovered by the probes containing AP-1 sites, but FAP p39 was also recovered. More extensive analysis revealed that several FRAs also associated with the AP-1 sites. Therefore, a set of inducible cellular genes encode proteins that associate with the same nucleic acid sequence element. This element has been shown to confer inducibility to heterologous promoters. In fact, this element resides in the promoter of the c-*fos* gene itself and has been shown to interact with Fos (the functional significance of which is being pursued with M. Gilman [Molecular Genetics of Eukaryotic Cells Section]).

Activation of Fos occurs in response to diverse stimuli. The process is typically very rapid and short-lived. However, the consequences, largely dependent on cell type and growth state, are remarkably varied. They include changes in growth status, differentiation status, and nerve cell depolarization. The demonstration of Fos association with a transcriptional control element suggests that Fos may exert effects on the regulation of different sets of cellular and viral genes. The facts that Fos is associated with other inducible cellular proteins, such as FAP p39, and that antigenically related proteins are also inducible and capable of recognizing AP-1 sites indi-

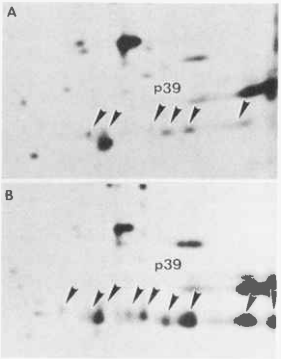

FIGURE 5 Fos-associated protein (p39) associates with AP-1 sites. (*A*) Region from the HR2D gel in which p39 migrates; the sample is the same as that presented in Fig. 4C. (*B*) Region from the HR2D gel in which p39 migrates; the sample is the same as that presented in Fig. 4D.

cate a very flexible although complex mechanism for differential regulation of gene expression.

None of these observations would have been made as directly and quantifiably as they have been without the use of DNAP assays in conjunction with HR2D gels. We suspect that this strategy will be useful in the detailed examination of protein-protein and nucleic acid interactions that is expected to lead to an exposition of the mechanisms controlling gene expression.

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STRUCTURE

This section includes three laboratories interested in the detailed structural properties of proteins. The Structure program is a new one that was organized when Drs. Jim Pflugrath and John Anderson joined Cold Spring Harbor Laboratory to set up a new X-ray crystallography laboratory. The other two groups that are associated with this program are those of Dr. Mark Zoller, whose laboratory is interested in the structure-function relationships of protein kinases, and Dr. Rich Roberts, whose laboratory has had a long-standing interest in restriction enzymes and their associated methylases.

The impetus to establish a Structure program came from a growing need for more detailed structural information about many of the proteins that are being studied by investigators at Cold Spring Harbor Laboratory. It has become apparent, both here and elsewhere, that a full interpretation of the results of experiments at the DNA and RNA levels requires the complementary information that can be obtained from protein biochemistry, site-specific mutagenesis, and structural investigations. Increasingly, we find that promising lines of research are being hindered by the absence of detailed structural information about proteins. At present, such information can only be obtained by rigorous protein purification, crystallization, and X-ray analysis.

Fortunately, advances in recombinant DNA technology now allow the ready cloning and overexpression of proteins of interest. This combined with new methods for purification offers the possibility that many proteins important for our work can now be obtained in quantities sufficient to permit their crystallization. The time is right to capitalize both on the new technology and on the possibilities for analysis and understanding that can be afforded by X-ray analysis. Over the next few years, we anticipate growing interactions between the members of the Structure program and all other members of the laboratory.

NUCLEIC ACID CHEMISTRY

R.J. Roberts A.S. Bhagwat, C. Marcincuk, J. Martling, J. Meyertons, B. Mollet, J. Posfai, A. Sohail, M. Wallace, K. Weule

Role of *Dcm* in Very Short Patch Repair

A.S. Bhagwat, A. Sohail [In Collaboration with M. Lieb, University of Southern California Medical School]

Mismatches in DNA such as

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-CTAGG-          -CTTGG-
-GGTCC-    or    -GGACC-
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are repaired in *Escherichia coli* in favor of the lower strand creating CC(A/T)GG sites. This process is referred to as very short patch (VSP) repair. The sequence generated by the repair process is the site of the only known DNA cytosine methylase in *E. coli*—*Dcm*. This protein methylates the second cytosine in the sequence at position 5. 5-Methyl-cytosines created by *Dcm* deaminate to thymine at a significant rate. This generates T/G mismatches that are the normal substrates for VSP repair. In other words, VSP repair is likely to prevent mutations that could arise by the deamination of 5-methyl-cytosine.

We cloned the gene that codes for *Dcm* a few years

ago and mapped it within the clones by BAL-31-mediated deletions. The ability of various deletions to complement a methylation-defective mutation in the chromosomal *dcm* gene (*dcm*6) was tested for this purpose. The information required for *dcm* expression was mapped within a 1.7-kb piece of DNA.

Recently, three groups showed that the *dcm*6 mutation leads to a defect in the VSP repair ability of the cell. The simplest explanation of this result is that *Dcm* is required for VSP repair. It has been suggested that *Dcm* could play a role in the recognition of T/G mismatches that are the substrate for VSP repair. Hence, we tested the BAL-31-generated deletions in a *dcm* clone for their ability to complement the VSP repair defect of *dcm*6.

Three classes of phenotypes were found for the deletions (Fig. 1). All methylation-defective (M^-) constructs were unable to complement the VSP repair defect of *dcm*6: They were VSR^-. This is consistent with the suggestion that *Dcm* is required for VSP repair. Most methylation-proficient (M^+) constructs were repair-proficient (VSR^+), but, unexpectedly, two M^+ constructs were found to be VSR^-. These contained the two smallest chromosomal inserts among the M^+ constructs (Fig. 1).

To understand the situation better, we sequenced the smallest chromosomal insert that had the phenotype M^+ VSR^+ (pDCM23). We also determined the endpoints of various BAL-31 deletions for which the methylation and repair phenotypes were already known. Fragments from various BAL-31 deletion constructs were cloned in bacteriophage M13 vectors and sequenced using the dideoxy chain-termination method.

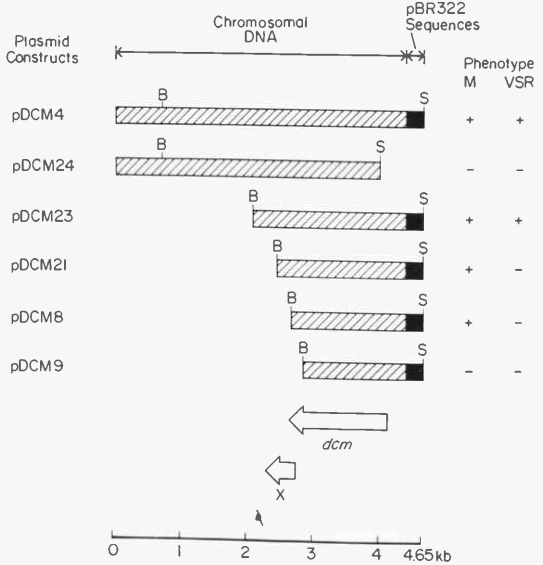

FIGURE 1 Phenotype of BAL-31 deletions in the *dcm* region. BAL 31 was used to generate deletions from either the *Bam*HI site or the *Sal*I site in pDCM4. Chromosomal DNA (striped lines) and a small segment of the vector, pBR322, are shown (heavy lines). Phenotypes M and VSR refer to the ability of the plasmids to complement the *dcm*6 mutation for methylation of CC(A/T)GG sites and for VSP repair, respectively. Open arrows represent the ORFs in the insert within pDCM23, as deduced by DNA sequencing. (B) *Bam*HI; (S) *Sal*I.

The largest open reading frame (ORF) in this sequence codes for a protein of 472 amino acids, and nearly all the BAL-31 deletions that truncate this ORF are M^-. The sequence of this ORF can be aligned with the sequence of the *Eco*RII methylase, which has the same sequence specificity as *Dcm*. On the basis of these criteria, we believe that this ORF is the *dcm* gene.

The plasmid pDCM21 contains all of the *dcm* gene, but has the phenotype M^+ VSR^-. Therefore, *dcm* is not sufficient for complementing the VSP repair defect of the *dcm6* mutation. Within the sequenced DNA, there exists a second reading frame partially overlapping with *dcm* and on the same strand as *dcm*. The protein coded by this ORF (named X temporarily) may also be required for VSP repair (Fig. 1). All BAL-31-generated deletions from the left that truncate or remove ORF X have the phenotype VSR^-. Deletions from the right presumably eliminate the common promoter of *dcm* and ORF X and lead to the M^- VSR^- phenotype regardless of the length of the deletion. Finally, we have made a construct that removes a restriction fragment internal to *dcm* that has the phenotype M^- VSR^+. This deletion does not affect the region 5′ to *dcm* or the ORF X. It is therefore possible that the presence of functional product X may be sufficient to complement the defect in VSP repair in a *dcm6* background. The *dcm6* mutation may be pleiotropic, i.e., affect both *dcm* and gene *X*.

We have apparently stumbled upon a new DNA repair gene in *E. coli*. The product of this gene appears to be essential for VSP repair, and its expression may be from the same promoter as *dcm*. If so, product X would be produced whenever *Dcm* is produced. Therefore, increased cytosine methylation in the cell would go hand in hand with increased VSP repair and C→T transitions would be avoided.

Hybrid Cytosine Methylase Genes

J. Meyertons

DNA methylases that transfer a methyl group to position 5 in cytosine share long stretches of sequence similarities. In addition, each methylase possesses at least one segment of approximately 100 amino acids that is unique. It is possible that the shared sequences among methylases represent common functional domains for the methyl transfer reaction, whereas the unique segment is involved in the recognition of the target DNA sequence. To identify such protein domains, fusions are being made between the *Hha*I and *Hpa*II methylases (recognition sequences G*CGC and C*CGG respectively, where *C is 5-methylcytosine). Over 40% of the residues in the two enzymes are identical, and the regions of amino acid sequence similarity between these two methylases appear in the same order in each protein. Hybrid methylases are being constructed by exchanging the functionally equivalent segments of the *Hha*I and *Hpa*II methylase genes. The two genes are being placed next to each other, and nuclease BAL-31-generated deletions will be used to fuse them. Methylase activity expressed by the resulting hybrids will be assayed by testing the resistance of plasmid DNA to either *Hha*I or *Hpa*II restriction endonuclease cleavage. The hybrid enzyme constructions could potentially generate a novel methylase capable of recognizing a new sequence. These studies will help to define the nature of DNA-cytosine methylase recognition of DNA.

Studies of the *Msp*I Methylase

B. Mollet

The *Msp*I restriction-modification system recognizes the sequence 5′-CCGG-3′. The complete system has been previously cloned and sequenced (P.-M. Lin and R.J. Roberts, in prep.). A fragment containing the methylase gene was subcloned next to a fragment containing the inducible pTac promoter, and this derivative overproduced the methylase to high levels. Our recent efforts have concentrated on developing a scheme for the purification of the *Msp*I methylase to homogeneity. The first problems encountered during purification resulted from the high levels of expression. It was found that inclusion bodies were present in *E. coli* cells expressing the methylase (Fig. 2). As a result, the methylase was severely aggregated and came out of solution very readily. For instance, attempts to remove nucleic acids by streptomycin sulfate precipitation resulted in the removal of most of the *Msp*I methylase protein. However, the methylase could be resolubilized by mild detergent treatment and was in fact considerably purified by this step. Further purification was achieved by DEAE-cellulose chromatography, oligonucleotide affinity chromatography, and FPLC on a Mono-Q support. The oligonucleotide affinity column was prepared from a polymerized oligonucleotide

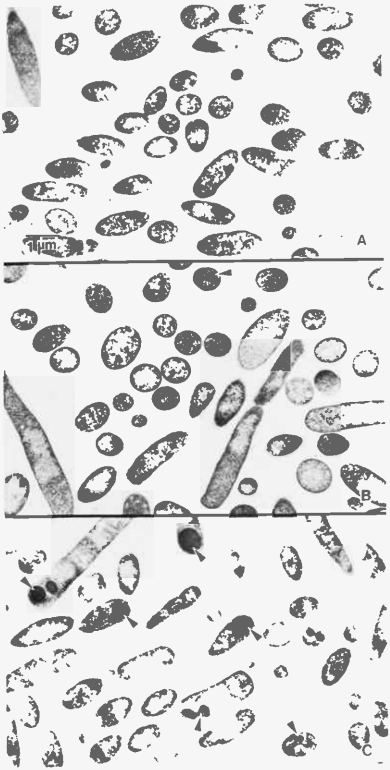

FIGURE 2 An electron micrograph of *E. coli* cells overproducing the *MspI* methylase. The dark objects, indicated by arrows, within many of the cells are well-formed inclusion bodies that are believed to contain the bulk of the methylase protein. We thank D. Spector (Cell Biology Section) for this figure.

(CCAGGCCGGCCTGGCCGG) containing the recognition sequence, which had been coupled to cyanogen-bromide-activated Sepharose 4B. It proved to be a very powerful step in the purification. This is the first time that oligonucleotide affinity columns have been used for the purification of methylases, and our results suggest that it will prove to be an extremely powerful method in other cases. We are currently trying to solve the remaining problems of aggregation and to remove the final impurities so that its crystallization might be attempted.

Computer Programs to Analyze DNA and Protein Sequences

J. Posfai

Many DNA sequences are available for the genes encoding restriction enzymes and their cognate methylases. In addition, several sequences are available for individual methylases. Among the restriction enzymes, comparisons reveal no obvious homologies that can be related to function. However, comparisons of the primary sequences of 13 bacterial DNA methyltransferases, which catalyze the formation of 5-methylcytosine, have been more rewarding. The overall similarity of the 13 sequences is not sufficient to allow any of the existing programs for multiple sequence alignment to produce a satisfactory global alignment. Consequently, pairwise dot-matrix comparisons were carried out, and individual alignments were produced. These formed the basis for a global alignment of all 13 sequences. Five regions of close local similarity were used as initialization points for this alignment, which was mainly carried out manually. Each of these conserved regions was also used in the PROFILE method (Gribskov et al., *Proc. Natl. Acad. Sci. 84:* 4355 [1987]) to build position-dependent weight matrices (Profiles). This approach could then produce a global alignment and allowed the manual alignment to be improved. Using these profiles as motifs of cytosine methylation, we have found that they clearly differentiate the known cytosine methylases from all other sequences in the PIR library. This approach is being extended in several ways. We have developed a set of new algorithms that allow us to experiment with motif construction and to use these motifs for predictive purposes. We have also developed routines that use consensus sequences to define relatedness and that will extract related sequences from protein databases. A database of protein sequence motifs is being set up that will facilitate the analysis of newly determined sequences. We are presently searching for motifs that will uniquely describe methylases that form N^6-methyladenosine and N^4-methylcytosine. Further experiments are aimed at defining motifs that may be of general use in distinguishing proteins that interact with *S*-adenosylmethionine.

Restriction Endonucleases

R.J. Roberts, J. Martling, K. Weule

The collection of restriction endonucleases continues to grow, and more than 930 such enzymes have now been isolated and characterized. 135 different specificities have been discovered among the type II enzymes. During the last year, more than 30 new enzymes have been isolated and characterized as part of a collaborative program with I. Schildkraut and D. Comb (New England Bio-Labs). Among these are seven valuable new specificities, including two enzymes (*Mse*I [TTAA] and *Ase*I [AATTAAT]) that recognize sequences containing only A or T within the recognition sequence. Of the 16 possible tetranucleotide palindromes, enzymes that recognize 13 of these sequences now exist (Table 1).

TABLE 1 Simple Tetranucleotide Palindromes

AATT	ACGT	AGCT	ATAT
*Tsp*EI	*Mae*II	*Alu*I	
CATG	CCGG	CGCG	CTAG
*Nla*III	*Hpa*II	*Fnu*DII	*Mae*I
GATC	GCGC	GGCC	GTAC
*Mbo*I	*Hha*I	*Hae*III	*Rsa*I
TATA	TCGA	TGCA	TTAA
	*Taq*I		*Mse*I

For many years, our group has been maintaining databases of information about restriction enzymes and methylases. Some of this information has been maintained in an electronic database manager, INFORMIX, that has allowed easy access, update, and dissemination of a small part of the information. However, a great deal of information was maintained in unlinked files and could only be accessed with difficulty. Recently, the Laboratory has acquired a new electronic database system called ORACLE, and a major effort has been under way for the last few months to transfer all of the restriction enzyme information into a single relational database managed by the ORACLE software. Once the transfer is complete and the appropriate user interfaces are written, access to information about restriction enzymes, their recognition sequences, commercial availability, etc., will be very much simplified. Not only will the information be available locally, but its dissemination through BITNET and other electronic networks can be automated. In particular, the specific data that are required by computer programs searching sequences for restriction enzyme recognition sites will be quickly available in electronic form.

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MACROMOLECULAR CRYSTALLOGRAPHY

J. W. Pflugrath R.M. Blumenthal A. Caleca J. Tropp
J. E. Anderson W. Brady T. Knapp J. Walter
J. Cairl J. Scuderi

A major objective of the Macromolecular Crystallography Group is to determine the structures of a number of biologically important proteins to atomic resolution in order to better understand their functions in cell processes such as growth control and development. Our studies are designed to benefit from and to complement the genetic and biochemical experiments performed by other groups at the

Laboratory. Together with these biochemical data, crystallographic models of protein kinases, oncogene products, and *trans*-acting factors will help us comprehend the roles of these macromolecules in cell division, in cell transformation, and, ultimately, in diseases such as cancer and acquired immunodeficiency syndrome (AIDS).

Our crystallographic experiments require well-formed crystals of the macromolecule being investigated. To grow crystals of a useful size (>0.3 mm on a side), one must have available several milligrams of the highly purified macromolecule. Although this does not guarantee that crystals can be produced, it is the minimal prerequisite before attempting to crystallize the molecule. To this end, a major part of our laboratory and time is devoted to producing and purifying to crystallographic homogeneity the proteins we are studying.

Once crystals are available, we are poised to collect and evaluate diffraction data on our Enraf-Nonius area detector. Installed this past year and controlled by software developed in our laboratory, it has yielded excellent data on test crystals of lysozyme in a fraction of the time required by normal diffractometer or film methods. Data are further analyzed on one of our three MicroVAX computers, and atomic models are built with the aid of our Evans & Sutherland PS 350 interactive three-dimensional computer graphics system.

Structure Determination of Yeast *RAS2*

J.W. Pflugrath, J. Scuderi, J. Tropp [in collaboration with J. Field and M. Wigler, Cold Spring Harbor Laboratory]

RAS2 from *Saccharomyces cerevisiae* is a structural and functional homolog of the mammalian *ras* oncogene product (see M. Wigler, Molecular Genetics of Eukaryotic Cells Section). We wish to compare the atomic structure of the yeast protein and the recently determined structure of human c-Ha-*ras* p21 (de Vos et al., *Science 239:* 888 [1988]). Because of amino acid sequence homology, we predict that the structure of yeast *RAS2* is very similar to that of human *ras*. We expect to see differences also, since the mammalian protein contains only 189 amino acids, whereas that of yeast has 322 amino acids. Of particular interest to us is the second half of the *RAS2* molecule, which contains the "extra" 130-odd residues. From an *Escherichia coli* expression system developed in M. Wigler's laboratory, we are currently isolating *RAS2* in sufficient quantity and purity to begin crystallization experiments.

Yeast cAMP-dependent Protein Kinase

J.W. Pflugrath, J. Scuderi [in collaboration with J. Kuret and M. Zoller, Cold Spring Harbor Laboratory]

We are investigating the structure of the yeast cAMP-dependent protein kinase in a complementary collaboration with M. Zoller and colleagues. The holoenzyme consists of a dimer of catalytic subunits (*TPK1*) and a dimer of regulatory subunits (*BCY1*). The results from X-ray crystallography will ideally complement the genetic results of Zoller's group, which highlight the nature of the protein-protein interactions among the four polypeptide chains. Our efforts are currently concentrated on obtaining the amounts of *BCY1* needed for crystallization. We are using the *E. coli*-expressed *BCY1* and a purification procedure based on a cAMP-agarose affinity column developed in the laboratory of M. Zoller (Johnson et al., *J. Biol. Chem. 262:* 8636 [1987]). When purification procedures for the holoenzyme or catalytic subunit are worked out, we intend to crystallize them also.

In closely related work with J. Kuret, we have obtained crystals of a deletion mutant of the regulatory subunit, *BCY1* ΔI, that are unsuitable for X-ray diffraction experiments. *BCY1* ΔI inhibits the catalytic subunit with the same kinetics as the wild-type regulatory subunit, yet cannot form dimers. We are trying other methods of crystallization in order to attain improved crystals.

Area Detector Software

J.W. Pflugrath [in collaboration with A. Messerschmidt, Max-Planck-Institut für Biochemie]

Many laboratories including our own have developed or purchased state-of-the-art area detectors in order to collect and evaluate X-ray diffraction data from macromolecules at a rate never before possible with either film methods or automated diffractometers. One impediment to the optimal use of these devices has been the lack of adequate software to control them. We have continued to develop our device-independent software system to fulfill this need. We

have improved our MADNES area detector program in a number of areas including searching, autoindexing, prediction and evaluation of reflections, and user-friendliness. Our success can be measured by the growing list of users that now include, among others, the MRC Laboratory of Molecular Biology, Cambridge; the EMBL, Heidelberg; the Department of Chemistry, Princeton University; the Department of Chemistry, University of California, Berkeley; the Rosenstiel Science Center, Brandeis University; the Laboratory of Molecular Biology, Birkbeck College, London; the Blackett Laboratory, Imperial College, London; and the Laboratory of Chemical Physics, University of Groningen, The Netherlands.

Purification of Bacterially Expressed c-*myc* Protein

J. Anderson, J. Cairl [in collaboration with N. Sullivan, Cold Spring Harbor Laboratory, and R. Watt, Smith Kline & French]

Acquisition of the three-dimensional structure of the c-*myc* protein will be a major advance in the study of this proto-oncogene product. The structure will be an invaluable aid to understanding c-*myc* function and perhaps to understanding the function of other nuclear oncogenes. As a step toward our goal of determining this structure, we purified overexpressed recombinant human c-*myc* protein from *Escherichia coli*. The bacteria expressing the c-*myc* protein were found by D. Spector (Cell Biology Section) to contain intracellular inclusion bodies corresponding to the c-*myc* protein. Accordingly, the c-*myc* protein is recovered in the insoluble pellet when the cell lysate is centrifuged. The purification involves detergent extraction of the pellet to eliminate some contaminants, urea solubilization of the c-*myc* protein, and anion-exchange chromatography, followed by removal of urea by dialysis. When this soluble c-*myc* protein, which is about 90% pure, was chromatographed on a Sephacryl S-200 column, it eluted in the void volume, indicating that it has an apparent molecular mass exceeding 250 kD, corresponding to at least three or four c-*myc* molecules.

We analyzed samples of this aggregated c-*myc* protein by SDS-PAGE, using sample buffer with or without dithiothreitol (DTT) as a reducing agent. The sample containing DTT had as its main component the 66-kD polypeptide corresponding to monomeric c-*myc* protein. The sample without DTT, however, had in addition to the 66-kD protein (which was present at lower levels than in the +DTT sample) a very high-molecular-weight polypeptide. Thus, the c-*myc* protein purified as we have described exists predominantly as a large aggregate that is at least partially mediated by *inter*molecular disulfide bonds. It is unlikely (although not impossible) that c-*myc* protein normally has intermolecular disulfide linkages, especially since at least one other protein that forms inclusion bodies when overexpressed in *E. coli*, prochymosin, is also cross-linked by disulfides (Schoemaker et al., *EMBO J. 4:* 775 [1985]). Some of the cysteine residues involved in these cross-links might participate in *intra*molecular disulfides in the native c-*myc* protein, so there is a good chance that the c-*myc* protein we have prepared is incorrectly folded. For structural studies, we need homogeneous native protein, and so we have decided to develop an alternative purification procedure.

We are currently working to increase the proportion of soluble c-*myc* protein produced in vivo, in collaboration with K. Chan and D. Spector (Cell Biology Section). If disulfide cross-links form in inclusion bodies, as suggested by Schoemaker et al. (*EMBO J.4:* 775 [1985]), then soluble protein that does not form inclusion bodies should not be cross-linked and would be more likely to be properly folded. Increasing the amount of soluble protein may require simply reducing the level of overexpression from the existing vector, or it might require changing the expression system and possibly the host organism as well. Once we have accomplished this, we will develop a protocol to purify the c-*myc* protein to homogeneity for crystallization and structural analysis.

Structural Studies of Restriction-modification Enzymes

J. Anderson, R.M. Blumenthal, J. Walter, A. Caleca [in collaboration with F. Barany, Cornell University Medical College, and I. Schildkraut, New England Bio-Labs]

Another aspect of our research concerns restriction endonucleases and methylases. These enzymes recognize specific DNA sequences and then cleave or methylate the DNA, respectively. Methylation by a particular methylase protects the site from cleavage by the corresponding endonuclease. Our goal is to solve the structures of several of these enzymes both

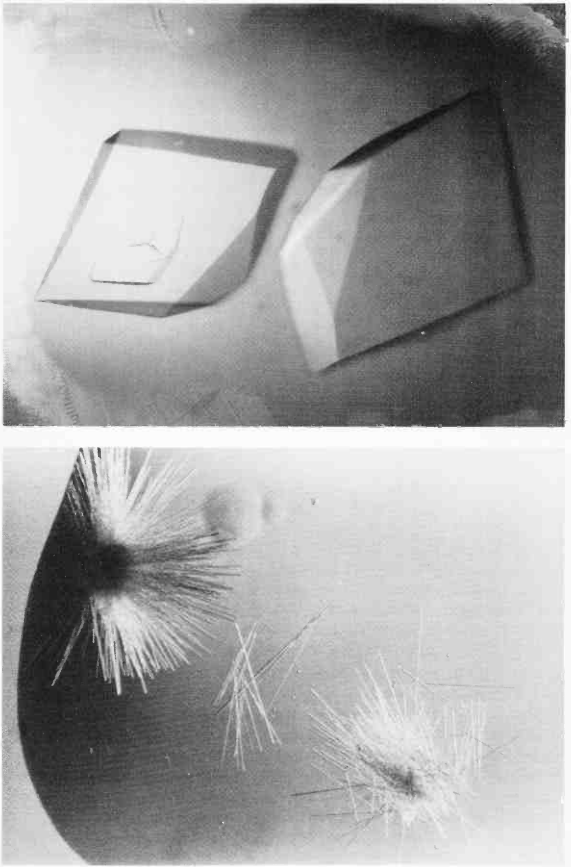

FIGURE 1 (*Top*) Crystals of *Hin*dIII endonuclease, each about 0.5 mm across, grown from 2.4 M ammonium sulfate at pH 7.8 and room temperature. (*Bottom*) Cocrystals of *Hin*dIII endonuclease and the oligonucleotide duplex

GTACAAGCTTCAT
ATGTTCGAAGTAC

grown from 1.75 M ammonium sulfate at pH 7.0 and room temperature. Rods are 0.6–0.7 mm long.

as free proteins and in complexes with oligonucleotides carrying their recognition sites. These structures will help us to understand how specific DNA sequences are recognized by these proteins and will also help us to understand their enzymatic mechanisms.

*Hin*dIII ENDONUCLEASE

These studies were carried out by J. Anderson, A. Caleca, J. Walter, and I. Schildkraut. The *Hin*dIII endonuclease recognizes the site AAGCTT and cleaves after the first adenine. Large, well-formed crystals of the endonuclease (Fig. 1, top) grow from ammonium sulfate at pH 7.8, but these crystals diffract at best only to about 7-Å resolution, which would not provide adequate structural detail. We are currently scanning for conditions that give crystals that diffract to higher resolution.

We have also set up cocrystallizations of the *Hin*dIII endonuclease with oligonucleotides carrying its recognition site. We have obtained several crystal forms, but none are yet large enough for X-ray analysis. One of the crystal forms is shown in Figure 1 (bottom). We are searching for conditions that will give larger cocrystals.

*Taq*I ENDONUCLEASE

These studies were carried out by J. Anderson, A. Caleca, and F. Barany. The *Taq*I endonuclease, from the thermophilic bacterium *Thermus aquaticus*, recognizes the DNA sequence TCGA and cleaves after the thymine. We have obtained two crystal forms for *Taq*I endonuclease, neither of which is yet large enough for X-ray analysis. Cocrystallization attempts have yielded very tiny needle crystals. We are continuing to search for conditions that will produce larger crystals both of the protein alone and of the protein with DNA.

Both *Taq*I and *Hin*dIII endonucleases are contaminated with small amounts of other polypeptides, some of which may be breakdown products of the intact molecules. Removal of these impurities might improve our chances of growing quality crystals of these proteins, and thus we are attempting to develop purification protocols to accomplish this.

*Pvu*II METHYLASE

These studies were carried out by R.M. Blumenthal and J. Walter. The *Pvu*II methylase recognizes the sequence CAGCTG, and methylates the internal cytosine at the N4 position. We have successfully overexpressed the methylase in *E. coli*. The overproducing strain expresses about 10% of the total soluble protein as *Pvu*II methylase. Most of the methylase protein stays in the supernatant when a whole-cell lysate is centrifuged. The methylase remains soluble when up to 1% polyethyleneimine is added as a precipitant to the supernatant of the cell lysate. Subsequent ion-exchange chromatography on DEAE-Sephacel yields protein that is about 75% pure. We are devising further steps to purify the methylase to homogeneity for crystallization studies.

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SITE-SPECIFIC MUTAGENESIS

M. Zoller K. Johnson L. Levin J. Kuret
M. Iacovacci C. Nicollette

Structure/Function of Yeast cAMP-dependent Protein Kinase

M. Zoller, K. Johnson, M. Iacovacci, L. Levin,
C. Nicollette, J. Kuret

Protein kinases play an important role in regulating cellular processes. Structural analysis of a number of these enzymes has identified separate catalytic (C) and regulatory (R) domains. The function of the R domain is to inhibit kinase activity. These domains can be on separate subunits, as in cAMP-dependent protein kinase, or within the same polypeptide, as in cGMP-dependent protein kinase, protein kinase C, and myosin light-chain kinase. It has been postulated that inhibition of phosphotransferase activity is maintained by occupation of the active site of the C domain by a pseudosubstrate sequence within the R domain. The association between the R and C domains has been shown to be extremely tight, with binding constants in the subnanomolar range. Activation of these enzymes occurs by dissociation of the R domain and is triggered by binding of small effectors (cAMP, cGMP, Ca^{++}, phospholipid) or activating proteins (calmodulin) to the R domain.

Comparisons have been made between the primary sequences of all known eukaryotic protein kinases. This analysis has shown that the C subunits (domains) belong to a family of proteins that stem from a common primordial structure. The family can be broken down into kinases that phosphorylate serine or threonine residues and proteins that phosphorylate tyrosine. The common structural features are an ATP-binding site and a protein-substrate-recognition site. However, only a few residues have been mapped to these structural domains. New protein kinases are always compared with the primary structure of cAMP-dependent protein kinase. The R subunits (domains) from the different protein kinases appear to be functionally similar, although unrelated structurally.

Although the primary structures of many protein kinases are known, many questions with respect to the structure and function of these enzymes still exist regarding the interactions between the R and C domains as well as the recognition of specific substrates. A three-dimensional structure of a protein kinase does not yet exist. We are interested in applying recombinant DNA technology to the study of protein kinases. cAMP-dependent protein kinase was chosen for these studies because it is the best-characterized protein kinase. *Saccharomyces cerevisiae* is being utilized in order to exploit the advantages of yeast molecular genetics for the isolation and characterization of mutant proteins. We have taken several approaches: (1) expression of the genes encoding the subunits of cAMP-dependent protein kinase, (2) genetic techniques to obtain interesting mutants, and (3) site-directed mutagenesis to test the role of specific amino acids.

In *S. cerevisiae*, as in higher eukaryotes, the holoenzyme of cAMP-dependent protein kinase is a tetrameric molecule composed of two C subunits and two R subunits. In the absence of cAMP, the R subunit binds tightly to the C subunit and inhibits catalytic activity. The effector cAMP binds to the R subunit, and the holoenzyme dissociates into two active C subunits and an R-subunit dimer. The C subunit phosphorylates specific protein substrates, thereby altering the biochemical properties of these proteins. As described by M. Wigler and co-workers (Molecular Genetics of Eukaryotic Cells Section), three genes in yeast, *TPK1*, *TPK2*, and *TPK3*, encode distinct C subunits, C_1, C_2, and C_3, respectively. All three proteins are structurally and functionally homologous to their mammalian counterparts. A single gene, *BCY1*, encodes the yeast R subunit. The yeast R subunit is homologous to mammalian R subunits, and it resembles the mammalian type II R subunit in its ability to act as a substrate for C. Synthesis of cAMP in *S. cerevisiae* is catalyzed by adenylate cyclase (*CYR1*) and is positively regulated by the *RAS1* and *RAS2* gene products. At least one of the *RAS* genes is normally required for viability, but both can be deleted in strains that lack a functional R subunit of cAMP-dependent protein kinase. In addition, studies by T. Toda, S. Cameron, and M. Wigler demonstrated that yeast cells require at least one of the *TPK* genes for viability.

Purification and Characterization of C_1, the C Subunit of *S. cerevisiae* cAMP-dependent Protein Kinase Encoded by *TPK1*

M. Zoller, K. Johnson, J. Kuret

We have purified and characterized the C subunit, C_1, encoded by the *TPK1* gene. To purify C_1 completely free of C_2 and C_3, a strain was constructed (in collaboration with T. Toda, S. Cameron, and M. Wigler [Molecular Genetics of Eukaryotic Cells Section]) that contained only the *TPK1* gene and genetic disruptions of the other two *TPK* genes. The cellular level of C_1 was increased by expressing the genes for C_1 (*TPK1*) and the yeast R subunit (*BCY1*) on multiple-copy plasmids within this strain. This resulted in increased expression of C_1 from 10 μg/liter to 400 μg/liter. Purification was accomplished by a two-column procedure in which cAMP-dependent protein kinase holoenzyme was chromatographed on Sephacryl-200, and then bound to an anti-R-subunit immunoaffinity column. Pure C_1 was released from the antibody column by addition of cAMP. The protein migrated on an SDS-polyacrylamide gel with a relative molecular weight of 52,000. Kinetic analysis showed that the apparent K_m values for ATP and Kemptide were 33 μM and 101 μM, respectively. The k_{cat} was determined to be 640 min^{-1}. The protein weakly autophosphorylated, incorporating less than 0.1 mole phosphate per mole of C subunit. Amino-terminal sequencing revealed that the protein was blocked. Recently, we have improved the expression of C_1 by use of a heterologous promoter (ADH1) and coexpression with R (Ala-241), a mutant form of *BCY1* that has a ten times higher affinity for the C subunit, compared with the wild-type R subunit. This resulted in a strain that expresses cAMP-dependent protein kinase at a level of approximately 5 mg/liter. This level should provide sufficient protein to begin crystallographic analysis of the yeast enzyme.

A Mutation in the C Subunit of cAMP-dependent Protein Kinase That Disrupts Regulation

L. Levin, J. Kuret, M. Zoller

A major question about protein kinases revolves around the identification of the contacts between the R and C domains that are important for regulation of enzymatic activity. To approach this problem, we have begun a project that maps these contacts by analysis of genetically isolated mutants. Our first mutant is an altered C subunit (*TPK1*) of yeast cAMP-dependent protein kinase. This mutant C subunit is no longer subject to regulation, yet it retains its catalytic activity. As discussed above, at least one of the *RAS* genes is normally required for viability, but both can be deleted in strains that lack a functional R subunit of cAMP-dependent protein kinase. On the basis of these studies, we predicted that mutations in the C subunit of cAMP-dependent protein kinase that disrupt regulation of catalytic activity would suppress a ras^{ts} defect. We isolated a dominant suppressor of a temperature-sensitive allele of *RAS2* that mapped to the *TPK1* gene (in collaboration with S. Powers, T. Michaeli, S. Cameron, and M. Wigler). DNA sequencing of the coding region of the mutant gene identified a single adenine-to-guanine transition in the *TPK1*-coding sequence, resulting in a substitution of alanine for threonine at amino acid 241 of C_1 (see Fig. 1A). This residue is conserved between the yeast and mammalian C subunits. Interestingly, this threonine is phosphorylated in both the yeast and mammalian proteins. Affinity-labeling experiments on the mammalian C subunit suggest that a neighboring residue is in close proximity to the protein-substrate-binding site.

To characterize the properties of the mutant C subunit, we purified the protein using the immunoaffinity procedure described above. Biochemical analysis of the mutant subunit indicates at least a 100-fold decreased affinity for the yeast R subunit. The mutant C subunit exhibits an approximately threefold increase in K_m values for ATP and peptide cosubstrates and is essentially unchanged in its catalytic rate. These results identify this threonine as an important contact between C and R subunits and suggest that the phosphate plays a direct role in the interaction. In addition, these studies suggest that this residue is only a minor contact in substrate recognition. Thr-241 and a number of other amino acids in the region from Asp-228 to Glu-252 are conserved in mammalian cGMP- and cAMP-dependent protein kinases and protein kinase C (see Fig. 1B). As discussed above, these protein kinases are composed of separate R and C domains and may be regulated by pseudosubstrate inhibition. The sequence conservation and apparent common regulatory mechanism

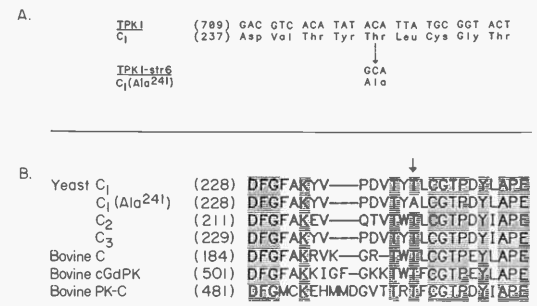

FIGURE 1 (*A*) The nucleotide sequence of *TPK1* and the predicted amino acid sequence of C_1 in the region surrounding the suppressor mutation. (*B*) Amino acid sequences of the mutant, C_1 (Ala-241), and wild-type yeast C_1 subunits, and other serine-threonine protein kinases in the region of the putative substrate-binding domain. Shading indicates absolutely conserved amino acids. The arrow points to the site of the substitution in C_1 (Ala-241). Numbers in parentheses indicate the first amino acid listed. Related protein kinases are the other two isozymes of the yeast C subunit (C_2 and C_3), the Cα subunit of the bovine cAMP-dependent protein kinase (bovine C), the bovine cGMP-dependent protein kinase (cGdPK), and bovine protein kinase Cα (PK-C).

of these protein kinases suggest that the functional role of this threonine is conserved. This mutant has been extremely useful in developing strains to obtain extragenic suppressors (e.g., mutant R subunits). One of the phenotypes of a strain containing C_1 (Ala-241) is that it is sensitive to heat shock, whereas a strain carrying the wild-type C subunit is resistant to heat shock. We have used this phenomenon to isolate and characterize R subunits that have a greater affinity for the C subunit than for the wild-type R subunit (discussed below).

A Yeast System to Express and Analyze Mutants of the cAMP-dependent Protein Kinase

L. Levin, M. Zoller

To purify mutant C subunits for biochemical analysis, we use a "*TPK* swap procedure" that provides a way to overexpress any mutant C subunit in the absence of the wild-type protein. This procedure was used by T. Toda and M. Wigler (Molecular Genetics of Eukaryotic Cells Section) to assess plasmids carrying portions of the *TPK1* gene. As depicted in Figure 2, the procedure involves introduction of a plasmid containing the mutant C subunit (*TPK1^m*) into a strain in which all three chromosomal *TPK* genes have been genetically destroyed. Since at least one *TPK* gene is required for viability, the strain is kept alive by a wild-type *TPK1* carried on a plasmid. The wild-type *TPK1* plasmid is marked by the *ADE8* gene (adenine auxotrophy); this strain requires leucine for growth. The plasmid with the mutant *TPK1^m* gene contains the *LEU2* gene (leucine auxotrophy). After selection for transformants that contain both plasmids, plasmid loss is induced by growth without selection for either plasmid. Individual colonies are tested for leucine or adenine auxotrophy, indicating which *TPK* gene has been maintained. Strains that have successfully swapped plasmids will now require adenine for growth, but not leucine. Since viability requires an active C subunit, mutant C subunits that cannot support growth will not swap. Expression from plasmids, rather than chromosomally integrated genes, improves the level of expression. Subsequently, an additional plasmid containing the *BCY1* gene encoding the yeast R subunit can be introduced in order to produce a strain that overexpresses both the C and R subunits. This facilitates purification of the mutant C subunit. Yeast offers the advantage over heterologous expression systems as being the natural environment for production of these proteins. In addition, this system is superior

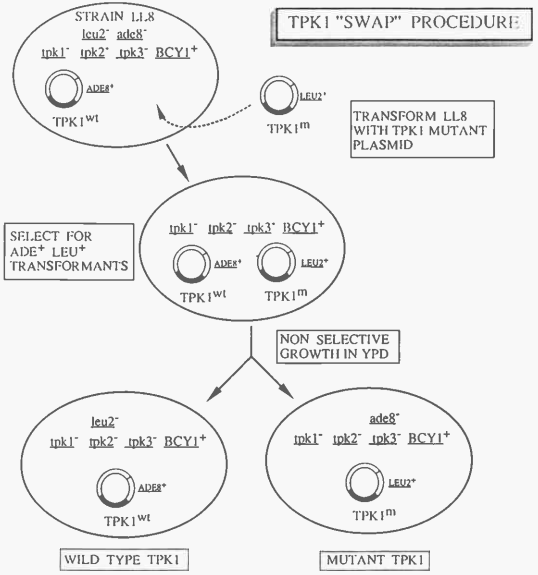

FIGURE 2 The *TPK* swap procedure: A yeast system to express and analyze mutants of the cAMP-dependent protein kinase. Strain LL8 (*tpk1*⁻, *tpk2*⁻, *tpk3*⁻, *BCY1*, YEp*ADE8*/*TPK1*, *leu2*⁻) is transformed with YEp*LEU2*/TPK1ᵐ, a plasmid that contains a mutant C-subunit gene. Transformants that have both plasmids are selected by growth on media lacking both adenine (for selection of wild-type *TPK1*) and leucine (for selection of mutant *TPK1*ᵐ). Plasmid loss is induced by growth in media without selection and then the surviving strains are tested for viability on selective media lacking either adenine or leucine.

to other eukaryotic expression systems that rely on the use of dominant mutants in order to accommodate the wild-type protein in the background.

The C Subunit of Mammalian cAMP-dependent Protein Kinase Can Replace the Yeast Protein In Vivo

K. Johnson, M. Zoller [in collaboration with L. Slice and S. Taylor, University of California at San Diego]

To test the functional relationships between the yeast and mammalian C subunits, we expressed the gene encoding the mammalian C subunit in yeast. The coding sequence of the yeast *TPK1* gene was replaced by the coding sequence for the mammalian gene such that the natural protein would be produced. This gene was carried on a yeast expression vector and introduced into yeast using the *TPK1* swap procedure outlined above. Preliminary results indicate that the mammalian C subunit can swap successfully with the yeast C subunit and maintain viability. These experiments indicate that the two proteins are structurally and functionally similar and broaden the conclusions we will obtain from the study of the yeast enzyme. Studies are in progress to determine whether the genetic tricks that we use to isolate mutations in the yeast cAMP-dependent protein kinase can be used in the mammalian protein. In addition, this re-

sult may provide a model system in which to reengineer protein kinase activity.

Mutagenesis of the R Subunit of Yeast cAMP-dependent Protein Kinase: Isolation of Site-directed Mutants with Altered Binding Affinity for the C Subunit

J. Kuret, K. Johnson, C. Nicolette, M. Zoller

Biochemical experiments and amino acid sequence comparisons on a number of different R subunits have identified several structural domains. One of these regions, termed the hinge, is a major determinant involved in the specific inhibition of catalytic activity in the inactive holoenzyme complex. To dissect this region further and to characterize the role of specific amino acids, oligonucleotide-directed mutagenesis was used to produce mutants in the hinge region of the yeast R subunit (see Fig. 3). Our initial studies were focused on Ser-145, which is part of the "pseudosubstrate" sequence in the hinge domain: ArgArgThrSerVal. The mutant proteins were expressed in *Escherichia coli*, purified, and analyzed in vitro for the ability to inhibit the activity of the purified yeast C subunit in the presence and absence of cAMP. When assayed in the absence of cAMP, the wild-type R subunit inhibited C with an IC_{50} (inhibition constant) of 49 nM. Replacement of amino acid residue Ser-145 with alanine or glycine produced mutants that were two- to tenfold better inhibitors of C, whereas replacement with glutamate, aspartate, lysine, or threonine produced mutants that were two- to fivefold worse inhibitors of C relative to wild-type R. When assayed in the presence of cAMP, all R subunits had a decreased affinity for the C subunit. These results suggest that the amino acid at position 145 of R contributes to R-C interaction and therefore influences the equilibrium of yeast protein kinase subunits in vitro. We have exploited these findings by expressing the Ala-145 variant in yeast in order to improve the level of expression of wild-type and mutant C subunits and facilitate their purification.

Crystallization of the Yeast R Subunit

J. Kuret, M. Zoller [in collaboration with J. Pflugrath, Cold Spring Harbor Laboratory]

To understand the structure of protein kinases on a molecular level, we have initiated a collaborative

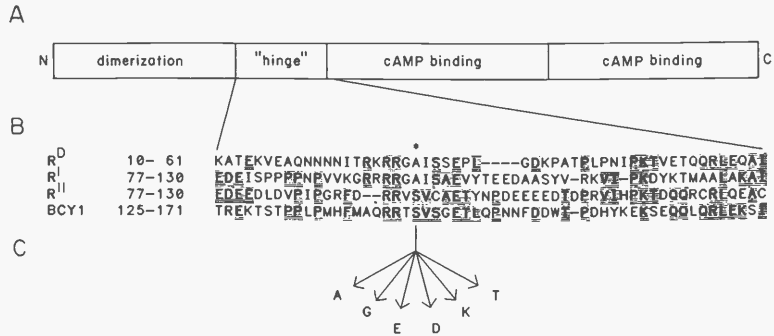

FIGURE 3 Structure of the hinge region of the wild-type R subunit and of the mutants. (*A*) Organization of functional domains along the R-subunit polypeptide chain. (*B*) Sequence homology in the hinge region of various R subunits including bovine R^I and R^{II}, *D. discoideum* R (R^D), and yeast R (*BCY1*). (*C*) Ser-145 of yeast R is part of the pseudosubstrate sequence Arg-Arg-X-Ser involved in inhibition of catalytic activity. To characterize the role of this serine in R-C interactions, it was changed to the amino acids indicated by oligonucleotide-directed mutagenesis. Each of the mutated proteins was expressed, purified, and then tested for the ability to inhibit C-subunit activity.

project with J. Pfulgrath on the determination of the three-dimensional structure of cAMP-dependent protein kinase. Our efforts over the past year have focused on the use of a relatively small (10-liter) fermentor for the production of the yeast R subunit (*BCY1*). Our experiments demonstrated that use of the fermentor can improve yields significantly. A 10-liter culture of *E. coli* expressing the yeast R subunit yielded 100–200 mg of protein in crude extracts. This represents a five times increase in yield of protein expressed per liter over shaker-flask methods. Now that we have optimized our expression system, our efforts have turned to the problem of obtaining protein of sufficient purity to obtain usable crystals. These studies are still in progress. We have recent evidence that a form of the protein in which the amino-terminal domain has been deleted can be obtained with less heterogeneity than the full-length R subunit. This form inhibits the C subunit with the same kinetics as the wild-type R subunit but does not form R-R dimers. An initial attempt to produce crystals with this preparation yielded small crystals unusable for X-ray analysis, yet provides an impetus that crystallization will be possible with futher work.

A Genetic System to Study Interactions between R and C Subunits: Isolation and Characterization of R Subunits with Increased Affinity for the C Subunit

C. Nicolette, L. Levin, M. Zoller

This screen utilizes the sensitivity to heat shock of a strain that contains C_1 (Ala-241) as the sole C subunit. The strain is sensitive to heat shock because the C subunit is not regulated. A strain that contains a mutant R subunit that regulates the activity of C_1 (Ala-241) will be heat-shock-resistant. We have already demonstrated that this screen can successfully identify tight binding variants using R (Ala-145) constructed by in vitro site-directed mutagenesis.

The procedure involves the transformation of this heat-shock-sensitive strain using a pool of plasmids that contain the randomly mutagenized *BCY1* gene. Initially, we are using a mutD strain for mutagenesis, but we are investigating other procedures to direct mutations randomly into the *BCY1* gene. Transformants are tested for resistance to heat shock, and the *BCY1*-containing plasmids are isolated from these heat-shock-resistant colonies. The mutation is identified by sequencing with oligonucleotide primers that span the *BCY1*-gene-coding sequence.

In the initial experiments, two strains of yeast were constructed, both of which contain C_1 (Ala-241). One strain contained the wild-type R subunit and the other contained the mutant R (Ala-145). Each strain was tested for sensitivity to heat shock. The control strain containing the wild-type R subunit was still heat-shock-sensitive, but the strain containing R (Ala-145) was resistant.

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NEUROSCIENCE

The research conducted by the Neuroscience Program at Cold Spring Harbor Laboratory continues to focus on the functional development of neurons. Our interests lie in neurons of the central nervous system, particularly in the brain where developmental disorders as well as degenerative neurological diseases can lead to disabilities in learning and memory. To make progress in this field, we have chosen a variety of cell-culture systems, both primary neurons and neural cell lines, to examine their differentiation and proliferation. We use protein purification and characterization to uncover factors that promote neuronal development. Molecular biology has given us the tools to study the gene regulation of these factors. Thus, the projects carried out in this context employ all the latest techniques in molecular and cellular biology, biochemistry, and genetics to uncover the mysteries of the brain.

NEURONAL GROWTH AND DIFFERENTIATION

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

We are interested in the mechanisms involved in the development, organization, and maintenance of connections within the central nervous system (CNS). There is a great deal of evidence indicating that soluble growth factors play a critical role in these processes. Our goals are to isolate and characterize these factors and to uncover experimentally their mechanisms of action on neurons. To this end, we have developed cell-culture systems for examining neurotrophic growth factors, proteins that elicit the outgrowth of projections, or neurites, from the cell. It is these neurites that become the axon and dendrites of the neuron that carry the signals from one cell to another.

Cellular Models of Neurite Outgrowth

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

Neuronal cell cultures can be used to identify and characterize soluble neurotrophic factors. A significant problem with these cultures, however, is the difficulty in obtaining pure neuronal populations. We are able to obtain cultures of pure CNS neurons in the virtual absence of nonneuronal cells by utilizing cells derived from 7-day-old chick embryo cerebral cortex. As shown in Figure 1, the extension of neuronal processes by these cells is highly dependent on the presence of soluble growth factors, such as that derived from bovine brain. At this stage of embryonic development, the cerebral cortical cells are postmitotic, having lost the ability to undergo cell division. The cells are able to generate or regenerate neurites under controlled conditions. In this neurite-extension assay, the cells are maintained in a serum-free, defined medium, which allows us to add known samples with a high degree of reproducibility. Within 24 hours, neurite outgrowth is prominent, and the cells are scored on the basis of their morphologies. Survival of the cells is very high, which allows us to distinguish neurite extension activity from survival functions.

Cell lines have been very useful to us in studying neurite outgrowth. The neuroblastoma cell line, neuro-2a, is derived from a murine tumor line known as C1300. The neuro-2a cells are an excellent parallel to the primary cerebral cortical cells because they respond to many of the same stimuli. The neuro-2a

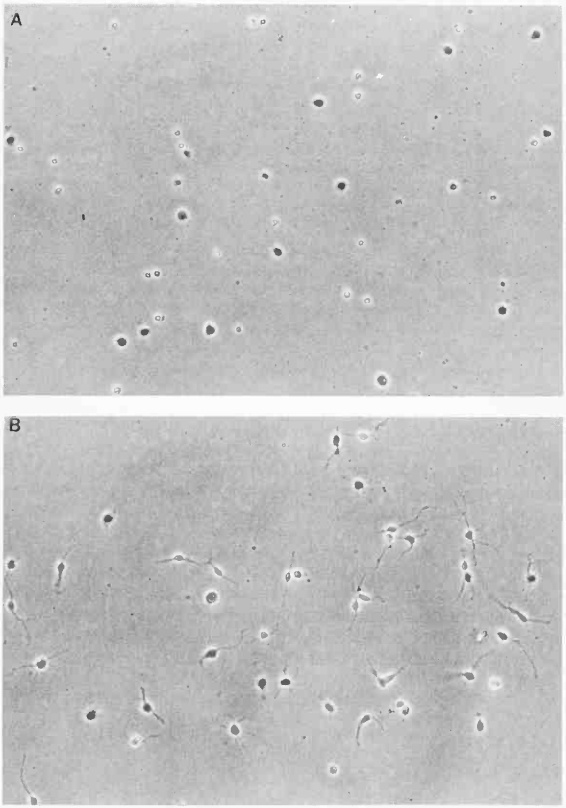


FIGURE 1 Chicken embryo cerebral cortical cells were maintained in defined media without addition (*A*) or with bovine-brain-derived neurotrophic factor (*B*).

cells offer an important advantage over the primary cells in that they can be manipulated to respond to stimuli very rapidly, hours rather than days. Another cell line, the C6 rat astrocytoma cell line, has been helpful as a model of glial cells that produce soluble neurotrophic substances. We prepare conditioned, serum-free media from these cells, which contain several strong neurite-promoting materials. Thus, cell cultures provide significant models for the production and response to soluble neurotrophic factors.

Soluble neurotrophic factors are likely to act through specific cell-surface receptors. These receptors would then activate selected second-messenger-generating systems, thereby initiating an intracellular response. We have used the neuro-2a cell-culture system to study the intracellular signals that mediate neurite outgrowth and neuronal differentiation.

Figure 2 shows the various morphological changes in neuro-2a cells caused by manipulation of components of the cAMP and inositol phospholipid second-messenger systems. The data indicate that cAMP, calcium ions, and the activation of protein kinase C may all be involved in the initiation of neurite outgrowth. We are currently probing specific biochemical events that mediate the profound morphological changes in neuro-2a cells caused by various pharmacological probes.

Purification of a Neurotrophic Factor

J.C. Figueiredo, D.R. Marshak

Using primary chick embryo cortical cultures, we have identified a factor from bovine brain that causes neurite outgrowth. We are currently purifying this factor to chemical homogeneity for structural analysis. To date, the preparation consists of (1) homogenization in hypotonic, neutral buffer, (2) heat treatment, (3) batch adsorption and elution on anion exchange resins, (4) column chromatography on anion exchange resin, (5) gel filtration, and (6) chromatography on heparin-Sepharose conjugates. The specific activity appears to increase 50,000-fold during this purification. This activity appears to be different from that of the S100β protein described below. Table 1 shows preliminary purification data and the resulting specific activity of the factor.

TABLE 1 Purification of Neurotrophic Activity from Bovine Brain

Procedure	Protein (mg)	Specific activity (units/mg protein)
Homogenate	200,000	not determined
Heat-treated supernatant	3,000	not determined
DE-52 batch	640	not determined
DE-52 column	150	666
Ultrage	8	1,100
Heparin-Sepharose	0.16	50,000

Mechanism of Action of S100β

D.R. Marshak, R. Tonner, J.C. Figueiredo

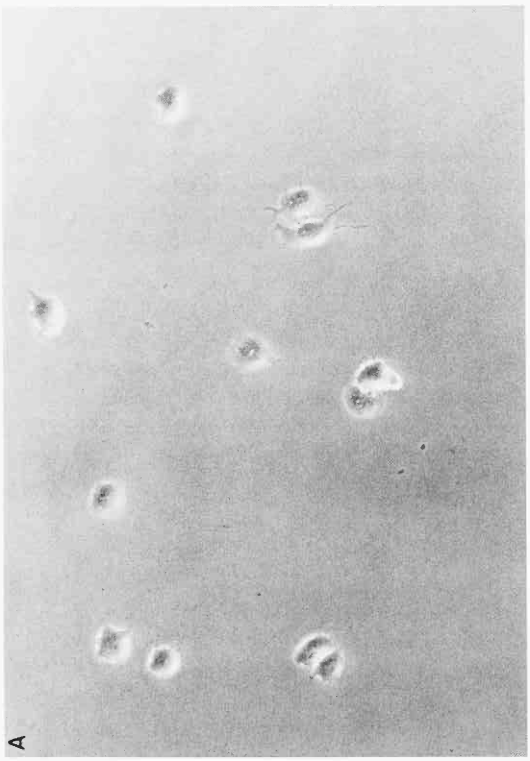
During the past several years, we have been investigating the role of the protein S100β in neuronal physiology. A disulfide form of the protein acts as a neurotrophic factor in both the neuro-2a and primary chick cortical cells. We have recently observed that S100β dimerizes and polymerizes under certain conditions in vitro (Fig. 3). Reduction and alkylation of cysteine residues obliterates the biological activity, and a structural analog, S100α, lacking one of the cysteine residues, also has no activity. We have conducted studies of chemical reduction and reoxidation with a variety of agents, including tetrathionate, ferricyanide, glutathione, and benzofuroxan. In some instances, formation of dimers and higher polymers occurs, but quantitative regeneration of biological activity has not yet been accomplished.

To study further the mechanism of action of S100β as a neurotrophic agent, we have developed a variety of reagents and methods. Antisera to the monomeric, oxidized protein and, alternatively, to the polymeric protein are being produced in rabbits to probe the native state of the protein (Fig. 3). Synthetic peptides comprising a large portion of the molecule have been synthesized. These synthetic peptides will be used to probe the functional domains of S100β and to prepare site-directed antisera. Using synthetic oligonucleotides, J. Figueiredo has isolated cDNA molecules for the protein from a rat brain library. We are currently using all of these reagents to identify the active form of S100β in cell culture, to characterize the receptor for the protein, and to begin to analyze the role of the protein in vivo. These studies have taken on new importance since the recent demonstration by another laboratory that the gene for S100β resides on human chromosome 21 in the region that is duplicated in Down's syndrome and in the vicinity of the gene for familial Alzheimer's disease. The role of this protein in neurological diseases and developmental disorders of the nervous system is still unknown, and our goal is to establish the function of this protein in the developing nervous system under normal and abnormal conditions.

Proliferation and Differentiation of Neurons

D.R. Marshak [in collaboration with D. Spector, Cold Spring Harbor Laboratory]

One of the central questions in neuronal devlopment is the relationship between the ability of a neuron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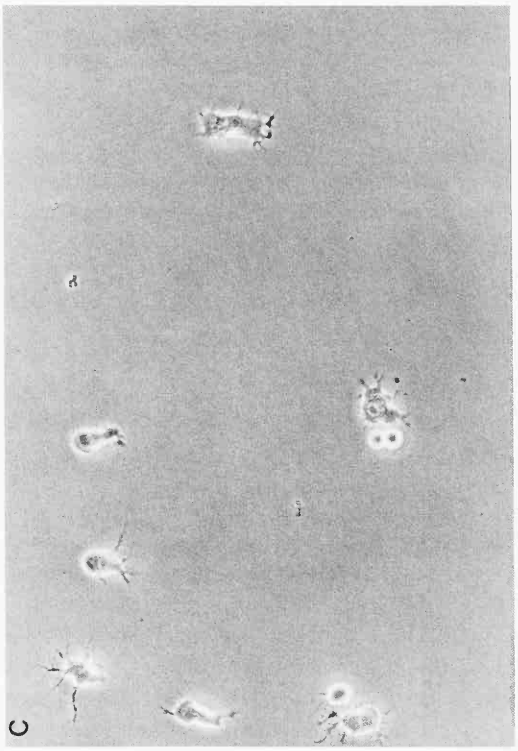
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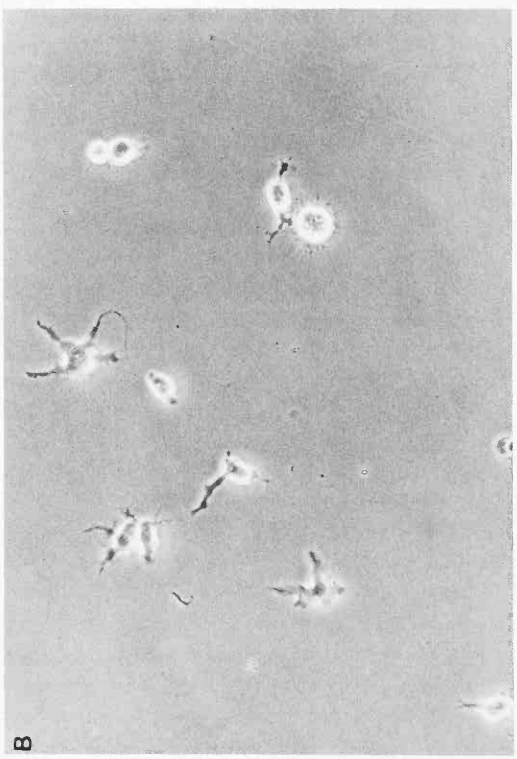
B

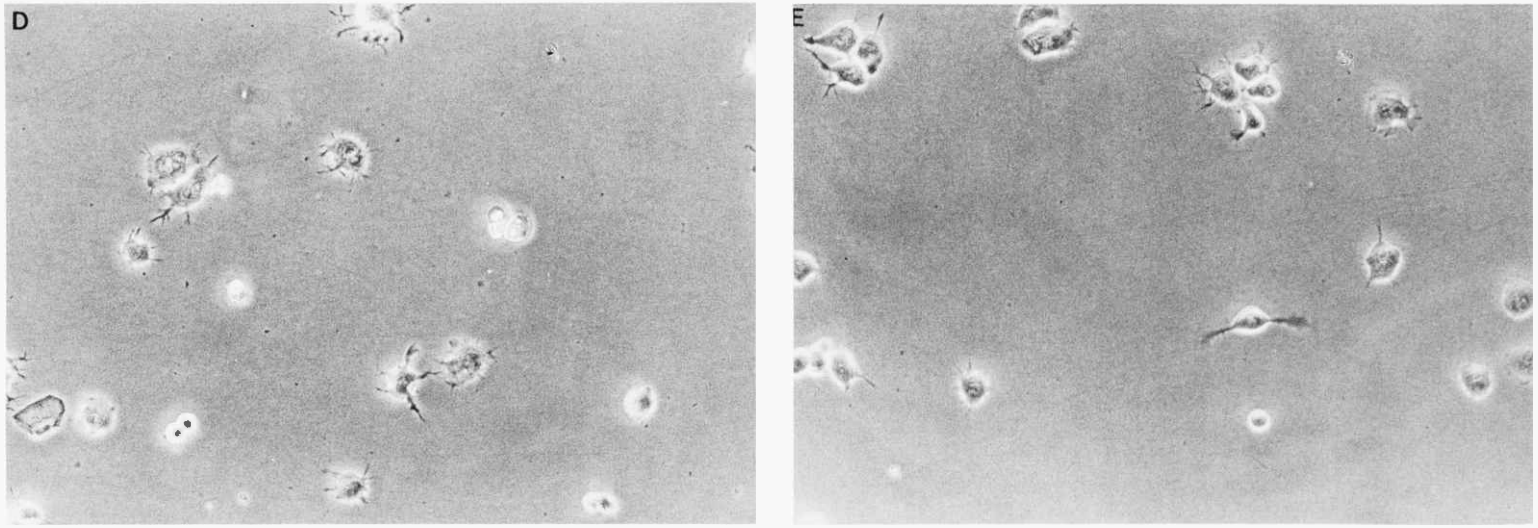

FIGURE 2 Neuro-2A cells were treated with no addition (*A*), 1 mM dibutyryl cAMP (*B*), 100 nM phorbal ester (TPA) (*C*), 100 nM calcium ionophore (A23187) (*D*), and both TPA and A23187 (*E*).

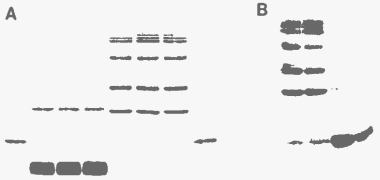

FIGURE 3 (*A*) Polyacrylamide gel electrophoresis of S100β (lanes *2,3,4*) showing dimer and monomer, S100β polymers (lanes *5,6,7*), and molecular-weight standards (lanes *1,8*). (*B*) Western blot using antisera to S100β showing polymers (lanes *1,2*) and monomer and dimer (lanes *3,4*).

to undergo cell division and its ability to differentiate into a functional, excitable neuron. We have begun to study this in the neuro-2a neuroblastoma cell-culture system. Under conditions of high density and in the presence of serum, these cells proliferate, whereas in serum-free media at low density, these cells send out processes that are characteristic of the developing neurons. We are using immunocytochemical techniques to localize markers of neuronal maturation and other proteins involved in proliferation. Figure 4 shows neurons stained with fluorescent antibody probes to tubulin (a marker of neurites) and the proliferating cell nuclear antigen (PCNA), which is involved in DNA replication. It is apparent from these experiments that PCNA staining is still visible even after prominent neurites have developed. However, we have not yet determined the extent of functional DNA replication during neuronal differentiation. It will be interesting to find out if differentiated neurons retain the ability to divide, as a potential therapy for degenerative neurological diseases.

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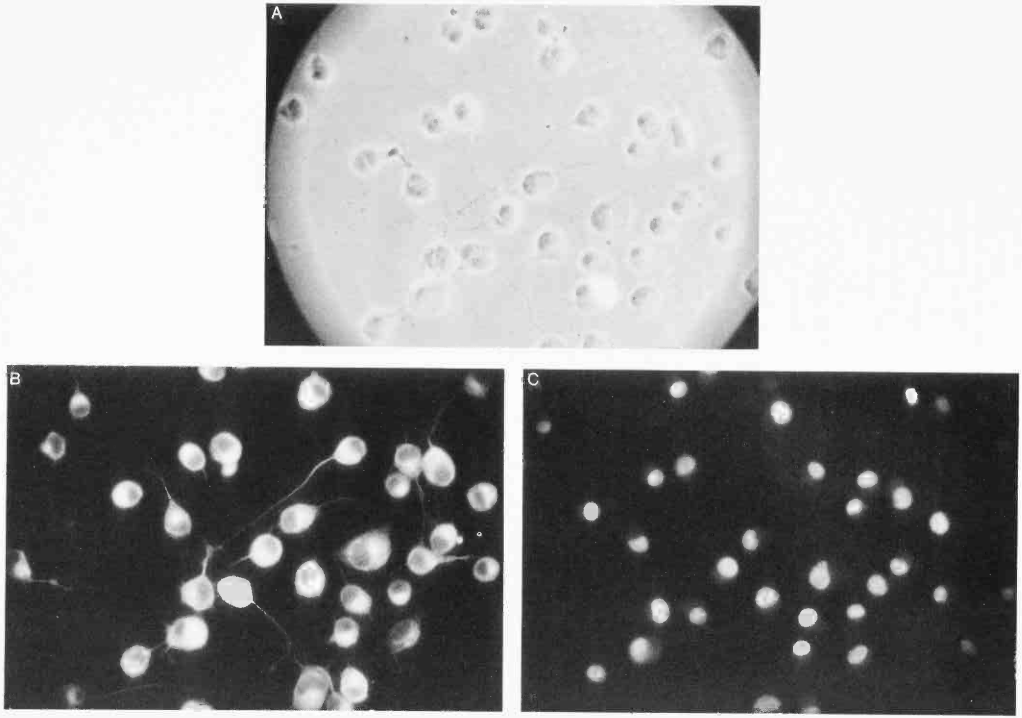
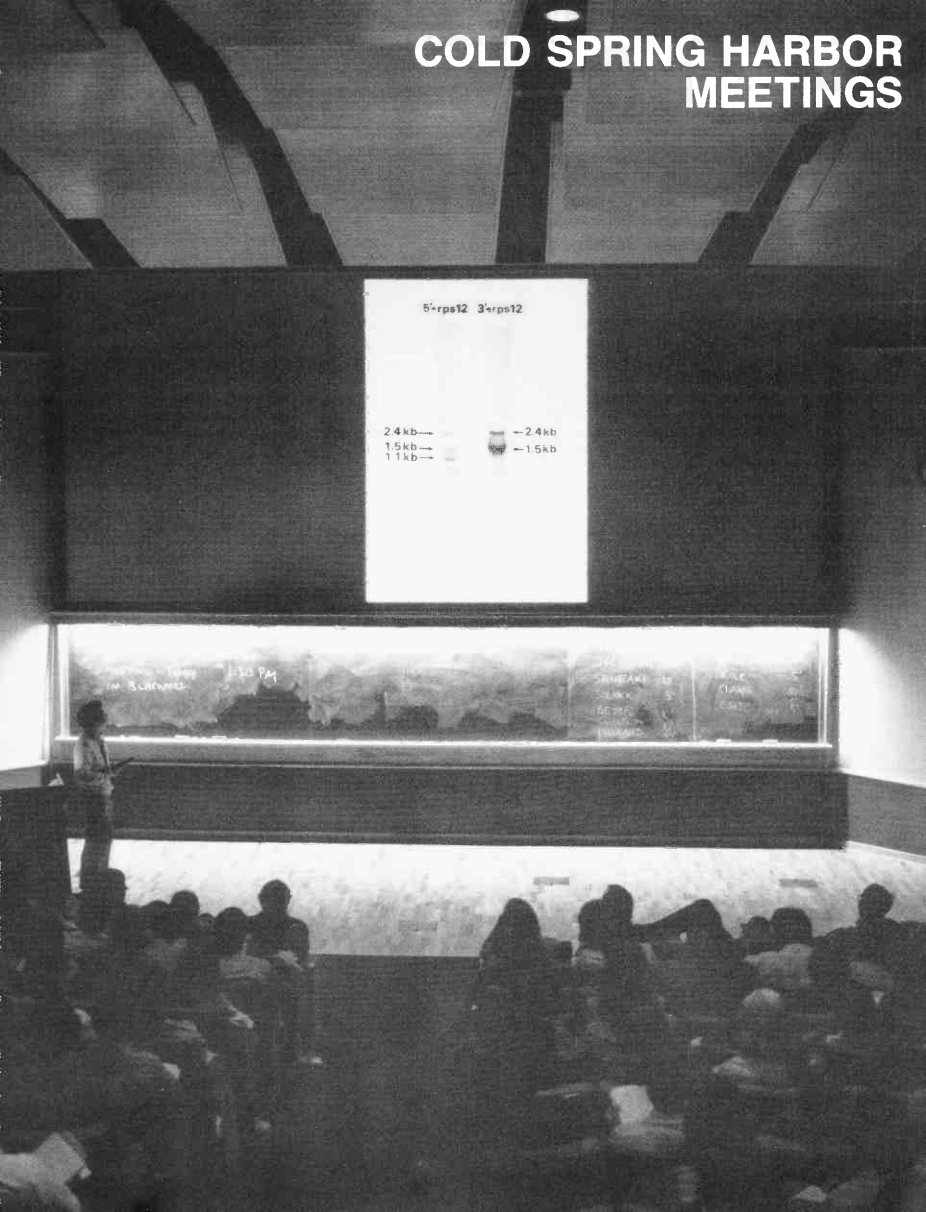


FIGURE 4 Neuro-2A cells growing processes in cultures. (*A*) Phase contrast; (*B*) immunostaining for tubulin; (*C*) immunostaining for PCNA.

COLD SPRING HARBOR MEETINGS



52nd COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

The Evolution of Catalytic Function

May 27—June 3

167 participants

For all too long, RNA never had the essential simplicity we wanted from it. Unlike DNA, the closer we got to it, the more complex it seemed. Initially, we focused on its informational properties, postulating that it carried the genetic information from DNA to the cytoplasmic sites of protein synthesis. It then seemed natural to believe that the main RNA molecules within ribosomes were the templates for the ordering of amino acids during protein synthesis. Quickly, however, this idea was overturned with the discovery first of transfer RNA and then of the true templates, messenger RNA itself. These seminal discoveries had the positive impact of permitting the basic outlines of protein synthesis to be established, as well as permitting the elucidation of the genetic code. At the same time, the dilemma arose of why RNA played so many different roles. In some profound way, the explanation had to lie in the molecular events that gave rise to the origin of life.

Rationally thinking about events so distant in the past, however, was then and still is a major intellectual challenge. Francis Crick rose to the occasion by postulating that RNA had to have an enzymatic role, as well as template and structural roles, functioning during the early stages of life as the enzyme for its own self-replication. Under this scheme, the first genetic molecule was RNA, with DNA coming later, after the essential outlines of the genetic code were established. But logical as this idea was, it had little impact, and for almost 20 years, thinking about the origin of life was regarded at best as a safe haven for minds unable to keep up with the extraordinary rush of a recombinant-DNA-driven assault on the nature of life as it exists today.

Then in 1982, the discovery of the first example RNA self-splicing suddenly led to the realization that RNA molecules do have the capability to act as catalysts. Over the past several years, many more examples of self-splicing have been found, and the fact that the catalytic activity of RNase P resides in its RNA component has been demonstrated. With Crick's conjecture now a fact and with the recent rapid progress in understanding today's enzymes and ribosomes, the time had thus arrived to bring together a diverse collection of pure chemists, biochemists, molecular biologists, and evolutionary biologists to discuss the evolutionary events that may have given rise to the living organisms that now exist on earth. As a title, we chose the Evolution of Catalytic Function to help assure that this Symposium would bring together minds that think in terms of chemistry, as well as about genetic information. The final program comprised 110 speakers, each speaking some 30 minutes. These presentations led to one of our intellectually most demanding meetings, since the talks were arranged to be longer than customary in view of the very diverse nature of the audience, which this year consisted of some 167 scientists. Helping to assure the success of the meeting were the wide-ranging opening talks by Daniel Koshland, Leslie Orgel, and Manfred Eigen as well as the gracefully lucid summary by Alan Weiner.

P. Grabowski, P.A. Sharp, N. Pace

R. Gumport, R. Symons

This meeting was supported in part by the Alfred P. Sloan Foundation, National Cancer Institute, a division of National Institutes of Health; National Science Foundation; U.S. Department of Energy; and the Lucille P. Mackey Charitable Trust.

Welcoming Remarks: J.D. Watson

SESSION 1 OPENING REMARKS

Koshland, D., Dept. of Biochemistry, University of California, Berkeley: Evolution of function in enzymes.

Orgel, L., Salk Institute for Biological Studies, San Diego, California: The earliest catalytic biopolymers—A speculative review.

Eigen, M., Max-Planck-Institut für biophysikalische Chemie, Göttingen, Federal Republic of Germany: New concepts to deal with evolution of nucleic acids.

SESSION 2 PRIMORDIAL SOUP

Chairman: L. Orgel, Salk Institute for Biological Studies

Miller, S.L., Dept. of Chemistry, University of California, San Diego, La Jolla: Which organic compounds could have occurred on the prebiotic earth?

Ferris, J.P., Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, New York: Prebiotic synthesis—Problems and challenges.

Westheimer, F., Dept. of Chemistry, Harvard University, Cambridge, Massachusetts: Why God chose phosphates.

Schwartz, A.W., Visscher, J., van der Woerd, R., Bakker, C.G., Laboratory for Exobiology, Faculty of Science, University of Nijmegen, The Netherlands: In search of RNA ancestors.

Joyce, G.F., Salk Institute for Biological Studies, La Jolla, California: Nonenzymatic template-directed synthesis of informational macromolecules.

Benner, S.A., MacPherson, L.J., Piccirilli, J.A., Moroney, S.E., Krauch, T., Laboratory for Organic Chemistry, Swiss Federal Institute of Technology, Zurich, Switzerland: The last riboorganism—Synthetic approaches to functional RNA molecules.

SESSION 3 ESSENTIALS OF CATALYSIS

Chairman: W.P. Jencks, Brandeis University

Jencks, W.P., Graduate Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Economics of enzyme catalysis.

Breslow, R., Dept. of Chemistry, Columbia University, New York: Artificial enzymes.

Bartlett, P.A., Marlowe, C.K., Giannousis, P.P., Hanson, J.E., Dept. of Chemistry, University of California, Berkeley: Phosphorus-containing peptide analogs as peptidase inhibitors.

Tramontano, A., Janda, K., Lerner, R., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Catalytic antibodies.

Schultz, P., Pollack, S., Jacobs,. J., Dept. of Chemistry, University of California, Berkeley: Catalytic antibodies.

Plückthun, A., Max-Planck-Institute for Biochemistry, Martinsried, Federal Republic of Germany: Engineering of antibodies with known three-dimensional structure.

C.-I. Brandon, A. Klug

Picnic

SESSION 4 RIBOSOMES

Chairman: M. Nomura, University of California, Irvine

Nomura, M., Thomas, M., Sor, F., Bolotin-Fukuhara, M., Cole, J.R., Matthews, L., Yamagishi, M., Dept. of Biological Chemistry, University of California, Irvine: Control of the synthesis of ribosomes and ribosomal components in bacteria – Translational regulation and feedback loops.

Nierhaus, K.H., Brimacombe, R., Gualerzi, C., Nowotny, V., Rheinberger, H.-J., Stöffler, M., Wittmann-Liebold, B., Wittman, H.G., Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany: Studies on structure, assembly, evolution, and function of ribosomes.

Oakes, M., Rivera, M., Soufer, D., Shankweiler, G., Scheinman, A., Lake, J., Molecular Biology Institute, University of California, Los Angeles: Evolving ribosome structure and function – Mapping sequences with DNA hybridization microscopy.

Weiss, R.B., Atkins, J.F., Smith, D.M., Gesteland, R.F., Dept. of Human Genetics, University of Utah Medical Center, Salt Lake City: Protein sequencing of frameshift junctions resulting from abnormal ribosome translocations.

Noller, H.F., Stern S., Moazed, D., Powers, T., Svensson, P., Weiser, B., Thimann Laboratories, University of California, Santa Cruz: rRNA – Architecture and active sites.

Gerbi, S.A.,[1] Jeppesen, C.,[1] Stebbins-Boaz, B.,[1] Ares, M.,[2] Rothschild, L.J.,[1] [1]Division of Biology and Medicine, Brown University, Providence, Rhode Island; [2]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Evolution of eukaryotic rRNA.

Moore, P.B.,[1,2,3] Capel, M.S.,[3] Kjeldgaard, M.,[4] Engelman, D.M.,[2,3] Depts. of [1]Chemistry, [2]Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut; [3]Dept. of Biology, Brookhaven National Laboratory, Upton, New York; [4]Division of Biostructural Chemistry, Dept. of Chemistry, Aarhus University, Denmark: Quaternary organization of the small ribosomal subunit from *E. coli*.

Yonath, A.,[1,2] Bartels, K.S.,[2] Frolow, F.,[1] Hope, H.,[3] Kratky, C.,[4] Leonard, K.R.,[5] Weinstein, S.,[1] Wittmann, H.G.,[6] [1]Weizmann Institute, Rehovot, Israel; [2]Max-Planck Research Unit, Hamburg, Federal Republic of Germany; [3]University of California, Davis; [4]University of Graz, Austria; [5]EMBL, Heidelberg, Federal Republic of Germany; [6]Max-Planck Institute, West Berlin, Federal Republic of Germany: Toward a molecular model for intact ribosomal particles.

SESSION 5 PUTTING A PROTEIN TOGETHER

Chairman: M. Levitt, Weizmann Institute

Levitt, M., Weizmann Institute, Rehovot, Israel: Computer simulation of protein dynamic in solution.

Karplus, M., Dept. of Chemistry, Harvard University, Cambridge, Massachusetts: Dynamics of proteins – Applications.

Shoemaker, K.R.,[1] Marqusee, S.,[1] York, E.J.,[2] Stewart, J.M.,[2] Baldwin, R.L.,[1] [1]Dept. of Biochemistry, Stanford University Medical Center, California; [2]Dept. of Biochemistry, University of Colorado Medical School, Denver: α-Helix formation by peptides in aqueous solution.

Chothia, C.,[1,2] Lesk, A.,[1,3] [1]Laboratory of Molecular Biology, Cambridge; [2]Dept. of Chemistry, University College London, England; [3]Biocomputing Program, EMBL, Heidelberg, Federal Republic of Germany: Protein evolution and protein structure.

McLachlan, A.D., MRC Laboratory of Molecular Biology, Cambridge, England: Gene duplication and the building up of protein structures.

SESSION 6 RNA: CATALYSIS. I.

Chairman: T. Cech, University of Colorado, Boulder

Cech, T., Barford, L., Been, M., Price, J., Tanner, N.K., Zaug, A., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: The *Tetrahymena* intervening sequence RNA as an enzyme.

Inoue, T., Salk Institute, La Jolla, California: Minimum requirement for group I IVS RNA catalysis.

Waring, R.B.,[1] Towner, P.,[1] Davies, R.W.,[1,2] [1]Dept. of Biochemistry and Applied Molecular Biology, UMIST, Manchester, England; [2]Allelix, Inc., Mississauga, Ontario, Canada: Role of exon-intron base pairing in splice-site selection by the self-splicing intron of *T. thermophila*.

Michel, F., Jacquier, A., Centre de Génétique moléculaire du CNRS, France: Long-range intron-intron and intron-exon pairings implied in self-splicing of class II introns.

Doudna, J.A., Cherry, J.M., Gerber, A.S., Szostak, J.W., Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Genetic dissection of a catalytic RNA.

Tabak, H.F.,[1] Arnberg, A.C.,[2] Van der Horst, G.,[1] Kwakman, J.H.J.M.,[1] Van der Veen, R.,[1] Winter, A.J.,[1] Grivell, L.A.,[1] Laboratory of Biochemistry, [1]University of Amsterdam, [2]University of Groningen, The Netherlands: Yeast mitochondrial self-splicing RNAs.

Peebles, C.L.,[1] Benaton, E.J.,[1] Jarrell, K.A.,[2] Dietrich, R.C.,[2] Uhl, T.M.,[2] Perlman, P.S.,[2] [1]University of Pittsburgh, Pennsylvania; [2]Ohio State University, Columbus: *cis* and *trans* reactions of mutant and wild-type forms of a group II intron.

SESSION 7 PROTEINS: STRUCTURE. I.

Chairman: A. Jones, Uppsala University

Kaiser, E.T., Rockefeller University, New York, New York: Toward the rational design and construction of enzymes.

Schulz, G.E., Institut für Organische Chemie und Biochemie der Universität, Freiburg, Federal Republic of Germany: Structural and functional relationships in the adenylate kinase family.

Achari, A.,[1] Scott, D.,[1] Barlow, P.,[1] Vidal, J.C.,[1] Brunie, S.,[2] Otwinowski, Z.,[1] Sigler, P.B.,[1] [1]Dept. of Biochemistry and Molecular Biology, University of Chicago, Illinois; [2]Ecole Polytechnique, Palaiseau, France: Facing up to membranes – Structure/function relationships in phospholipases.

Quiocho, F.A., Vyas, N.K., Sack, J.S., Vyas,. M.N., Howard Hughes Medical Institute, Depts. of Biochemistry and Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas: Atomic protein structures reveal basic features of binding of sugars, sulfate, leucine, and calcium.

Steitz, T.A., Beese, L., Freemont, P., Friedman, J., Sanderson, M., Derbyshire, V., Joyce, C., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Structural studies of Klenow fragment – An enzyme with two active sites.

Klug, A., MRC Laboratory of Molecular Biology, University Postgraduate Medical School, Cambridge, England: "Zinc-fingers" – A novel protein fold.

Eisenberg, D., Almassy, R.J., Janson, C.A., Chapman, M., Suh, S.W., Smith, W.W., Cascio, D., Molecular Biology Institute and Dept. of Chemistry and Biochemistry, University of California, Los Angeles: Glutamine synthetase and RuBisCO D.

Branden, C., Uppsala Biomedical Center, Swedish University of Agricultural Sciences: Structure and formation of RuBisCO.

J. Knowles

A. Klug

C. Chothia

D. Eisenberg

SESSION 8 RNA: TEMPLATES

Chairman: J. Darnell, Rockefeller University

Biebricher, C.K., Max-Planck Institut für Biophysikalische Chemie, Göttingen, Federal Republic of Germany: Replication and evolution of short-chained RNA species replicated by Qβ replicase.

Priano, C.,[1] Kramer, F.R.,[2] Mills, D.R.,[1] [1]Institute of Cancer Research and Dept. of Genetics and Development, Columbia University College of Physicians and Surgeons, [2]Public Health Research Institute, New York, New York: RNA bacteriophage replication—Requirement for secondary structure formation during RNA replication.

Hall, T.C.,[1] Marsh, L.E.,[1] Rao, A.L.N.,[1] Dreher, T.W.,[2]Griffing, L.R.,[1] [1]Biology Dept., Texas A&M, College Station; [2]Dept. of Agricultural Chemistry, Oregon State University, Corvallis: Promoter, aminoacylation, and nucleotidyl transferase function of BMV RNA.

Wimmer, E., Dept. of Microbiology, State University of New York, Stony Brook: Molecular events leading to poliovirus genome replication.

Krug, R.M., St. Angelo, C., Broni, B., Meyers, L., Agris, C.H., Shapiro, G., Memorial Sloan-Kettering Cancer Center, New York, New York: Transcription and replication of influenza virion RNA in the nucleus of infected cells.

Lai, M.M.C.,[1,2] Makino, S.,[1] Soe, L.,[1] Shieh, C.-K.,[1] Chang, M.-F.,[1] Stohlman, S.,[1,2] Depts. of [1]Microbiology, [2]Neurology, University of Southern California School of Medicine, Los Angeles: Leader RNA-primed transcription of coronavirus.

Wertz, G.,[1] Howard, M.,[1] Patton, J.,[2] Davis, N.,[3] [1]Dept. of Microbiology, University of Alabama, Birmingham; [2]University of South Florida, Tampa; [3]North Carolina State University, Raleigh: Switch from transcription to replication on a viral RNA template.

Bellocq, C., Raju, R., Kolakofsky, D., Dept. of Microbiology, University of Geneva Medical School, Switzerland: Translational requirement of LaCrosse virus mRNA synthesis.

SESSION 9 PROTEIN SYNTHESIS AND THE GENETIC CODE

Chairman: T. Cavalier-Smith, Kings College, London

Weiner, A.M., Maizels, N., Yale School of Medicine, New Haven, Connecticut: The tRNA synthesome model—Were tRNA synthetases the first step in the evolution of protein synthesis?

Reanney, D., La Trobe University, Victoria, Australia: Genetic error and genome design.

Cavalier-Smith, T., Dept. of Biophysics, Cell and Molecular Biology, Kings College, London, England: Cellular basis for molecular evolution.

Fitch, W.M., Dept. of Biological Sciences, University of Southern California, Los Angeles: The phylogeny of tRNA sequences provides evidence for ambiguity reduction in the origin of the genetic code.

Jukes, T.H.,[1] Osawa, S.,[2] Muto, A.,[2] Lehman, N.,[1] [1]Space Sciences Laboratory, University of California, Berkeley; [2]Dept. of Biology, Nagoya University, Japan: Evolution of anticodons—Variations in the genetic code.

Osawa, S.,[1] Jukes, T.H.,[2] Muto, A.,[1] Yamao, F.,[1] Ohama, T.,[1] Andachi, M.,[1] [1]Dept. of Biology, Nagoya University, Japan; [2]Space Sciences Laboratory, University of California, Berkeley: Role of GC/AT-biased mutation pressure in evolution of eubacterial genetic code.

Ozeki, H.,[1] Umesono, K.,[1] Inokuchi, H.,[1] Kohchi, T.,[2] Sano, T.,[2] Ohyama, K.,[2] [1]Dept. of Biophysics, [2] Research Center for Cell and Tissue Culture, Kyoto University, Japan: Genetic system of chloroplasts.

P.A. Sharp

F.M. Richards

C. Chothia

J.D. Watson

SESSION 10 RNA: ANATOMY

Chairman: A. Klug, Medical Research Council, Cambridge

Moras, D., Institute de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Conformational changes and dynamics of tRNA.

R. Basavappa,[1] Wagner, T.,[1] Schevitz, R.W.,[1] Podjarny, A.D.,[1] Sussman, J.,[2] Sigler, P.B.,[1] [1]Dept. of Biochemistry, and Molecular Biology, University of Chicago, Illinois; [2]Weizmann Institute of Science, Rehovot, Israel: Structure/function analysis of eukaryotic initiator tRNA.

Turner, D.H., Sugimoto, N., Jaeger, J.A., Longfellow, C.E., Freier, S.M., Kierzek, R., Dept. of Chemistry, University of Rochester, New York: Improved parameters for prediction of RNA structure.

Tinoco, I., Jr., Hardin, C.C., Walker, G.T., Davis, P., Dept. of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley: RNA conformation from A to Z.

DORCAS CUMMINGS LECTURE

Ernest Mayr, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts: Evolutionary Theory.

M. Nomura, P.A. Sharp

W. Gilbert, R. Lerner

SESSION 11 RNA: CATALYSIS. II.

Chairman: S. Altman, Yale University

Altman, S., Baer, M., Bartkiewicz, M., Gold, H., Lawrence, N., McCorkle, G., Vioque, A., Dept. of Biology, Yale University, New Haven, Connecticut: Properties of M1 RNA, the catalytic RNA subunit of ribonuclease P from *E. coli*, and its analogs from other organisms.

Pace, N.R., Reich, C., James, B.D., Waugh, D.S., Olsen, G.J., Pace, B., Liu, J., Aprison, E.Z., Dept. of Biology and Institute for Molecular and Cellular Biology, Indiana University, Bloomington: Structure and catalytic function in *B. subtilis* RNase P RNA.

Forster, A.C., Jeffries, A.C., Sheldon, C.C., Symons, R.H., Dept. of Biochemistry, University of Adelaide, Australia: Self-cleavage reactions of viroid and virusoid RNAs.

Epstein, L., Florida State University, Tallahassee: Transcripts of newt satellite DNA self-cleave in vitro.

Uhlenbeck, O.C., Sullivan, F.X., Sampson, J.R., Behlen, L.S., DiRenzo, A.B., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Characterization of RNA self-cleavage reactions.

Shvedova, T.A., Korneeva, G.A., Otroschenko, V.A., Venkstern, T.V., USSR Academy of Sciences, Moscow: Catalytic activity of the nucleic acid component of rabbit muscle branching enzyme.

Sharp, P.A., Konarska, M.M., Grabowski, P.J., Lamond, A., Seiler, S., Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Splicing of precursors to mRNAs.

Krämer, A., Frendewey, D., Lahr, G., Frick, M., Keller, W., Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Components involved in the assembly and the catalytic activity of spliceosomes formed on nuclear mRNA precursors in vitro.

SESSION 12 PROTEINS: COFACTORS

Chairman: M. Perutz, Medical Research Council, Cambridge

Perutz, M.F.,[1] Fermi, G.,[1] Luisi, B.,[1] Shaanan, B.,[2] Liddington, R.C.,[3] [1]MRC Laboratory of Molecular Biology, Cambridge, England; [2]Dept. of Structural Chemistry, Weizmann Institute, Rehovot, Israel; [3]Dept. of Chemistry, York University, England: Stereochemistry of cooperative mechanisms in hemoglobin.

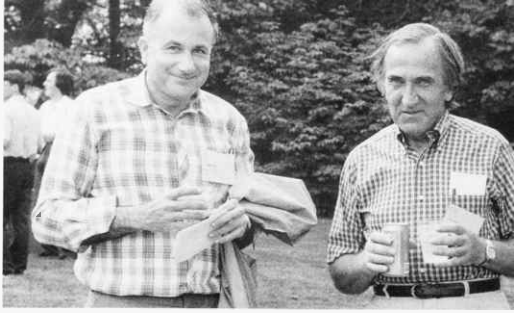
Bruice, T.C., Dept. of Chemistry, University of California, Santa Barbara: Porphyrin iron (III) mixed function oxidases. An evolutionary end point for transition metal (III) reactions with oxygen donors.

Frey, P.A., Moss, M.L., Institute for Enzyme Research, Graduate School, and Dept. of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison: *S*-Adenosylmethionine and the mechanism of hydrogen transfer in the lysine 2,3-aminomutase reaction.

Berg, J.M., Dept. of Chemistry, Johns Hopkins University, Baltimore, Maryland: Metal ions in proteins – Structural and catalytic roles.

Lin, A.I., Ashley, G.W., Harris, G., Ator, M., Stubbe, J., Dept. of Biochemistry, University of Wisconsin, Madison: Comparison between the *L. leichmannii* and *E. coli* ribonucleotide reductases.

P. Carter, D. Helfman



J. Ninio, L. Orgel

SESSION 13 PROTEINS: STRUCTURE. II.

Chairman: F. Richards, Yale University

Richards, F., Yale University, New Haven, Connecticut: Internal packing and protein structural classes.

Kretsinger, R.H., Dept. of Biology, University of Virginia, Charlottesville: Calcium coordination and the calmodulin fold divergent vs. convergent evolution.

Creighton, T.E., MRC Laboratory of Molecular Biology, Cambridge, England: Biosynthesis, processing, and folding of bovine trypsin inhibitor.

DeGrado, W.,[1] Ho, S.,[1] Eisenberg, D.,[2] Regan, L.,[1] [1]E.I. du Pont de Nemours & Company, Wilmington, Delaware; [2]Molecular Biology Institute, University of California, Los Angeles: Design, synthesis, and characterization of a four-helix protein.

Carrell, R.W., Pemberton, P., Boswell, J.R., Dept. of Haematological Medicine, University of Cambridge, England: The serpins – Evolution and redesign of a family of protease inhibitors.

Stackhouse, T.,[1] Onuffer, J.J.,[1] Matthews, C.R.,[1] Ahmed, S.A.,[2] Miles, E.W.,[2] [1]Dept. of Chemistry, Pennsylvania State University, University Park; [2]NIADDKD, National Institutes of Health, Bethesda, Maryland: Role of protein folding in the evolution of protein sequences.

Wells, J.A.,[1] Carter, P.J.,[1] Cunningham, B.C.,[1] Graycar, T.P.,[2] Estell, D.A.,[2] [1]Dept. of Biocatalysis, Genentech, Inc., [2]Research Dept., Genecor, Inc., South San Francisco, California: Evolution of specificity and catalysis in subtilisin.

Laskowski, M., Jr., Kato, I., Kohr, W.J., Park, S.J., Tashiro, M., Whatley, H.E., Dept. of Chemistry, Purdue University, West Lafayette, Indiana: Possible positive Darwinian selection in evolution of protein inhibitors of serine proteinases.

SESSION 14 EVOLUTIONARY TREES

Chairman: R. Doolittle, University of California, San Diego, La Jolla

Olsen, G.J., Dept. of Biology, Indiana University, Bloomington: The earliest phylogenetic branchings – Comparing rRNA-based evolutionary trees inferred with various techniques.

Lake, J.A., Molecular Biology Institute, University of California, Los Angeles: rRNA genes of the eukaryotic nucleus evolved from those of sulfur-metabolizing prokaryotes.

Li, W.H., Sourdis, J., Wolfe, K.H., Sharp, P.M., Center for Demographic and Population Genetics, University of Texas, Houston: Reconstruction of phylogenetic trees and estimation of divergence times under nonconstant rates of evolution.

Penny, D.,[1] Hendy, M.D.,[2] Henderson, I.M.,[1] Depts. of [1]Botany and Zoology, [2]Mathematics and Statistics, Massey University, Palmerston North, New Zealand: Estimating rates and accuracy on evolutionary trees.

Miyata, T.,[1] Hayashida, H.,[1] Kuma, K.,[1] Mitsuyasu, K.,[1] Yasunaga, T.,[2] [1]Dept. of Biology, Faculty of Science, Kyushu University, Fukuoka, [2]Institute of Physical and Chemical Research, Wako, Japan: Male-driven molecular evolution.

Doolittle, R.F., Dept. of Chemistry, University of California, San Diego, La Jolla: Reconstructing the evolution of vertebrate blood coagulation from a consideration of the amino acid sequences of clotting proteins.

Goodman, M.,[1] Czelusniak, J.,[1] Koop, B.,[1] Tagle, D.,[1] Slightom, J.,[1,2] [1]Wayne State University, Detroit, [2]Upjohn, Kalamazoo, Michigan: Globins – A case study in molecular phylogeny.

Stewart, C.-B.,[1,2] Schilling, J.W.,[3] Wilson, A.C.,[1,4] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Hormone Research Institute and Dept. of Biochemistry and Biophysics, University of California, San Francisco; [3]California Biotechnology, Mountain View; [4]Dept. of Genetics, Cambridge University, England: Convergent evolution of lysozymes.

SESSION 15 PROTEINS: FUNCTION

Chairman: J. Knowles, Harvard University

Hermes, J.D., Blacklow, S.C., Knowles, J.R., Dept. of Chemistry, Harvard University, Cambridge, Massachusetts: Evolution of enzyme efficiency.

Alber, T.C., Davenport, R.C., Jr., Lolis, E.M., Ringe, D., Petsko, G.A., Dept. of Chemistry, Massachusetts Institute of Technology, Cambridge: Yeast triose phosphate isomerase—Lessons on catalysis from crystallography and site-directed mutagenesis.

Fersht, A.R., Dept. of Chemistry, Imperial College of Science and Technology, London, England: Dissection of the anatomy of an enzymatic reaction.

Higaki, J.,[1] Gibson, B.W.,[1] Craik, C.S.,[1,2] Depts. of [1]Pharmaceutical Chemistry, [2]Biochemistry and Biophysics, University of California, San Francisco: Genetic analysis of the active site of trypsin—Replacement of Ser-195 with Cys and Asp-102 with Asn.

Mowbray, S., Thorsness, P., Koshland, D.E., Jr., Dept. of Biochemistry, University of California, Berkeley: Transmission of regulatory conformational changes through proteins.

Benkovic, S.J., Fierke, C., Kuchta, R., Dept. of Chemistry, Pennsylvania State University, University Park: Implications for protein function from kinetic analysis.

Ninio, J., Institute Jacques Monod, Paris, France: Kinetic devices and enzyme accuracy.

SESSION 16 EXONS AND INTRONS

Chairman: W. Gilbert, Harvard University

Gilbert, W., Harvard University, Cambridge, Massachusetts: Introns/exons in evolution.

Doolittle, W.F., Dept. of Biochemistry, Dalhousie University, Halifax, Nova Scotia: What introns have to tell us.

Gō, M., Nosaka, M., Dept. of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan: Protein architecture and origin of introns.

Blake, C.C.F., Harlos, K., Holland, S.K., Laboratory of Molecular Biophysics, University of Oxford, England: Exon and domain evolution in the proenzymes of blood coagulation and fibrinolysis.

Belfort, M., Wadsworth Center for Laboratories and Research and School of Public Health Sciences, State University of New York, New York State Department of Health, Albany: Genetics and distribution of group I introns in the T-even phages.

Shub, D.A., Dept. of Biological Sciences, State University of New York, Albany: A family of self-splicing group I introns in *E. coli* bacteriophage T4.

Summary: A. Weiner, Yale University School of Medicine

Regulation of Liver Gene Expression

April 29 – May 3

ARRANGED BY

Georg Fey, Scripps Clinic and Research Foundation
Gerald Fuller, University of Alabama, Birmingham

229 participants

Attended by 250 scientists from the United States and abroad, the Regulation of Liver Gene Expression meeting focused on mechanisms of tissue-specific gene expression in the liver, on hormonal and developmental regulation of liver gene expression, on conditions of maintaining differentiated hepatic cells in culture, and on the expression of the liver acute-phase genes. Much attention has been focused on the recent development of tissue-specific cell-free transcription systems from rat liver nuclei (Schibler). Using this system, *trans*-acting control factors of the albumin gene and of several other liver-specific genes have been identified and partially purified. For several liver genes, tissue specificity is achieved by cooperative binding of a series of *trans*-acting factors in the promoter upstream regions (Schibler, Yaniv, Klein-Hitpass, Wahli). Hepatocyte-stimulating factor (HSF), a monokine responsible for the induction of liver acute-phase genes during acute and chronic inflammation, was purified (Fuller). Gauldie reported that HSF is identical to interferon $\beta 2$. Impressive progress has been made with the genetic characterization of *trans*-acting loci controlling liver gene expression (Fournier, Weiss, Gluecksohn-Waelsch), and an extinguisher locus of the tyrosine amino transferase gene has been mapped by somatic-cell genetics. Other genetic loci controlling the expression of the α-feto protein gene (*rif* and *raf*; Camper and Tilghman) have been characterized in detail. Retroviral vectors have been used successfully to transfect primary hepatocyte cultures (Wolff, Wilson, Woo). Bennett and collaborators reported on the successful establishment of a novel hepatoblast culture system. These cultures, unlike other known hepatocyte cultures, undergo a program of differentiation and tissue-specific gene expression that mimics events during early embryogenesis in vivo. Gohda and collaborators reported the discovery of a novel human hepatocyte growth factor in the plasma of patients with fulminant hepatic failure. The hormone-binding and DNA-binding domains of the glucocorticoid receptors were defined in a beautiful series of mutagenesis experiments described by Ringold. Liao reported that the rat serum amyloid A gene transcripts lack an exon, which is present in the rat gene and in the equivalent mouse and human SAA gene transcripts. Mice and humans are afflicted by serum amyloidosis due to the deposition of amyloid fibrils, whereas rats do not suffer from this condition. Probably the alternative splicing pathway is responsible for the absence of this disorder in rats. In the keynote presentation, Tjian demonstrated the power and elegance of cell-free transcription systems and biochemical purification procedures in defining transcriptional cofactors. Several transcription factors have been purified, and the cloning and mutagenesis of SP1

were reported. The next conference on the regulation of liver gene expression has been scheduled for 1989 in Cold Spring Harbor.

This meeting was supported in part by American Hoechst Corporation, Inc., Bechman Instruments, Inc., Bethesda Research Laboratory, Bio-Rad (Chemical Division), Calbiochem Biochemicals/Behring Diagnostics (division of Hoechst), HaakeBuchler Instruments, Inc., The March of Dimes Birth Defects Foundation, the following divisions of the National Institutes of Health: NIAID and NIDDK; Dr. James Pittman, School of Medicine at University of Alabama, Birmingham, Stratagene Cloning Systems, Whatman, Inc., Codon, and Behring.

SESSION 1 TISSUE-SPECIFIC EXPRESSION OF LIVER GENES

Chairman: U. Schibler, University of Geneva

Lichtsteiner, S., Gorski, K., Mueller, C., Wuarin, J., Schibler, U., Dept. of Molecular Biology, University of Geneva, Switzerland: Transcription factors and promoter elements involved in the liver-specific expression of the albumin gene.

Cereghini, S., Blumenfeld, M., Raymondjean, M., Carranca, A.G., Yaniv, M., Dept. of Molecular Biology, CNRS, Institut Pasteur, Paris, France: Factors involved in control of tissue-specific expression of the albumin gene.

Cortese, R., De Simone, E., Frain, M., Hardon, E., Morrone, G., Oliviero, S., Paonessa, G., EMBL, Heidelberg, Federal Republic of Germany: Cell-specific expression of genes coding for human plasma proteins.

Johnson, P., Landschulz, W., LaMarco, K., Graves, B., McKnight, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Identification of a DNA-binding protein from rat liver nuclei that activates viral gene expression in vitro.

Costa, R.H., Lai, E., Darnell, J.E., Jr., Rockefeller University, New York, New York: Regulatory sequences for the mouse transthyretin (pre-albumin) gene include a cell-specific enhancer.

Leff, T., Reue, K., Das, H.K., Breslow, J., Laboratory of Biochemical Genetics and Metabolism, Rockefeller University, New York, New York: Negative control elements regulate cell-type-specific expression of the human apoprotein-B and -CIII genes in vivo and in vitro.

Levy-Wilson, B., Blackhart, B.D., Mahley, R.W., McCarthy, B.J., Gladstone Foundation Laboratories, University of California, San Francisco: Structure and regulation of the human apolipoprotein-B gene.

Schorpp, M., Wagner, U., Ryffel, G.U., Kernforschungszentrum Karlsruhe, Federal Republic of Germany: A DNA element in the 5′-flanking region of the *X. laevis* albumin genes confers tissue-specific expression in mouse hepatoma cells.

SESSION 2 MODULATION OF LIVER GENE EXPRESSION

Chairman: G. Schütz, German Cancer Research Center, Heidelberg

Schütz, G., Jantzen, H.-M., Strähle, U., Klock, G., Gloss, B., Stewart, F., Becker, P., Schmid, W., German Cancer Research Center, Heidelberg, Federal Republic of Germany: Glucocorticoid regulation of expression of the tyrosine aminotransferase gene.

Granner, D., Dept. of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee: Mechanisms involved in the hormonal regulation of transcription of the PEPCK gene.

Karin, M., Imagawa, M., Chiu, R., Bodner, M., Dana, S., Dept. of Pharmacology, University of California School of Medicine, San Diego, La Jolla: Regulation of gene expression by hormone-responsive and tissue-specific *trans*-acting factors.

Klein-Hitpass, L., Cato, A.C.B., Ryffel, G.U., Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, Federal Republic of Germany: Estrogen-responsive DNA element—Sequence requirements and interaction with steroid hormone receptors.

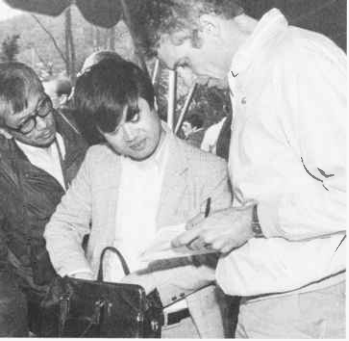
Shapiro, D.J., Barton, M.C., Weiler, I.J., Lew, D., McKearin, D.M., Keller, M.J., Blume, J., Nielsen, D., Dept. of Biochemistry, University of Illinois, Urbana: Estrogen receptor regulation in vitellogenin and retinol-binding protein gene expression.

Whitlock, J.P., Jr., Durrin, L.K., Jones, P.B.C., Dept. of Pharmacology, Stanford University School of Medicine, California: Regulation of gene expression by dioxin.

Duester, G., Knoll, D., Dept. of Biochemistry, Colorado State University, Fort Collins: Regulation of human liver ADH gene expression by glucocorticoids.

Wahli, W., Martinez, E., ten Heggeler-Bordier, B., Hipskind, R., Seiler-Tuyns, A., Walker, P., Corthésy, B., Institut de Biologie Animale, Université de Lausanne, Switzerland: *cis*- and *trans*-Acting elements of the estrogen-regulated *Xenopus* vitellogenin genes.

J. Kushner, S. Macintyre



G. Fuller, L. Holland

SESSION 3 POSTER SESSION

Aiello, L.P., Shia, M.A., Pilch, P.F., Farmer, S.R., Dept. of Biochemistry, Boston University School of Medicine, Massachusetts: Characterization and hepatic expression of rat α_1-inhibitor III.

Amrani, D.L.,[1,2] Nimmer, D.,[2] Bergtrom, G.,[2] [1]Dept. of Medicine, University of Wisconsin Medical School, [2]Dept. of Biology, University of Wisconsin, Milwaukee: Glucocorticoids regulate production of plasma fibronectin at the transcriptional and posttranscriptional levels.

Andus, T.,[1] Bauer, J.,[1] Ganter, U.,[2] Northoff, H.,[2] Männel, D.,[3] Tran-Thi, T.-A.,[1] Decker, K.,[1] Heinrich, P.C.,[1] [1]University of Freiburg; [2]Blutspendezentrale des DRK, Ulm; [3]Institut für Immunologie, Heidelberg, Federal Republic of Germany: Biosynthesis of HSF in human monocytes.

Andus, T.,[1] Geiger, T.,[1] Bauer, J.,[1] Kunz, D.,[1] Heisig, M.,[1] Northoff, H.,[2] Tran-Thi, T.-A.,[1] Decker, K.,[1] Gerok, W.,[1] Heinrich, P.C.,[1] [1]University of Freiburg; [2]Blutspendezentrale des DRK, Ulm, Federal Republic of Germany: Induction of acute-phase protein synthesis – Studies on the regulation of rat α_2-macroglobulin in vivo and in hepatocyte primary cultures.

Aycock, R.S.,[1] Raghow, R.,[2] Seyer, J.M.,[3] Depts. of [1]Medicine, [2]Pharmacology, [3]Biochemistry, University of Tennessee, Memphis and Veterans Administration Medical Center: Enhanced expression of genes coding for interstitial procollagen chains by a hepatic fibrogenic factor.

Bade, E.G., Feindler, S., Graf, H., Fakultät für Biologie, Universität Konstanz, Federal Republic of Germany: Modulation of liver epithelial cell gene expression by EGF.

Baffet, G.,[1] Desvergne, B.,[1] Etienne, P.L.,[1] Deugnier, Y.,[1] Defer, N.,[2] Guguen-Guillouzo, C.,[1] [1]Hôpital Pontchaillou, Rennes, [2]Institut de Pathologie et Biologie Cellulaires et Moleculaires, Paris, France: Oncogene activation in human hepatocarcinomas.

Bauer, J., Ganter, U., Andus, T., Richter, I., Gebicke-Haerter, P., Andreesen, R., Löhr, G., Heinrich, P.C., Gerok, W., University of Freiburg, Federal Republic of Germany: Comparison of α_2-macroglobulin synthesis in rat hepatocytes, rat astrocytes, and human mononuclear phagocytes.

Bauer, J.,[1] Gebicke-Haerter, P.,[1] Andus, T.,[1] Ganter, U.,[1] Northoff, H.,[2] Heinrich, P.C.,[1] Gerok, W.,[1] [1]University of Freiburg, [2]DRK-Blutspendezentrale, Ulm, Federal Republic of Germany: Secretion of HSF by rat astrocyte primary cultures.

Bernuau, D., Poliard, A., Feldmann, G., Laboratoire de Biologie Cellulaire, INSERM, Paris, France: In situ hybridization analysis of AFP mRNA expression in rat liver during regeneration after partial hepatectomy.

Bertolotti, R., Lutfalla, G., Armbruster, L., Centre de Génétique Moléculaire, CNRS, France: Liver-specific anti-oncogenes and extinguishers – Gene balance, shuttle vectors, and expression libraries.

Bhat, N.K., Fujiwara, S., Fisher, R.J., Papas, T.S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Regulation of *ets* loci during early phase of murine hepatic regeneration.

Bigot, Y.,[1] Ng, K.,[2] Maigné, J.,[2] Deschatrette, J.,[2] Meunier-Rotival, M.,[2] [1]BEAS, Tours, [2]Hôpital de Bicêtre, France: Localization and sequence of interspersed repeats in the mouse serum albumin gene.

Büchler, W., Walter, U., Lohmann, S.M., Dept. of Medicine, University of Würzburg, Federal Republic of Germany: Evidence for participation by the C subunit of cAMP-dependent protein kinase in the mechanism of cAMP-regulated gene expression in hepatocytes.

Chou, C.-K.,[1,2] Tsai, T.-F.,[2] Birnbaum, M.J.,[3] Rosen, O.M.,[3] Su, T.S.,[1,2] [1]Dept. of Medical Research, Veterans General Hospital, [2]Institute of Microbiology and Immunology, National Yang-Ming Medical College, Taipei, Taiwan, R.O.C.; [3]Memorial Sloan-Kettering Cancer Center, New York, New York: Expression of glucose transporter mRNA in a human hepatoma cell line but not in normal human liver.

Coe, J.E., Ross, M.J., Rocky Mountain Laboratories, Hamilton, Montana: Paradoxical effect of steroid hormones on hamster liver – Syrian vs. Armenian hamsters.

Poster Session

Corcos, L.,[1] Rousset, J.-P.,[1] Wiebel, F.J.,[2] Weiss, M.C.,[2] [1]Dept. of Molecular Biology, Institut Pasteur, Paris, France; [2]Institute of Biochemistry and Toxicology, Dept. of Toxicology, Munich, Federal Republic of Germany: Selection of hepatoma cells deficient in some cytochrome P450 molecular species.

Courtois, G., Morgan, J., Fourel, G., Crabtree, G., Dept. of Pathology, Stanford University School of Medicine, California: Tissue-specific expression of the β-fibrinogen gene requires the interaction of a liver-specific protein(s) with the β promoter.

Czaja, M., Weiner, F., Giambrone, M., Zern, M., Liver Research Center, Albert Einstein College of Medicine, Bronx, New York: Antifibrogenic effects of IFN-γ in in vitro and in vivo models.

Czaja, M., Weiner, F., Giambrone, M., Zern, M., Liver Research Center, Albert Einstein College of Medicine, Bronx, New York: Molecular studies of ceruloplasmin deficiency in Wilson's disease.

Danan, J.L., Jose, M., Poliard, A., Tratner, I., Poiret, M., Nahon, J.L., Sala-Trepat, J.M., Laboratoire d'Enzymologie, CNRS, France: Rat AFP gene regulation – Analysis of the promoter region and its interaction with liver nuclear extracts.

Daniel, V.,[1] Sharon, R.,[1] Tichauer, Y.,[1] Sarid, S.,[2] Depts. of [1]Biochemistry, [2]Biophysics, Weizmann Institute of Science, Rehovot, Israel: Mouse liver glutathione *S*-transferase Ya subunit – Gene structure and sequence.

Decaux, J.F.,[1] Levrat, F.,[1] Girard, J.,[2] Kahn, A.,[1] [1]INSERM, Paris, [2]CNRS, Meudon, France: Gene expression in primary culture of rat hepatocytes.

Delhaye, M.,[1] Gulbis, B.,[2] Mairesse, N.,[2] Galand, P., Free University of Brussels, Belgium: Characterization of γ-glutamyl transferase from neoplastic and nonneoplastic liver tissues in man and during rat liver hepatocarcinogenesis.

Dickson, P.W., Aldred, A.R., Marley, P.D., Schreiber, G., Dept. of Biochemistry, University of Melbourne, Parkville, Australia: Expression of the transthyretin gene in the liver and the choroid plexus.

Feinstein, S.I., Leff, T., Breslow, J.L., Laboratory of Biochemical Genetics and Metabolism, Rockefeller University, New York, New York: DNA sequences within 250 bases upstream from the cap site control liver-specific expression of the apolipoprotein-AI gene.

Fujita, M.,[1] Choi, H.,[2] Watanabe, T.,[1] Montgomery, K.,[1] Rosenberg, L.C.,[2] Reid, L.M.,[1] Depts. of [1]Molecular Pharmacology, [2]Connective Tissue Research, Albert Einstein College of Medicine, Bronx, New York: Heparin and heparin proteoglycan restore transcription of tissue-specific genes in primary liver cultures.

Gauldie, J.,[1] Baumann, H.,[2] Richards, C.,[1] [1]Molecular Virology and Immunology Program, McMaster University, Hamilton, Canada; [2]Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Human HSF and recombinant IL-1 and TNF stimulate distinct sets of acute-phase reactant proteins in vitro.

Georgatsou, E., Fourel, G., Tosi, M., Meo, T., Unité d'Immunogénétique, Institut Pasteur, Paris, France: Promoter differences in the murine genes encoding C4 and its testosterone-dependent isoform C4-Slp.

Gheradi, E., Bowyer, D.E., Dept. of Pathology, University of Cambridge, England: Tissue-specific expression of the LDL receptor revealed by antireceptor monoclonal antibodies.

S. Sell, K. Fournier

Goldman, N.D., Li, S.-P., Washington, G.C., Liu, T.-Y., CDB, FDA, Bethesda, Maryland: Biosynthesis of CRP in liver cell culture is regulated by a cytokine produced by monocytes and certain T lymphocytes.

Grant, S.G., Stephenson, D.A., Chapman, V.M., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Characterization of the hierarchical regulation of murine ornithine carbamoyltransferase.

Grayson, D.R., Darnell, J.E., Dept. of Molecular Cell Biology, Rockefeller University, New York, New York: A cell-type-specific enhancer is located upstream of the murine α_1-antitrypsin promoter.

Grundmann, U., Amann, E., Zettlmeissel, G., Küpper, H., Dept. of Molecular Biology, University of Marburg, Federal Republic of Germany: Characterization of cDNA coding for human factor XIIIa.

Guigoz, Y., Werffeli, P., Favre, D., Juillerat, M., Wellinger, R., Honegger, P., University of Lausanne, Switzerland: Aggregate culture of fetal rat liver cells—Differentiation and maintenance of liver-specific gene expression.

Guille, M.J., Arnstein, H.R.V., Dept. of Biochemistry, King's College, London, England: Estrogen alteration of vitellogenin mRNA stability—An investigation of the molecular mechanism.

Hattori, M., Tsuchiya, Y., Ito, T., K. Hayashida, Sakaki, Y., Research Laboratory for Genetic Information, Kyushu University, Fukuoka, Japan: Rat α_2-macroglobulin gene—Structure of the promoter and protein binding to the gene.

Herrero-Zabaleta, M.E., Knoll, B.J., Sell, S., Dept. of Pathology and Laboratory Medicine, University of Texas Medical School, Houston: DNA sequences involved in the regulation of the rat AFP gene—Unusual features of the enhancer region.

Holland, L.J., Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City: Unique pattern of regulation of ferritin gene expression by estrogens in *Xenopus* liver.

Höppner, W., Rasmussen, U.B., Wohlrab, H., Seitz, H.J., Universität Hamburg, Federal Republic of Germany and Harvard Medical School, Boston, Massachusetts: Hormonal regulation of adenine nucleotide translocator gene expression in different tissues oi the rat.

Höppner, W., Seitz, H.J., Institut für Physiologische Chemie, Universitäts-Frankenhaus Eppendorf, Hamburg, Federal Republic of Germany: Thyroid hormone action on P-enolpyruvate carboxykinase gene expression in rat liver cells.

Ichihara, A., Nakamura, T., Institute of Enzyme Research, University of Tokushima School of Medicine, Japan: Terminal differentiation of neonatal hepatocytes in primary culture requires contact with adult hepatocytes.

Itakura, K., Gertson, P.W., Carr, B.I., City of Hope National Medical Center, Duarte, California: Expression of EGF receptors during regeneration of liver and chemical hepatocarcinogenesis.

Iynedjian, P.B., Institute of Clinical Biochemistry, University of Geneva School of Medicine, Switzerland: Regulation of glucokinase mRNA in liver.

Jackson, M.R., Coughtris, M.W.H., Corser, R.B., Harding, D., Silson, S.M., Burchell, B., Dept. of Biochemistry, University of Dundee, Scotland: Expression and genetic deficiencies of hepatic microsomal UDP-glucuronyltransferases.

Jacob, S.T., Maguire, K.M., Garg, L.C., Webb, M.L., Dept. of Pharmacology and Cell and Molecular Biology Center, Pennsylvania State University College of Medicine, Hershey: Accurate and efficient transcription of mouse metallothionein I gene in a fractionated nuclear extract from rat liver and a rat hepatoma.

Jameel, S., Siddiqui, A., Dept. of Microbiology and Immunology, University of Colorado Medical School, Denver: A human liver-cell-specific enhancer and *trans*-acting factors.

Jefferson, D.M., Reid, L.M., Dept. of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York: Hormonal modulation of stability of individual mRNA species in primary liver cultures.

Jefferson, D.M.,[1] Zern, M.A.,[2] Weiner, F.R.,[2] Czaja, M.J.,[2] Reid, L.M.,[1] Depts. of [1]Molecular Pharmacology, [2]Medicine, Albert Einstein College of Medicine, Bronx, New York: Effects of DEX on tissue-specific gene expression in cultured hepatocytes.

Johnston, D.E., Jefferson, D.M., Dept. of Physiology, Medicine, and Pediatrics, Tufts University School of Medicine and New England Medical Center, Boston, Massachusetts: Further purification and characterization of a serum factor that modulates hepatocyte-specific mRNA half-life.

SESSION 4 TRANS-REGULATION OF LIVER GENES

Chairmen: **M. Weiss,** Pasteur Institute, Paris
K. Fournier, University of Southern California, Los Angeles

Herbomel, P., Ott, M.-O., Mottura-Rollier, A., Heard, J.-M., Yaniv, M., Weiss, M.C., Dept. of Molecular Biology, CNRS, Institut Pasteur, Paris, France: DNA targets for positive and negative regulation of albumin transcription.

Fournier, K., University of Southern California, Los Angeles: Trans-regulation of liver-specific gene expression in hepatoma-hybrid cells.

Babiss, L.E., Herbst, R.S., Bennett, A., Darnell, J.E., Rockefeller University, New York, New York: Albumin gene expression—Required DNA sequences and proteins that bind to them.

Ng, K.,[1] Maigné, J.,[1] Fougère-Deschatrette, C.,[2] Meunier-Rotival, M.,[1] Deschatrette, J.,[1] [1]Hôpital de Bicêtre, France; [2]Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Expression of the mouse serum albumin gene introduced into differentiated and dedifferentiated rat hepatoma cells—Effect of reversion and somatic hybridization.

Warren, H., Pachnis, Z., Tilghman, S., Princeton University, New Jersey: Regulation of genes under the control of *raf*, a *trans*-acting locus in the mouse.

Latimer, J.J., Berger, F.G., Baumann, H., Dept. of Molecu-

lar and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: α_1-Antitrypsin expression is induced during puberty in the kidney of *M. caroli*.

Majmudar, G., Roux, J., Grange, T., Pictet, R., Institut Jacques Monod, Paris, France: Localization of a negative regulatory element involved in the tissue-specific expression of tyrosine amino transferase gene.

Palmer, D.,[1] Muglia, L.,[2] Rothman-Denes, L.B.,[1,2] Depts. of [1]Biochemistry and Molecular Biology, [2]Molecular Genetics and Cell Biology, University of Chicago, Illinois: Rat AFP-negative regulatory element.

Shaul, Y., Ben-Levy, R., Faktor, O., Dickstein, R., Berger, I., Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Liver-cell-specific activity of HBV enhancer element is mediated by a cellular *trans*-acting factor(s).

Vaulont, S., Cognet, M., Lone, Y.C., Kahn, A., INSERM, Paris, France: Structure and chromatin conformation of the rat L-type pyruvate kinase gene.

Venetianer, A., Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary: Activation of AFP synthesis in rat hepatoma cells with reduced sensitivity to glucocorticoids.

SESSION 5 REGULATION OF LIVER GENES BY MONOKINES AND PEPTIDE HORMONES

Chairman: G. Fuller, University of Alabama, Birmingham

Fuller, G.M., Grenett, H., Bunzel, R.J., Dept. of Cell Biology and Anatomy, University of Alabama, Birmingham: Isolation, characterization, antibody production, and cloning of the hepatocyte-stimulating factor from a mouse macrophage cell line.

Baumann, H., Onorato, V., Jahreis, G.P., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: HSF of human keratinocytes.

Darlington, G.J., Wilson, D.R., Baylor College of Medicine, Houston, Texas: An in vitro model of the human acute-phase response.

Ganapathi, M.K., Mackiewicz, A., Schultz, D., Samols, D., Brabenec, A., Hu, S.-I., Macintyre, S.S., Kushner, I., Case Western University, Cleveland Metropolitan General Hospital, Ohio: Regulation of synthesis of human and rabbit acute-phase proteins by monokines.

Perlmutter, D.H., Depts. of Pediatrics and Cell Biology, Washington University School of Medicine and Children's Hospital, St. Louis, Missouri: Regulation of human hepatic acute-phase gene expression involves several mediators and mechanisms.

Rogers, J.T.,[1] Glass, J.,[2] Auron, P.E.,[1] [1]Massachusetts Institute of Technology, Cambridge; [2]Dept. of Hematology, Louisiana State University, Shreveport: Translational induction of H and L ferritin in human hepatoma (Hep-G2) cells by IL-1β.

Shapiro, J., Mortensen, R.F., Dept. of Microbiology, Ohio State University, Columbus: Induction of the mouse acute-phase reactant serum amyloid P component by recombinant monokines IL-1 and TNF.

Sipe, J.D., Arthritis Center, Boston University School of Medicine, Massachusetts: Variable expression of murine SAA genes in liver and peripheral sites during acute and chronic inflammation.

Reid, L.M., Fujita, M., Choi, H., Watanabe, T., Montgomery, K.T., Jefferson, D.M., Rosenberg, L.C., Albert Einstein College of Medicine, Bronx, New York: Both extracellular matrix and hormones are needed for expression of tissue-specific mRNAs in primary liver cultures.

SESSION 6 EVOLUTION OF PLASMA PROTEIN GENES

Chairmen: W. Gilbert, Harvard University
E. Davie, University of Washington, Seattle

Marchionni, M., Harvard University, Cambridge, Massachusetts: The Intron theory of genes.

Davie, E.W., Dept. of Biochemistry, University of Washington, Seattle: Molecular biology of blood coagulation factors.

Hill, R.E., Hastie, N.D., MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, Scotland: Accelerated evolution in the reactive center regions of serine protease inhibitors—A case of positive Darwinian selection.

Yang, F., Adrian, G.S., Naylor, S., Bowman, B.H., Dept. of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio: Evolution and expression of plasma protein genes

Prowse, K.R., Baumann, H., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Multiple inflammation-inducible α_1-acid glycoprotein forms correlate with multiple genes in *M. caroli*.

Held, W.,[1] Gallagher, J.,[1] Hohman, C.,[1] Kuhn, N.,[1] Sampsell, B.,[2] Hughes, R., Jr.,[1] [1]Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, [2]Dept. of Biology, State University College, Buffalo, New York: Identification and characterization of functional genes encoding the mouse major urinary proteins.

Derman, E.,[1] Shahan, K.,[1] Shi, Y.,[2] Denaro, M.,[1] [1]Public Health Research Institute, Inc., New York, [2]Dept. of Cell Biology, New York University Medical School, New York: Coordinate and divergent regulation of mouse urinary protein gene isotypes.

Bishop, J.O., Spiegelberg, T., Dept. of Genetics, University of Edinburgh, Scotland: Hormonal effects on the expression of tissue-specific genes in cultured mouse hepatocytes and in liver.

SESSION 7 DEVELOPMENTAL REGULATION OF GENE EXPRESSION

Chairman: S. Tilghman, Princeton University

Camper, S., Tilghman, S., Princeton University, New Jersey: Regulation of the α-fetoproteins in cells and mice.

Gluecksohn-Waelsch, S., Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Developmental regulation of expression of a group of liver-specific genes in the mouse.

Bélanger, L.,[1] Guertin, M.,[1] LaRue, H.,[1] Turcotte, B.,[1] Chevrette, M.,[1] Wrange, O.,[2] [1]Université Laval, Quebec, Canada; [2]Karolinska Institute, Stockholm, Sweden: Developmental and hormonal regulation of the AFP gene in rat liver.

Burch, J., Evans, M., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Identification of upstream elements that mediate the estrogen-dependent transcription of the major vitellogenin gene in the avian liver.

Camoretti-Mercado, B.,[1] Muglia, L.,[1] Mellovitz, B.,[2] Rothman-Denes, L.B.,[1] Ross, S.R.,[2] Depts. of [1]Molecular Genetics and Cell Biology, [2]Biological Chemistry, University of Chicago, Illinois: Developmental regulation of rat AFP gene in transgenic mice.

Wolff, J.,[1] Saint Louis, D.,[2] Verma, I.,[2] Skelly, H.,[3] Leffert, H.,[3] Yee, J.-K.,[1] Friedmann, T.,[1] Depts. of [1]Pediatrics, [3]Medicine, University of California, [2]Salk Institute, Molecular Biology and Virology Laboratory, La Jolla: Retroviral gene transfer and expression in primary adult rat hepatocytes.

Briand, P., Cavard, C., Grimber, G., Laboratoire de Biochimie Génétique, Hôpital Necker, Paris, France: Mouse enzyme deficiency corrected by gene transfer.

Kelsey, G.D.,[1] Povey, S.,[1] Bygrave, A.E.,[2] Lovell-Badge, R.H.,[2] [1]MRC Human Biochemical Genetics Unit, [2]MRC Mammalian Development Unit, London, England: Species- and tissue-specific expression of human α_1-antitrypsin in transgenic mice.

West, A., Fenton, W.A., Bennett, J., Rasmussen, J., Levy, E., Horwich, A., Rosenberg, L.E., Dept. of Human Genetics, Yale University, New Haven, Connecticut: Isolation of the human ornithine transcarbamylase gene and characterization of its 5′-flanking sequence.

H. Tsubouchi, Y. Daikohara, E. Gohda

SESSION 8 POSTER SESSION

Kwon, B., Haq, A., Molecular Genetics Laboratory, Guthrie Research Institute, Sayre, Pennsylvania: Isolation of a mouse tyrosinase gene that maps at the mouse albino locus.

Lagacé, M., Howell, B.W., Shore, G.C., Dept. of Biochemistry, McGill University, Montreal, Canada: Rat carbamyl phosphate synthetase 1 gene. 5′-flanking sequence and tissue-specific transcriptional regulation in vitro.

Lamers, W.H., van Roon, M.A., Westenend, P.J., Zonneveld, D., Moorman, A.F.M., Charles, R., Dept. of Anatomy and Embryology, University of Amsterdam, The Netherlands: Inducibility of hepatocyte-specific enzymes in embryonic rat hepatocytes.

Latruffe, N., Kanté, A., Bailly, A., Adami, P., Cherkaoui-Malki, M., Université de Franche-Comté, Laboratoire de Biochimie, CNRS, Besançon, France: Biogenesis of BDH; a ketone-bodies-converting enzyme.

Lernhardt, W., La Jolla Cancer Research Foundation, California: Expression of a truncated C3 mRNA in T-helper lymphocytes and T-cell hybridomas.

Liu, J.-K., Zaret, K.S., Section of Biochemistry, Brown University, Providence, Rhode Island: Multiple tissue-specific DNase-I-hypersensitive sites exist simultaneouosly on the serum albumin gene in mouse liver chromatin.

Lucotte, G., CNTS-Institute, Laboratory of Molecular Genetics, Paris, France: Variations of some human plasma protein genes.

Ma, K.T.,[1] Woodworth, C.D.,[2] Isom, H.C.,[2] Liao, W.S.L.,[1]

[1]Dept. of Biochemistry and Molecular Biology, M.D. Anderson Hospital and Tumor Institute, Houston, Texas; [2]Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Stimulation of the acute-phase response in SV-40-immortalized rat hepatocytes.

Macintyre, S., Samols, D., Kushner, I., Case Western Reserve University, Cleveland Metropolitan General Hospital, Ohio: Regulation of intracellular transport of CRP in cultured rabbit hepatocytes.

Machiewicz, A., Ganapathi, M.K., Schultz, D., Kushner, I., Case Western Reserve University, Cleveland Metropolitan General Hospital, Ohio: Regulation of acute-phase protein glycosylation by monokines.

Maeda, S.,[1] Wakasugi, S.,[1] Murakami, T.,[1] Yamamura, K.,[2] Shimada, K.,[1] [1]Dept. of Biochemistry, [2]Institute for Medical Genetics, Kunamoto University Medical School, Japan: Expression of the pre-albumin (transthyretin) gene in fetal and adult mice.

Mairesse, N.,[1] Delhaye, M.,[2] Galand, P.,[3] [1]Campus Erasme, [2]Erasme Hospital, [3]School of Medicine, University of Brussels, Belgium: Changes in the pattern of neosynthesized proteins in preneoplastic and neoplastic liver during experimental hepatocarcinogenesis in rats.

Mantle, T.J., Rigney, E., Boyce, S.I., Dept. of Biochemistry, Trinity College, Dublin, Ireland: Testosterone regulation of the expression of glutathione *S*-transferase YFYF (7-7) in mouse liver—Effect of phenobarbital.

Mardon, H.J., Baralle, F.E., Sir William Dunn School of Pathology, University of Oxford, England: Expression of fibronectin in regenerating liver.

Meyer, S., Höppner, W., Seitz, H.J., Institut für Physiologische Chemie, Universität Hamburg, Federal Republic of Germany: Direct effect of glucose on P-enolpyruvate carboxykinase gene expression in rat liver cells.

Milward, L., Yeoh, G.C.T., Dept. of Physiology, University of Western Australia, Perth: Expression of AFP by cultured fetal rat hepatocytes.

Minderop, R.H., Höppner, W., Seitz, H.J., Institut für Physiologische Chemie, Universität Hamburg, Federal Republic of Germany: Regulation of hepatic glucokinase gene expression—Role of carbohydrates, glycocorticoid, and thyroid hormones.

Mira, E., Castaño, J.G., Faculty of Medicine, University of Madrid, Spain: Cloning of a rat-liver-specific mRNA under insulin control.

Moorman, A.F.M., de Groot, C.J., Janzen, J.W.G., Charles, R., Lamers, W.H., Dept. of Anatomy and Embryology, University of Amsterdam, The Netherlands: Reciprocal regulation of carbamyl phosphate synthetase and glutamine synthetase gene expression in rat liver.

Nebes, V.L., Morris, S.M., Jr., Dept. of Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh, Pennsylvania: Hormonal regulation of mRNA levels for five urea cycle enzymes in rat hepatocytes cultured in serum-free medium.

Noda, C., Nakamura, T., Ichihara, A., Enzyme Science Center, University of Tokushima, Japan: Regulation of liver-specific gene expression by glucocorticoids—Possible involvement of a *trans*-acting regulatory protein induced by glucocorticoids.

Northemann, W.,[1] Gehring, M.,[2] Braciak, T.,[1] Hudson, G.,[1] Shiels, B.,[1] Heinrich, P.,[3] Hanson, R.,[4] Fey, G.,[1] [1]Dept. of Immunology, Research Institute of Scripps Clinic, [2]Agouron Pharmaceuticals, Inc., La Jolla, California; [3]University of Freiburg, Federal Republic of Germany; [4]Case Western Reserve University, Cleveland, Ohio: Structure and expression of the rat acute-phase genes coding for α_2-macroglobulin and α_1-inhibitor III.

Panduro, A., Shalaby, F., Shafritz, D., Liver Research Center, Albert Einstein College of Medicine, Bronx, New York: Changing patterns of transcriptional and posttranscriptional control of liver-specific gene expression during rat development.

Paul, D., Höhne, M., Hoffmann, B., Fraunhofer Institute for Toxicology, Hannover, Federal Republic of Germany: Growth control of cultured hepatocytes, their immortalization and transformation in vitro.

Prowse, K.R., Baumann, H., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Localization of the *cis*-acting element through which HSF and IL-1 enhance expression of the rat α_1-acid glycoprotein gene.

Ramadori, G.,[1] Mitsch, A.,[1] Rieder, H.,[1] Tobias, P.,[2] Ulevitch, R.,[2] Meyer zum Büschenfelde, K.-H.,[1] [1]Dept. of Internal Medicine, University of Mainz, Federal Republic of Germany; [2]Research Institute of Scripps Clinic, La Jolla, California: SAA gene expression in mouse and rabbit hepatocytes.

Ramadori, G.,[1] Rieder, H.,[1] Knittel, T.,[1] Dienes, H.P.,[2] Meyer zum Büschenfelde, K.-H.,[1] [1]Dept. of Internal Medicine, [2]Institute of Pathology, University of Mainz, Federal Republic of Germany: Fibronectin biosynthesis by fat-storing cells of rat liver. Comparison with fibronectin from hepatocytes.

B. McCarthy, J. Bishop

Poster Session

Ramadori, G.,[1] Tobias, P.,[2] Mitsch, A.,[1] Rieder, H.,[1] Ulevitch, R.,[2] Meyer zum Büschenfelde, K.-H.,[1] [1]Dept. of Internal Medicine, University of Mainz, Federal Republic of Germany; [2]Research Institute of Scripps Clinic, La Jolla, California: Synthesis of a new acute-phase reactant; lipopolysaccharide-binding protein by rabbit hepatocytes.

Raymondjean, M., Cereghini, S., Yaniv, M., Dept. of Molecular Biology, CNRS, Institut Pasteur, Paris, France: Four rat proteins interacting with the albumin promoter are detected by gel-retardation assays.

Robinson, G.S.,[1] Bellas, B.E.,[1] Ben-Ze'ev, A.,[1] Bucher, N.L.R.,[2] Farmer, S.R.,[1] Depts. of [1]Biochemistry, [2]Pathology, Boston University Medical School, Massachusetts: Cellular matrices and hepatic gene expression.

Roux, J., Grange, T., Majmudar, G., Pictet, R., Institut J. Monod, Paris, France: Study of the rat tyrosine aminotransferase gene. Control region involved in tissue-specific repression.

Shieh, S.-Y.,[1] Lee, S.-C.,[1,2] [1]Institute of Biological Chemistry Academia Sinica; [2]School of Medicine, National Taiwan University, Republic of China: Cloning the mouse α_1-acid glycoprotein cDNA and its gene expression during liver regeneration.

Shiels, B.,[1] Northemann, W.,[1] Gehring, M.,[2] Fey, G.,[1] [1]Dept. of Immunology, Research Institute of Scripps Clinic, [2]Agouron Pharmaceuticals, Inc., La Jolla, California: Alterations in poly(A) tail length of α_1-acid glycoprotein mRNA during acute inflammation.

Smith, J.D., Breslow, J.L., Laboratory of Biochemical Genetics and Metabolism, Rockefeller University, New York, New York: Functional domains that regulate the tissue-specific expression of the human apolipoprotein-E gene.

Spolski, R.J.,[1] Aprison, B.S.,[2] Sanchez, A.,[3] Wangh, L.J.,[3] [1]Ontario Cancer Institute, Toronto, Canada; [2]Dept. of Biology, Indiana University, Bloomington; [3]Dept. of Biology, Brandeis University, Waltham, Massachusetts: Estrogen-dependent DNA synthesis and division of frog liver cells.

Spray, D.C.,[1] Fujita, M.,[2] Saez, J.C.,[1] Choi, H.,[3] Watanabe, T.,[2] Hertzberg, E.,[4] Rosenberg, L.C.,[3] Reid, L.M.,[2] Depts. of [1]Neuroscience, [2]Molecular Pharmacology, [3]Orthopedic and Connective Tissue Research, Albert Einstein College of Medicine, Bronx, New York; [4]Dept. of Biochemistry, Baylor College, Houston, Texas: Proteoglycans and glycosaminoglycans induce gap-junction synthesis and function in primary liver cultures.

Stephens, A.W., Siddiqui, A., Hirs, C.H.W., Depts. of Biochemistry, Biophysics, and Genetics, and Microbiology and Immunology, University of Colorado Medical School, Denver: Expression of a functionally active human antithrombin III.

Strain, A.J., Frazer, A., Hill, D.J., Dept. of Pediatrics, Clinical Sciences Centre, University of Sheffield, England: TGFβ inhibits DNA synthesis in hepatocytes isolated from both normal and regenerating rat liver.

Strain, A.J., Hill, D.J., Milner, R.D.G., Dept. of Pediatrics, Clinical Sciences Centre, University of Sheffield, England: Regulation of DNA synthesis in primary human fetal hepatocytes by IGF-I and TGFβ.

Szpirer, J.,[1] Islam, Q.,[2] Levan, G.,[2] Szpirer, C.,[1] [1]Dept. of Molecular Biology, Université Libre de Bruxelles, Belgium; [2]Dept. of Genetics, University of Gotelborg, Sweden: Localization of rat genes coding for liver-specific functions.

Szpirer, C., Rivière, M., Molne, M., Szpirer, J., Dept. de Biologie Moléculaire, Université Libre de Bruxelles, Belgium: Correlation between expression and unmethylation at a specific site of the mouse AFP gene.

Ting, L.P.,[1] Tu, C.L.,[1] Chou, C.K.,[1,2] [1]Graduate Institute of Microbiology and Immunology, National Yang-Ming Medical College, [2]Dept. of Medical Research, Veterans General Hospital, Taiwan, Republic of China: Induction of expression of three heat-shock protein genes in human hepatoma cells by insulin.

Weber-Benarous, A., Kerner, N., Ginot, F., Kahn, A., INSERM, Paris, France: Retrovirus-mediated transfer and tissue-specific expression of rat aldolase-B gene.

Weiner, F.R.,[1] Czaja, M.J.,[1] Jefferson, D.M.,[2] Giambrone, M.-A.,[1] Tur-Kaspa, R.,[1] Reid, L.M.,[2] Zern, M.A.,[1] Depts. of [1]Medicine, [2]Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York: Effects of DEX on in vitro collagen gene expression.

Weinstat, D.L., Montigny, W.J., Zaret, K.S., Section of Biochemistry, Brown University, Providence, Rhode Island: Temperature-sensitive hepatocyte-derived cell lines are dependent on mutiple factors for high-level expression of mouse serum albumin mRNA.

Williams, S.,[1] Grant, S.,[2] Lusis, A.,[3] Kinniburgh, A.,[1] Depts. of [1]Human Genetics, [2]Cellular and Molecular Biology, Roswell Park Memorial Institue, Buffalo, New York; [3]Dept. of Medicine and Microbiology, University of California, Los Angeles: Effect of dietary cholesterol on the level of apolipoprotein-AIV mRNA in mouse liver.

Wilson, D.R., Kelly, J.H., Darlington, G.J., Baylor College of Medicine, Houston, Texas: Elements for tissue-specific expression of human albumin reside in the 5′-flanking sequence.

Wilson, R.H., Gibbs, C.S., Houston, J.K., Hussain, A., Dept. of Genetics, University of Glasgow, Scotland: Structure and expression of a mammalian glutamine synthetase gene.

Wu, L.C., Yu, C.Y., Morley, B.J., Cambell, R.D., MRC Immunochemistry Unit, Dept. of Biochemistry, Oxford University, England: Regulation of expression of the complement component genes encoded in the MHC.

Zakin, M.M., Schaeffer, E., Brunel, F., Boissier, F., Cohen, G.N., Unité de Biochimie Cellulaire, Institut Pasteur, Paris, France: Human transferrin gene—Organization, evolution, and specific transcriptional signals.

Tijian, R., Kadonaga, J., Briggs, M., Mitchell, P., Bohmann, D., Heberlein, U., England, B., Biggin, M., Dept. of Biochemistry, University of California, Berkeley: Regulation of eukaryotic transcription by the interplay of promoter and enhancer binding proteins in mammalian cells and *Drosophila*.

SESSION 9 REQUIREMENTS FOR HEPATIC DIFFERENTIATION IN TISSUE CULTURE

Chairmen: **J.E. Darnell, Jr.,** Rockefeller University
H. Leffert, University of California, San Diego, La Jolla

Bennett, A.L.,[1] Paulson, K.E.,[1] Miller, R.E.,[2] Darnell, J.E.,[1] [1]Dept. of Molecular Cell Biology, Rockefeller University, New York, New York; [2]Dept. of Medicine, Cleveland Veterans Administration Medical Center and Case Western Reserve University School of Medicine, Ohio: Phenotypic heterogeneity in differentiating 13-day fetal hepatoblast cultures that mimics in vivo differentiation.

Leffert, H.L.,[1] Koch, K.S.,[1] Shapiro, I.P.,[1] Skelly, H.,[1] Wolff, J.,[2] Yee, J.-K.,[2] Friedmann, T.,[2] Depts. of [1]Medicine, [2]Pediatrics, University of California, La Jolla, San Diego: Growth control of rat hepatocytes in primary culture.

Carr, B.I., Dept. of Medical Oncology, City of Hope Medical Center, Duarte, California: Serum factor(s) control the level of ^{125}I-labeled TGFβ binding in primary adult rat hepatocytes.

Chou, J.Y., Wan, Y.-J.Y., NIC, National Institutes of Health, Bethesda, Maryland: In vitro regulation of liver maturation.

Ichihara, A., Nakamura, T., Enzyme Science Center, University of Tokushima, Japan: Terminal differentiation of neonatal rat hepatocytes in primary cultures requires contact with adult hepatocytes.

Isom, H.C., Woodworth, C.D., Georgoff, I., Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Liver-specific gene expression in hepatocytes and immortalized hepatocyte cell lines.

Marceau, N., Nõel, M., Germain, L., Hôtel-Dieu de Québec Hospital and Laval University, Canada: Expression of hepatocytic functions in cultured ductular oval cells.

Gohda, E.,[1] Tsubouchi, H.,[2] Nakayama, H.,[1] Hirono, S.,[1] Takahashi, K.,[2] Sakiyama, O.,[2] Hashimoto, S.,[2] Daikuhara, Y.,[1] [1]Dept. of Biochemistry, Kagoshima University Dental School, [2]Second Dept. of Internal Medicine, Kagoshima University, Japan: Human hepatocyte growth factor in plasma from patients with fulminant hepatic failure.

Liang, T.J., Plant, P.W., Diamond, L., Grieninger, G., L.D.

J. Gauldie, G. Fay, N. Northemann

E. Bade, M. Guille

Kimball Research Institute, New York Blood Center, New York: Chicken embryo hepatocyte culture—A model for dissecting the regulatory role of specific agents in the expression of hemopexin and other liver-specific gene products during hepatic development.

Dunsford, H.A., Karnasuta, C., Hunt, J.M., Sell, S., University of Texas Medical School, Houston: Monoclonal antibodies define different lineages of chemically induced hepatocellular carcinoma in rats.

Kelly, J.H., Dept. of Pathology, Baylor College of Medicine, Houston, Texas: Transcriptional control of albumin and AFP synthesis in the human hepatoblastoma line, Hep-G2.

Lhadi, C., Corlu, A., Baffet, G., Bourel, D., Kneip, B., Guguen-Guillouzo, C., Hôpital Pontchaillou, Rennes, France: Regulation of hepatocyte functions by cell-cell interactions—Involvement of a plasma membrane protein.

Schuetz, E.,[1] Omiecinski, C.,[2] Muller-Eberhard, U.,[3] Kleinman, H.,[4] Guzelian, P.,[1] [1]Medical College of Virginia, Richmond; [2]University of Washington, Seattle; [3]Cornell Medical College, New York, New York; [4]National Institutes of Health, Bethesda, Maryland: Regulation of gene expression in adult rat hepatocytes cultured on a basement-membrane matrix.

Carlin, C.R., Simon, D., Mattison, J., Knowles, B.B., Wistar Institute of Anatomy and Virology, Philadelphia, Pennsylvania: Expression and biosynthetic variation of the EGF receptor in human hepatocellular carcinoma-derived cell lines.

SESSION 10 REGULATION OF LIVER ACUTE PHASE GENES

Chairmen: **G. Fey,** Institute of Scripps Clinic
G. Ringold, Stanford University

Ringold, G., Klein, E., Northrop, J., Danielsen, M., Dept. of Pharmacology, Stanford University School of Medicine, California: Transcriptional regulation of MMTV and α_1-acid glycoprotein genes by the glucocorticoid receptor.

Schreiber, G., Dickson, P.W., Aldred, A.R., Fung, W.P., Thomas, T., Cole, T., Birch, H., de Jong, F., Dept. of Biochemistry, University of Melbourne, Australia: Expression and regulation of plasma protein genes in tissues associated with extracellular compartments.

Crabtree, G.R.,[1] Courtois, G.,[1] Campbell, L.,[1] Chodosh, L.,[3] Carthew, R.,[3] Fuller, G.,[2] Sharp, P.,[3] Morgan, J.,[1] [1]Stanford University, California; [2]University of Albama, Birmingham; [3]Massachusetts Institute of Technology, Boston: Intracellular and extracellular factors controlling the expression of the fibrinogen genes.

Woo, S.L.C., Shen, R.-F., Li, Y., Sifers, R.N., Clift, S., DeMayo, J., Wang, H., Tsai, S.Y., Dept. of Cell Biology, Howard Hughes Medical Institute and Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas: Tissue-specific expression and regulation of the human α_1-antitrypsin gene.

Ciliberto, G., Rüther, U., Arcone, R., Wagner, E., EMBO, Heidelberg, Federal Republic of Germany: Acute-phase induction of the human CRP gene in transgenic mice.

Rienhoff, H.,[1,2] Groudine, M.,[1] [1]Howard Hughes Medical Institute, University of Washington, [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Control of SAA gene expression.

Fowlkes, D.M.,[1] Lund, P.K.,[2] Blake, M.,[1] Snouwaert, J.,[1] [1]Dept. of Pathology, [2]Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill: Regulation of fibrinogen production by steroids is indirect.

Hu, S.-I.,[1] Samols, D.,[1] Schultz, D.,[2] Macintyre, S.,[2] Kushner, I.,[2] Depts. of [1]Biochemistry, [2]Medicine, Case Western Reserve University, Cleveland Metropolitan Hospital, Ohio: Hepatocytes have a special intracellular mechanism to regulate the secretion of CRP.

Liao, W.S.L., Li, X., Caldwell, C.K., Dept. of Biochemistry and Molecular Biology, M.D. Anderson Hospital and Tumor Institute, Houston, Texas: Expression and structural analysis of rat SAA gene.

Fey, G.,[1] Northemann, W.,[1] Shiels, B.,[1] Gehring, M.,[2] Braciak, T.,[1] Hudson, G.,[1] [1]Dept. of Immunology, Research Institute of Scripps Clinic, [2]Agouron Pharmaceuticals, Inc., La Jolla, California: Structure and acute-phase regulation of the rat liver α-macroglobulin gene family.

C. elegans

May 6—May 10

ARRANGED BY

Jonathan Hodgkin, MRC Laboratory of Molecular Biology
Robert Horvitz, Massachusetts Institute of Technology
Susan Strome, Indiana University
William Wood, University of Colorado, Boulder

299 participants

The growing number of nematode researchers attending this fifth Cold Spring Harbor meeting on *C. elegans* attests to its growing popularity as a developmental system. Most of the topics covered were old favorites: embryogenesis, postembryonic lineages, muscle organization, neuroanatomy, sex determination, dosage compensation, and genome organization. In addition to the genetic approaches to studying these topics described in past years, this year's meeting included an impressive array of molecular analyses as well. First and foremost was the ongoing cloning and mapping of the *C. elegans* genome; as of the end of 1987, 90% of the genome has been mapped to 680 "contigs" (stretches of overlapping, contiguous clones) of 100 kb average length. In addition, the identification of a growing list of transposable elements and the availability of "mutator strains" in which transposable elements hop at high frequency in the germ line have enabled investigators to tag and clone specific genes of interest. Three laboratories reported the generation of transgenic nematodes, enabling analysis of expression of genes that have been manipulated prior to introduction into worms. An unexpected finding was that many nematode RNAs undergo *trans*-splicing, resulting in the addition of a common leader sequence; this occurs in addition to *cis*-splicing. Most participants agree that this was the best *C. elegans* meeting yet.

This meeting was supported in part by The National Science Foundation and the following divisions of the National Institutes of Health: Division of Research Resources, National Institute of General Medical Sciences, National Institute on Aging.

SESSION 1 EMBRYOGENESIS

Chairman: P. Sternberg, California Institute of Technology

Hyman, A., MRC Laboratory of Molecular Biology, Cambridge, England: Centrosome movements in the early divisions of *C. elegans*.

Hill, D., Strome, S., Dept. of Biology, Indiana University, Bloomington: Role of microfilaments in *C. elegans* zygotes.

Morton, D., Kemphues, K., Section of Genetics and Development, Cornell University, Ithaca, New York: New mutations affecting cleavage pattern and cytoplasmic localization.

Schierenberg, E., Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany: Early cell-cell interaction in the embryo of *C. elegans*.

Ito, K., McGhee, J.D., Dept. of Medical Biochemistry, University of Calgary, Canada: Parental DNA strands segregate randomly during development.

Edgar, L., McGhee, J., Dept. of Medical Biochemistry, University of Calgary, Alberta, Canada: DNA synthesis and control of gut-specific gene expression in early embryos.

Schnabel, R., Schnabel, H., MRC Laboratory of Molecular Biology, Cambridge, England: Embryonic lethal mutations of *C. elegans*.

Mains, P., Sulston, I., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Dominant maternal-effect lethal mutations.

Levitan, D., Giambarella, H., Telfer, A., Stinchcomb, D., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Molecular genetics of maternal-effect lethal mutations.

Carter, P.W., Kemphues, K.J., Section of Genetics and Development, Cornell University, Ithaca, New York: Progress toward cloning *zyg-9* and *zyg-11*.

Yuan, J., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic and molecular genetic studies of *ced-3* and *ced-4*.

SESSION 2 GENOME ORGANIZATION AND TRANSCRIPTION

Chairman: T. Blumenthal, Indiana University

Coulson, A., Sulston, J., Albertson, D., Fishpool, R., MRC Laboratory of Molecular Biology, Cambridge, England: The thousand-island genome.

Huang, X.-Y., Barrios, L., Vonkhorporn, P., Hecht, R., Dept. of Biochemical and Biophysical Sciences, University of Houston, Texas: GAPDH gene family in *C. elegans*.

Ward, S.,[1] Burke, D.J.,[1] Hogan, E.,[1] Sulston, J.E.,[2] Coulson, A.R.,[2] Albertson, D.G.,[2] Ammons, D.,[3] Klass, M.,[3] [1]Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland; [2]MRC Laboratory of Molecular Biology, Cambridge, England; [3]Dept. of Biology, University of Houston, Texas: Genomic organization and sequence conservation of transcribed major sperm protein genes.

Bird, D.M., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Molecular characterization of *ama-1 IV* and environs.

Honda, S., Abruzzeze, R.V., Epstein, H.F., Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Transcription of selected genes during development.

Nelson, D.W., Honda, B.M., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Structure, organization, and expression of the 5S rRNA genes of *C. elegans* and *C. briggs*ae.

Dixon, D.K., Jones, D., Candido, E.P.M., Dept. of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, Canada: Alterations in the chromatin structure of the 16-kD heat-shock polypeptide (hsp16) genes of *C. elegans* following heat induction.

Hartman, P.S.,[1] Marshall, A.,[1] Dwarakanath, V.,[1] Mitchell, D.,[2] [1]Dept. of Biology, Texas Christian University, Fort Worth; [2]University of Texas System Cancer Center, Smithville: Biochemistry of DNA repair in *C. elegans*.

Mello, C., Dunaief, J., Stinchcomb, D., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: *C. elegans* segregators – Sequence requirements for the nucleation of a yeast spindle attachment site.

Starr, T., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Molecular studies in the region around *dpy-14*.

Rosenbluth, R., Rogalski, T., Johnsen, R., Heschl, M., McKim, K., Turner, L., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Twenty major zones define *LGV(left)*.

SESSION 3 POSTER SESSION

Zetka, M.C., Rose, A.M., University of British Columbia, Vancouver, Canada: Effect of *rec-1* on the accumulation of mutations and transposable elements.

Wojcik, A., Ozeran, D., Lanier, W., BIOSIS, Palo Alto, California: Is migration of dauer larvae induced?

Wheaton, V.I.,[1] Hedgecock, E.M.,[2] Otsuka, A.J.,[1] [1]Dept. of Genetics, University of California, Berkeley; [2]Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: Progress in cloning a novel dumpy gene.

Weston, K.,[1] Yochem, J.,[2] Seydoux, G.,[2] Greenwald, I.,[2] [1]MRC Laboratory of Molecular Biology, Cambridge, England; [2]Dept. of Biology, Princeton University, New Jersey: *lin-12* update.

Waring, D., Kenyon, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Genes affecting the "ray vs. alae" decision.

Wadsworth, W.G., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Acidification of intracellular pH accompanies the recovery of dauer larvae.

Vahidi, H., Curran, J., Nelson, D.W., Webster, J.M., Honda, B.M., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: 5S rRNA homologous sequences in the rDNA of *Meloidogyne* species.

Turner, L.M., Rosenbluth, R.E., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: γ-irradiation in *C. elegans*.

Tullis, G., Dalley, B., Golomb, M., Division of Biological Sciences, University of Missouri, Columbia: RNA polymerase II in mutant strains of *C. elegans* and in dauer larvae.

Trent, C., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Molecular cloning of the *her-1 V* gene.

Thomas, J., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Cell interactions between the somatic gonad and the vulval equivalence group, the sex myoblasts, and the HSN neurons.

M. Stern, S. McIntire, E. Hedgecock

Tabuse, Y., Miwa, J., NEC Fundamental Research Laboratories, Kawasaki, Japan: Phorbol ester TPA does not affect early embryogenesis of *C. elegans*.

Tabuse, Y., Nishiwaki, K., Miwa, J., NEC Fundamental Research Laboratories, Kawasaki, Japan: Molecular cloning of the *tpa-1* gene.

Strome, S., Beanan, M., Dept. of Biology, Indiana University, Bloomington: Characterization of a temperature-sensitive germ-line proliferation mutant.

Storfer, F.A., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Characterization of nullo-*X* embryos.

Stewart, H.I., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Ultraviolet mutagenesis in the nematode *C. elegans*.Stern, B.D.,[1] Ishii, N.,[1] Culotti, J.G.,[2] Hedgecock, E.M.,[1] [1]Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Dept. of Molecular Immunology and Neurobiology, Mt. Sinai Hospital Research Institute, Toronto, Canada: Progress in the molecular cloning of genes affecting neural development.

Spence, A., Shen, M., Hodgkin, J., MRC Laboratory of Molecular Biology, Cambridge, England: Walking to sex-determining genes *fem-1*, *mab-3*, and *tra-1*.

Sosnowski, B., Hogan, E., Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Developmental localization of a sperm-specific protein and isolation of its gene.

Siddiqui, S.,[1,2] Culotti, J.,[3] [1]Northwestern University, Evanston, Illinois; [2]Toyohashi University of Technology, Japan; [3]Mt. Sinai Research Institute and Hospital, Toronto, Canada: Antibodies against mouse neural cell adhesion molecule, N-CAM, stain male-specific neurons in *C. elegans*.

Shoemaker, J., Friedman, D., Fitzpatrick, P., Johnson, T.E., University of California, Irvine: Deficiency mapping of the *age-1* locus in *C. elegans*.

Shakes, D.C., L'Hernault, S.W., Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Analysis of spermiogenesis-defective mutants.

Sebastiano, M., Bazzicalupo, P., International Institute of Genetics and Biophysics, CNR, Naples, Italy: Putative gene for a vitelline membrane protein in *C. elegans*.

Schnabel, H., Schnabel, R., MRC Laboratory of Molecular Biology, Cambridge, England: *sot-1* ("sore-throat"), a gene required for pharyngeal differentiation in the embryo.

Schedin, P., Jonas, P., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Possible function for *her-1* in maintaining differentiated states of the intestine and germ line in adult males.

Schauer, I.E., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Transcription in early embryos of *C. elegans*.

Sassa, T., Hosono, R., Kuno, S., Dept. of Biochemistry, Kanazawa University, Japan: Enzymatic analysis of ChAT from a temperature-sensitive mutant of *C. elegans*.

Sanicola, M., Roberts, S.B., Childs, G., Emmons, S., Albert Einstein College of Medicine, Bronx, New York: Molecular characterization of the *C. elegans* histone gene family.

T. Blumenthal, P. Sternberg

Rogalski, T.M., Riddle, D.L., Dept. of Biological Sciences, University of Missouri, Columbia: Lethal mutations and chromosomal rearrangements affecting the *ama-1* gene.

Reape, T., Burnell, A., Dept. of Biology, St. Patrick's College, Kildare, Ireland: Investigation of enzyme activity in the dauer larva of *C. elegans* on returning to food.

Ray, A., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Changes in protein phosphorylation associated with dauer larva recovery.

Rand, J.B., Johnson, C.D., Dept. of Zoology, University of Wisconsin, Madison: Suppression of Ace-lethality by *cha-1* and *unc-17* mutations.

Quarantillo, B., Donahue, L., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Molecular analysis of *X*-chromosome dosage compensation continued.

Prenger, J.,[1] Boettcher, J.,[1] Weinmann, R.,[2] Golomb, M.,[1] [1]Division of Biological Sciences, University of Missouri, Columbia; [2]Wistar Institute, Philadelphia, Pennsylvania: Mapping epitopes and functional domains on RNA polymerase II from *C. elegans*.

Prasad, S., Baillie, D.L., Simon Fraser University, Burnaby, Canada: Molecular analysis of the region around *unc-22* on linkage group IV of *C. elegans*.

Plenefisch, J., Villeneuve, A., Hsu, D., Meyer, B., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Suppressin' 'n' enhancin' *her-1(n695sd)*.

Dreyfus, D.H., Emmons, S.W., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Properties of a polymorphic foldback family in *C. elegans*.

SESSION 4 SEX, GAMETES AND DOSAGE COMPENSATION

Chairman: C. Kenyon, University of California, San Francisco

Villeneuve, A., Meyer, B., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: *sdc-1* – A link between sex determination and dosage compensation.

Miller, L., Meyer, B., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: *y9* – An XO-specific lethal mutation that interacts with both dosage-compensation and sex-determination mutations.

Meneely, P.M., Nordstrom, K.D., Fred Hutchinson Cancer Research Center, Seattle, Washington: X-chromosome duplications elevate X-linked gene expression.

Manser, J., Trent, C., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: *sup-26III* – A regulator of *her-1*?

Schedl, T., Barton, M.K., Kimble, J., University of Wisconsin, Madison: Genetic analysis of germ-line sex determination.

Rosenquist, R., Lawson, D., Kimble, J., University of Wisconsin, Madison: Molecular analysis of the sex-determination gene, *fem-3*.

Okkema, P.G., Kimble, J., Dept. of Biochemistry, University of Wisconsin, Madison: Molecular analysis of *tra-2*, a sex-determination gene in *C. elegans*.

L'Hernault, S.W., Shakes, D.C., Hogan, E., Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Cloning, genetics, and phenotypic analysis of the *spe-17* gene.

Blumenthal, T., Zucker, E., Dept. of Biology, Indiana University, Bloomington: Molecular analysis of the vitellogenin genes. 68

Sharrock, W., Sutherlin, M., Dept. of Biochemistry, University of Minnesota, St. Paul: Supramolecular organization of yolk proteins in *C. elegans* embryos.

Shen, M.M., MRC Laboratory of Molecular Biology, Cambridge, England: *mab-3*, a gene controlling yolk production and a sex-specific lineage.

SESSION 5 NEUROBIOLOGY

Chairman: J. Culotti, Mt. Sinai Research Institute, Toronto

Johnson, C.D., Dept. of Zoology, University of Wisconsin, Madison: Inter- and intraspecies variance of the anatomy of the nematode nervous system.

Durbin, R.M., Thomson, J.N., MRC Laboratory of Molecular Biology, Cambridge, England: Cell interactions in the developing nervous system.

Hedgecock, E.,[1] Culotti, J.,[2] Hall, D.,[3] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Mount Sinai Hospital Research Institute, Toronto, Canada; [3]Albert Einstein College of Medicine, Bronx, New York: *uncs-5*, *-6*, and *-40* act in combination to guide migrating cells and axons along the circumferential coordinate of the body wall.

Desai, C.,[1] Garriga, G.,[1] McIntire, S.,[1] Thomson, N.,[2] Horvitz, R.,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]MRC Laboratory of Molecular Biology, Cambridge, England: Genes involved in the development of the HSN neurons.

Li, C., Chalfie, M., Dept. of Biological Sciences, Columbia University, New York, New York: External cues necessary for vulval innervation.

McIntire, S.,[1] White, J.,[2] Horvitz, R.,[1] [1]Massachusetts Institute of Technology, Cambridge; [2]MRC Laboratory of Molecular Biology, Cambridge, England: Identified GABAergic neurons and mutations affecting GABA expression and axonal outgrowth in *C. elegans*.

Way, J., Chalfie, M., Dept. of Biological Sciences, Columbia University, New York, New York: Cloning the *mec-3* gene.

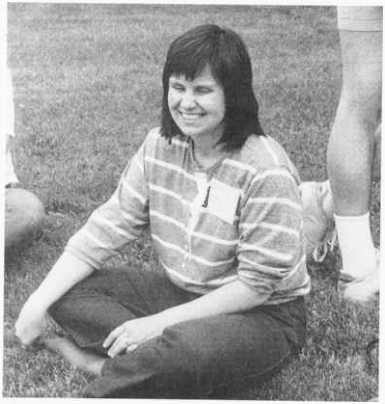
Wolinsky, E.J., Chalfie, M., Dept. of Biological Sciences, Columbia University, New York, New York: A Tc*1* insertion at the *deg-1* locus.

Nawrocki, L.W., MRC Laboratory of Molecular Biology, Cambridge, England: Molecular characterization of a neural mutant, *unc-24*.

Siddiqui, S.,[1,2] Aamodt, E.,[2] Rastinejad, F.,[2] Culotti, J.,[2,3] [1]Toyohashi University of Technology, Japan; [2]Northwestern University, Evanston, Illinois; [3]Mt. Sinai Research Institute and Hospital, Toronto, Canada: Differential expression of tubulin isotypes in *C. elegans* nervous system.

Cowden, C.,[1] Sithigorngul, P.,[1] Guastella, J.,[2] Stretton, A.O.W.,[1] [1]Dept. of Zoology, [2]Neurosciences Training Program, University of Wisconsin, Madison: Neuropeptide diversity in *Ascaris*.

Avery, L., Horvitz, H.R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Function of the pharynx.



A. Rose

B. Herman, J. Rand, C. Johnson

J. Miwa, B. Horvitz

SESSION 6 POSTER SESSION

Peters, K., Rose, A.M., University of British Columbia, Vancouver, Canada: Genetic and molecular analysis of *bli-4*.

Papp, A., Ambros, V., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: A genetic characterization of *lin-29* – Isolation of extragenic suppressors.

Otsuka, A.J.,[1] Wheaton, V.I.,[1] Jeyaprakash, A.,[1] Hartshorne, T.,[1] Hedgecock, E.M.,[2] [1]Dept. of Genetics, University of California, Berkeley; [2]Dept. of Cell Biology, Roche Institute, Nutley, New Jersey: Progress in cloning *unc-44*.

Niebur, E., Erdös, P., Institut de physique théorique, Université de Lausanne, Switzerland: On the control of locomotion of nematodes.

Nelson, G., Shubert, W., Marshall, T., Jet Propulsion Laboratory, Caltech, Pasadena, California: Genetic and developmental effects of heavy-ion radiation in *C. elegans*.

Munakata, N., National Cancer Center Research Institute, Tokyo, Japan: Mutations causing hypersensitivity to alkylating chemicals.

Morgan, P.G.,[1] Sedensky, M.M.,[1] Meneely, P.M.,[2] Cascorbi, H.F.,[1] [1]Dept. of Anesthesiology, University Hospitals of Cleveland, Case Western Reserve University School of Medicine, Ohio; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Response of *C. elegans* to volatile anesthetics.

Moerman, D.G., Rioux, S., Waterston, R.H., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Attempts to identify the *unc-52* gene by Tc*1* insertional mutagenesis.

Miller, D.M., Dept. of Zoology, North Carolina State University, Raleigh: Transposon mutagenesis to isolate genes regulating nematode neuromuscular development.

Milanick, M., Bennett, K., Dept. of Microbiology, University of Missouri, Columbia: Monoclonal antibodies to *Ascaris* recognize the P granules of *Caenorhabditis*.

Meheus, L., Vanfleteren, J., Laboratorium voor Morfologie en Systematiek der Dieren, Rijksuniversiteit Gent, Belgium: Age-related changes in the two-dimensional gel pattern of the nuclear protein of *C. elegans*.

Mancillas, J.R., MRC Laboratory of Molecular Biology, Cambridge, England: Spontaneous mutations affecting backward movement in *C. elegans*.

Mancebo, R., Babity, J.M., Harris, L.J., Starr, T., Rose, A.M., University of British Columbia, Vancouver, Canada: Low-copy-number alterations in the Tc*1* pattern of the Bristol strain, CB51 (*unc-13*).

Maine, E., Kimble, J., University of Wisconsin, Madison: *glp-1* suppressors.

McKim, K.S., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Analysis of the left third of *LGI* using the reciprocal translocation *szT1(I;X)*.

McKim, K.S.,[1] Clark, D.V.,[2] Johnsen, R.C.,[2] Rose, A.M.,[1] Baillie, D.L.,[2] [1]Dept. of Medical Genetics, University of British Columbia, Vancouver, [2]Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Tc1-induced lethal mutations.

L'Hernault, S.W., Shakes, D.C., Varkey, J., Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Genetic analysis of spermatogenesis in *C. elegans*.

Levin, J., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Progress in the cloning of *unc-93* and related genes.

Lerner, K., Goldstein, P., Dept. of Biological Sciences, University of Texas, El Paso: Electron microscopic autoradiographic analysis of decondensed chromatin regions on the chromosomes of *C. elegans*.

La Volpe, A., Bazzicalupo, P., International Institute of Genetics and Biophysics, CNR, Naples, Italy: Molecular organization of some repetitive DNA families.

Khosla, M., Honda, B.M., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Initiator methionine tRNA genes in *C. elegans*.

Kaminuma, T., Minamikawa, R., Kurita, N., Suzuki, I., Tokyo Metropolitan Institute of Medical Science, Japan: Development of a computer system for tracking embryo development of nematodes.

Johnson, C., Dept. of Zoology, University of Wisconsin, Madison: Variants of *Ascaris* with mirror-image neuronal morphologies.

Johnsen, R.C., Baillie, D.L., Simon Fraser University, Burnaby, Canada: Analysis of EMS and formaldehyde-induced lethals on *LGV(left)*.

Jeyaprakash, A.,[1] Wheaton, V.I.,[1] Hedgecock, E.M.,[2] Otsuka, A.J.,[1] [1]Dept. of Genetics, University of California, Berkeley; [2]Dept. of Cell Biology, Roche Institute, Nutley, New Jersey: Progress toward cloning unc-*104*.

Howell, A.M., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Canada: Essential genes in the *sDP2* region of *LGI*.

Hosono, R., Sassa, T., Kuno, S., Dept. of Biochemistry, Kanazawa University, Japan: AchE levels more closely reflect the *cha-1* gene defects than ChAT activity.

Hoskins, R., MRC Laboratory of Molecular Biology, Cambridge, England: Polymorphisms associated with spontaneous reversion of two alleles of unc-*31*.

Hodgkin, J., MRC Laboratory of Molecular Biology, Cambridge, England: Two genes, *mab-1* and *mab-11*, affecting genital morphogenesis and expression of sex-determination genes; analysis of *tra-2* suppressors.

Heschl, M.F.P., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Comparison of some hsp70-related genes.

Herman, R.K., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Further analysis of unc-*3*, *mec-4(e1611)*, and *daf-6* in genetic mosaics.

Herman, R.K., Kari, C.K., Schuyler, M.K., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: More *X*-chromosome duplications; recombination between duplication and chromosome.

Herman, M., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Lasers and cellular debris.

Herman, M., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Intercellular interactions in the male preanal equivalence group P(9-11).p.

Hartshorne, T.A.,[1] Chao, M.,[2] Otsuka, A.J.,[1] [1]Dept. of Genetics, University of California, Berkeley; [2]Dept. of Cell Biology and Anatomy, Cornell University Medical College, New York, New York: Vertebrate nervous system components may be present in C. *elegans*.

Harris, L.J., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: A Tc*1*-homologous C. *briggsae* repetitive element.

Hall, D.H., Hedgecock, E., Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York and Roche Institute of Molecular Biology, Nutley, New Jersey: Cell-guidance errors in the midbody in unc-*5*, unc-*6*, and unc-*40*.

Graham, R.W., Candido, E.P.M., Dept. of Biochemistry, University of British Columbia, Canada: Characterization of the ubiquitin gene from C. *elegans*.

SESSION 7 POSTEMBRYONIC DEVELOPMENT AND AGING

Chairman: S. Emmons, Albert Einstein College of Medicine

Austin, J., Kimble, J., University of Wisconsin, Madison: Genetic and developmental analysis of *glp-1*.

Kim, S., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Molecular genetics of vulval cell lineages.

Thomas, J., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genes that interact with *lin-12* in specifying cell fates.

Kenyon, C., Costa, M., Lee, T., Waring, D., Dept. of Biochemistry, University of California, San Francisco: Control of pattern formation in the lateral hypodermis.

Finney, M., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Molecular biology of unc-*86*.

Ruvkun, G., Giusto, J., Dept. of Genetics, Harvard Medical School and Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Molecular analysis of *lin-14*.

Liu, Z., Ambros, V., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Temporal control of an alternativepostembryonic developmental pathway.

Politz, S.M., Estevez, M., Donkin, S., Herman, D., O'Brien, P., School of Applied Biology, Georgia Institute of Technology, Atlanta: Identification of mutants with altered expression of adult surface antigens.

Link, C., Wood, W.B., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Approaches to studying male tail morphogenesis.

Friedman, D., Johnson, T., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Genetic analysis of *age-1*, a gene that increases mean and maximum life span in C. *elegans*.

K. Nelson

S. Burgess, J. Hodgkin

SESSION 8 TRANSFORMATION, TRANSPOSITION, AND SUPPRESSION

Chairman: J. Kimble, University of Wisconsin

Fire, A.,[1] Waterston, R.,[2] [1]Carnegie Institution, Baltimore, Maryland; [2]Washington University, St. Louis, Missouri: Expression of reintroduced muscle genes.

Collins, J., Forbes, B., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: Activation of a new transposable element in mutator strains.

Levitt, A., Emmons, S.W., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: A second transposon in *C. elegans*.

Mori, I., Moerman, D.G., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Regulation of germ-line transposition and excision of Tc*1*.

Eide, D., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: Structures of germ-line and somatic Tc*1* excision.

Ruan, K.-S., Emmons, S.W., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Chromosomal product of Tc*1* somatic excision.

Kiff, J., Moerman, D.G., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Rearrangements are associated with Tc*1* excision from the *unc-22* locus.

Plasterk, R., Bakker, A., Dept. of Biochemistry, University of Leiden, The Netherlands: Transposition of Tc*1* in vitro.

Pulak, R., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: Formation of spontaneous deletions in eukaryotes.

Kondo, K.,[1] Hodgkin, J.A.,[2] Waterston, R.,[1] [1]Washington University, St. Louis, Missouri; [2]MRC Laboratory of Molecular Biology, Cambridge, England: $tRNA^{TRP}$ amber suppressors of *C. elegans*.

Edgley, M.L., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: *Caenorhabditis* genetics center.

SESSION 9 POSTER SESSION

Freyd, G., Kim, S., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Identification of a Tc*1* in *lin-11*.

Foltz, N., Johnson, T., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Life span and self-fertility polygenes in *C. elegans*.

Fodor, A.,[1] Andrássy, I.,[2] [1]Institute of Genetics, BRC of Hungary Academy of Science, [2]Szeged, Dept. of Zoology, Eötvös L. University, Budapest, Hungary: Description of a new strain (BA 40-02) of *C. remanei*, a *Caenorhabditis* species of real female-male sex determination.

Fitzpatrick, P., Johnson, T., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Attempts to separate long life and reduced fertility using four-factor crosses.

Firth, J.D., Baillie, D.L., Simon Fraser University, Barnaby, Canada: Molecular analysis within the *unc-43-unc-22* interval of *C. elegans*.

Federhen, S., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic control of vulval morphogenesis.

Emmons, S.W., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Mechanism of male tail morphogenesis.

Ellis, R.E., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutations that prevent specific cell deaths.

Edgar, L., McGhee, J., Dept. of Medical Biochemistry, University of Calgary, Alberta, Canada: Tracking down gut determinants – Expression of gut-specific markers involves microfilaments at first cleavage and is related to early-cleavage planes.

Edgar, L., Dept. of Medical Biochemistry, University of Calgary, Alberta, Canada: *nob*, an embryonic mutant affecting only posterior development.

Driscoll, M., Ferguson, C., Hóm, N., Way, J., Savage, C., Chalfie, M., Dept. of Biological Sciences, Columbia University, New York, New York: Progress in cloning *mec* genes.

De Long, L., Casson, L., Meyer, B.J., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A morphological assay for levels of X-linked gene expression.

Cummins, C., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: MLC genes of *C. elegans*.

Cruzen, M., Johnson, T., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Lysozomal enzyme activity as a biomarker of aging using long-lived mutants of *C. elegans*.

Costa, M., Kenyon, C., Dept. of Biochemistry, University of California, San Francisco: Progress in molecular cloning of *mab-5*.

Coohill, T., Schubert, W., Marshall, T., Nelson, G., Jet Propulsion Laboratory, Caltech, Pasadena, California: Responses of radiation-sensitive mutants to high and low LET ionizing radiation.

Clark, S., Edwards, K., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Suppressors of *lin-1*.

Clark, D.V., Rogalski, T.M., Donati, L.M., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Organization of essential genes on *LGIV (right)*.

Chisholm, A., MRC Laboratory of Molecular Biology, Cambridge, England: Lineage alteration in *mab-9*.

Cheng, N., Kemphues, K., Section of Genetics and Development, Cornell University, Ithaca, New York: Further studies of *par-2*.

Cassada, R., Zoological Institute, University of Freiburg, Federal Republic of Germany: Bordering and burrowing and the *bor-1* gene.

Cassada, R., Zoological Institute, University of Freiburg, Federal Republic of Germany: A spontaneous alleie of *emb-3*.

Carr, S., Wood, W.B., Dept. of Molecular and Cellular Development, University of Colorado, Boulder: Purification of putative P-granule proteins.

Burr, A.H., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Computer-video system for tracking nematodes.

Burns, R.H., Russell, R.L., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Effects of AChE inhibitors on movement rates of wild-type and ChAT-deficient *C. elegans*.

Burgess, S.M., Trent, C., Wood, W.B., Dept. of Molecular and Cellular Development, University of Colorado, Boulder: Analysis of *ct31*, a dominant *X*-linked suppressor of *her-1(n695)V*.

Bullerjahn, A.M.E., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Fine-structure map of *ama-1*, an essential gene encoding an RNA polymerase II subunit.

Bird, D.McK., Albert, P.S., Von Mende, N., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Molecular cloning of *dpy-13 IV*.

Bhatt, H., Hedgecock, E., Roche Institute of Molecular Biology, Nutley, New Jersey: A monoclonal antibody to the PVP interneurons.

Bejsovec, A.M., Anderson, P., University of Wisconsin, Madison: Molecular and ultrastructural analysis of dominant *unc-54* mutations.

Beh, C.T., Ferrari, D.C., McGhee, J.D., Dept. of Medical Biochemistry, University of Calgary, Alberta, Canada: A gut-specific acid phosphatase in *C. elegans*—Expression, biochemistry, and genetics.

Barton, M.K., Kimble, J., University of Wisconsin, Madison: More about *fog-1*.

Bakker, A., Ampt, J., Plasterk, R., Dept. of Biochemistry, University of Leiden, The Netherlands: Expression of the Tc*1* transposase.

Bagchi, S., Gross, R., Rubin, C.S., Dept. of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York: cAMP-dependent protein kinase II in *C. elegans*—Enzyme characterization and isolation of cDNAs for the catalytic and regulatory subunits.

Babity, J.M., Rose, A.M., University of British Columbia, Vancouver, Canada: Cloning and fine-structure mapping of the *dpy-5* (*I*) gene.

Avery, L., Horvitz, H.R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A cell that dies during wild-type development can become a functional neuron in a *ced-3* mutant.

Arasu, P., Perler, F., New England Biolabs, Beverly, Massachusetts: A cloned antigenic determinant that appears to be unique to human lymphatic filarial nematodes.

Aamodt, E.,[1] Culotti, J.,[2] Holmgren, R. [1]Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois; [2]Dept. of Molecular Immunology and Neurobiology, Mt. Sinai Hospital Research Institute, Toronto, Canada: Microtubule cross-linking protein from *C. elegans*.

SESSION 10 MUSCLE BIOLOGY

Chairman: A. Rose, University of British Columbia

Waterston, R.H., Washington University, St. Louis, Missouri: Essential genes involved in muscle formation.

Benian, G.M.,[1,2] Mori, I.,[1] Moerman, D.G.,[1] Waterston, R.H.,[1] [1]Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri; [2]Dept. of Pathology, Emory University School of Medicine, Atlanta, Georgia: Selected sequencing of *unc-22* reveals possible function.

Kagawa, H.,[1,2] Gengyo, K.,[1] Karn, J.,[2] Brenner, S.,[2] [1]Dept. of Biology, Okayama University, Japan; [2]MRC Laboratory of Molecular Biology, Cambridge, England: Cloning and sequencing of the *unc-15* paramyosin gene of *C. elegans*.

Epstein, H.F., Berliner, G., Ortiz, I., Casey, D., Baylor College of Medicine, Houston, Texas: Thick-filament assembly.

Venolia, L., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Isolation and characterization of *unc-45* lethal mutations.

Krause, M.,[1] Bektesh, S.,[1] Van Doren, K.,[1] Hirsh, D.,[2] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Synergen, Boulder, Colorado: Evidence for a *trans* spliced leader sequence on actin RNA in *C. elegans*.

Barstead, R.J., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Characterizing a clone that putatively codes for the muscle protein p107b.

Stern, M.J., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutations affecting sex muscle development.

Lye, R.J., Porter, M.E., Scholey, J.M., McIntosh, J.R., University of Colorado, Boulder: Identification of a cytoplasmic microtubule-based motor in *C. elegans*.

RNA Processing

May 13—May 17

ARRANGED BY

Thomas Cech, University of Colorado, Boulder
Christine Guthrie, University of California, San Francisco
Hugh D. Robertson, Rockefeller University

377 participants

The excitement generated during the 1987 RNA Processing meeting can be gleaned from a list of the new advances announced at that meeting that were not anticipated at the 1986 meeting.

1. Identification of the complete set of small nuclear RNAs (snRNAs) required for nuclear mRNA splicing in yeast, their relationship to the mammalian snRNAs, and in two cases their sites of binding to pre-mRNA. This is a major advance, since only a few years ago it was not at all clear that there were yeast snRNAs equivalent to mammalian U1, U2, U4, U5, and U6 snRNAs. Now the powerful genetic methods available in yeast can be applied to the dissection of mRNA splicing in vivo and in vitro.
2. Evidence for *trans*-splicing (splicing together of RNA segments from different primary transcripts) for a series of actin mRNAs from the nematode worm. Combined with the earlier finding of *trans*-splicing in trypanosomes, it now appears that *trans*-splicing might be a general phenomenon in eukaryotes.
3. Sequencing of the functionally essential RNA subunit of a primer endonuclease involved in mammalian mitochondrial DNA replication and identification of RNA in the terminal deoxyribonucleotidyltransferase involved in telomere generation in *Tetrahymena*. These findings pave the way for studies designed to determine the function of the RNA in these ribonucleoprotein enzymes.
4. Identification and genetic proof of two RNA tertiary interactions that hold the 5′ exon into place for group II mitochondrial mRNA self-splicing. These tertiary interactions explain the molecular basis for group II RNA *trans*-splicing in vitro; analogous interactions are likely to be involved in plant chloroplast mRNA *trans*-splicing in vivo.
5. Determination of the RNA secondary structure of the RNA enzyme, RNase P, and new information about the portion of the pre-tRNA substrate with which it interacts. Both advances are necessary prerequisites for understanding structure-function relationships for this RNA enzyme.

The rapid pace of development in the areas described above is of course mainly due to the skill and ingenuity of individual research groups. Another key ingredient has been the excellent communication and critical evaluation of ideas and data provided by the annual RNA processing meetings.

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SESSION 1 RNA STRUCTURE AND FUNCTION

Chairman: P. Moore, Yale University

Milligan, J.F., Groebe, D., Witherell, G., Uhlenbeck, O.C., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: In vitro transcription of synthetic DNA using T7 RNA polymerase.

Schimmel, P., Edwards, H., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Specific recognition in vivo of an *S. cerevisiae* mitochondrial transfer RNA by an *E. coli* enzyme.

Lipson, S.E., Cimino, G.D., Hearst, J.E., Dept. of Chemistry, University of California, Berkeley: Structure of M1 RNA as determined by psoralen cross-linking.

Zwieb, C., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Determining the three-dimensional folding of the 7SL RNA—Functional implications.

Brow, D.A., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: snRNA interactions—The U4/U6 association is conserved in yeast.

Parker, K.A., Steitz, J.A., Dept. of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, Connecticut: Structural analyses of the U3 RNP reveal a conserved sequence available for base-pairing with pre-rRNA.

Stroke, I., Weiner, A., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Psoralen cross-linking of rat U3 RNA to the external transcribed spacer region of pre-rRNA.

Arenas, J.,[1] Phillips, B.,[2] Hurwitz, J.,[2] [1]Albert Einstein College of Medicine, Bronx, [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Isolation and characterization of an RNA helicase from HeLa cells.

SESSION 2 3′ ENDS AND POLYADENYLATION

Chairman: M. Wickens, University of Wisconsin

Wickens, M., Bardwell, V., Conway, L., Fox, C., Sheets, M., Zarkower, D., Dept. of Biochemistry, University of Wisconsin, Madison: Cleavage and polyadenylation of SV40 late pre-mRNAs in vitro.

Zhang, F., Cole, C.N., Molecular Genetics Center and Dept. of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire: Identification of a complex involved in processing of *tk* RNA 3′ ends in vitro.

Moore, C.L.,[1] Scolnik-David, H.,[2] Sharp, P.A.,[2] [1]Dept. of Molecular Biology, Tufts University Medical School, Boston, [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Assembly of precursor RNA containing the L3 poly(A) site into RNA-protein complexes.

Green, T., Noll, G., Vattay, A., Hart, R., Dept. of Biological Sciences, Rutgers University, Newark, New Jersey: Poly(A) site sequence requirements for in vitro cleavage activity and UV cross-linking to a protein factor.

Wilusz, J., Shenk, T., Dept. of Molecular Biology, Princeton University, New Jersey: Identification of a 64-kD nuclear protein that interacts specifically with the polyadenylation signal.

Christofori, G., Kramer, A., Keller, W., Division of Molecular Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Is there an snRNP involved in the 3′ cleavage and polyadenylation of mRNA precursors?

Takagaki, Y., Ryner, L.C., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: Partial purification of the factors involved in mRNA 3′-end cleavage and polyadenylation.

Gilmartin, G.M., McDevitt, M.A., Nevins, J.R., Rockefeller University, New York, New York: Multiple factors are required for pre-mRNA 3′ processing in vitro.

Proudfoot, N.J., Gil, A., Johnson, M.R., Whitelaw, E., Sir William Dunn School of Pathology, University of Oxford, England: Termination of transcription and 3′-end processing in eukaryotic genes transcribed by RNA polymerase II—The signals involved and their role in gene regulation.

Labhart, P., McStay, B., Reeder, R.H., Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington: rRNA 3′-end formation requires sequences at both 5′ and 3′ ends of the gene region.

Sarkar, N., Taljanidisz, J., Shen, P., Boston Biomedical Research Institute and Dept. of Biological Chemistry, Harvard Medical School, Massachusetts: 3′-terminal polyadenylation of mRNA in prokaryotes.

Posters:

Agris, C.H., Plotch, S.J., Krug, R.M., Memorial Sloan-Kettering Cancer Center, New York, New York: The block in splicing of influenza virus NS1 mRNA occurs after spliceosome formation.

Aloni, Y., Kessler, M., Resnekov, O., Seiberg, M., Regimov, N., Ben-gal, E., Amster-Choder, O., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Attenuation in the regulation of gene expression in viruses and animal cells.

Amero, S.A., Elgin, S.C.R., Dept. of Biology, Washington University, St. Louis, Missouri: An RNA-binding protein from *Drosophila* is associated with specific developmentally regulated loci.

Andersen, J.,[1] Zhao, K.,[1] Inouye, M.,[2] Delihas, N.,[1] [1]Dept. of Microbiology, State University of New York School of Medicine, Stony Brook; [2]Dept. of Biochemistry, Robert Wood Johnson Medical School, UMDNJ, Piscataway, New Jersey: Expression of the antisense RNA gene, *micF*, is regulated in response to temperature and osmolarity of the growth media.

Subbarao, M.N., Apirion, D., Dept. of Microbiology, Washington University, St. Louis, Missouri: 10Sa RNA—A small stable RNA of *E. coli*; cloning and processing.

Ares, M., Giglio, L., Chung, J.-S., Weiner, A.M., Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Two distinct factors bind to adjacent sites in the human U2 enhancer.

Baer, M.L.,[1] Jobling, S.A.,[1] Liem, K., Jr.,[1] Gehrke, L.,[1,2] [1]Harvard-Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Anatomy and Cellular Biology, Harvard Medical School, Boston, Massachusetts: Characterization of a specific mRNA-protein interaction using the mobility band-shift assay.

Baer, M., Kirsebom, L., Altman, S., Dept. of Biology, Yale University, New Haven, Connecticut: Differential effects on tRNA suppressor function of mutations in RNase P and selection of second-site revertants in the gene for M1 RNA.

Ruohola, H.,[1] Baker, S.M.,[2] Hazen, J.,[2] Parker, R.,[3] Platt, T.,[2] [1]Dept. of Cell Biology, Yale University, New Haven, Connecticut; [2]Dept. of Biochemistry, University of Rochester Medical Center, New York; [3]Dept. of Biochemistry and Biophysics, University of California, San Francisco: Orientation-dependent 3′-end formation—*CYC1* transcription terminator fragments inserted in the actin intron of yeast.

Bark, C., Weller, P., Zabielski, J., Janson, L., Pettersson, U., Dept. of Medical Genetics, Biomedical Center, University of Uppsala, Sweden: Transcription of a mouse U6 RNA gene by RNA polymerase III is enhancer-dependent.

Belasco, J.,[1] Beatty, J.T.,[2] Cohen, S.N.,[3] [1]Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Microbiology, University of British Columbia, Vancouver, Canada; [3]Dept. of Genetics, Stanford University, California: A determinant of mRNA stability that governs differential expression of *R. capsulatus* photosynthesis genes.

Bovenberg, R.A.L., Baas, P.D., Jansz, H.S., University of Utrecht, The Netherlands: Processing of CALC-I RNA in vitro.

Buck, G.A., McCarthy, C., Dept. of Microbiology and Immunology, Virginia Commonwealth University, Richmond: Small RNAs sharing sequence homology with the primary transcript of the trypanosome mRNA spliced leader.

Carmichael, G., Marlor, C., Adami, G., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Role of the polyoma late leader exon length in RNA splicing and stability.

Rodrigues, M.L., Castaño, J.G., University of Madrid, Spain: 5′ and 3′ pre-tRNases from Ehrlich ascites cells.

Lavery, D., Chen-Kiang, S., Dept. of Microbiology, Mount Sinai School of Medicine, New York, New York: Repressed synthesis of adenovirus E1A mRNAs in human lymphoid cells is regulated posttranscriptionally.

Christensen, M.,[1] Junio, M.,[1] Fuxa, K.,[1] Pierron, G.,[2] [1]Dept. of Biology, Texas A & M University, College Station; [2]Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Does the processing of mRNA and rRNA involve related RNA-packaging proteins?

Chu, F.K., Maley, G.F., Maley, F., Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany: Structural studies of a prokaryotic self-splicing RNA.

Clark, M.W., Campbell, J., Abelson, J., California Institute of Technology, Pasadena: The *rna*1 mutation disrupts the location of proteins found in the yeast nucleolus.

Collins, R.A., Dept. of Botany, University of Toronto, Canada: Conservation of secondary structure outside of the "core" in a large subclass of group I introns.

Connelly, S., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: Sequences required for efficient cleavage and polyadenylation of the SV40 early transcript also function as an RNA polymerase II termination signal.

Craig, N.,[1] Kass, S.,[2] Sollner-Webb, B.,[2] [1]Dept. of Biological Science, University of Maryland, Catonsville; [2]Dept. of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland: Cell-free processing of mouse rRNA—Analysis of nucleotide and biochemical determinants.

Perea, J., Creusot, F., Banroques, J., Becam, A.M., Delahodde, A., Goguel, V., Mendelzon, D., Jacq, C., Centre de Génétique Moléculaire du CNRS, laboratoire associé a l'Université P. et M. Curie, France: Intron-encoded bI4 maturase and splicing of two mitochondrial group I introns of *S. cerevisiae*.

Davis, N.W., Gegenheimer, P., Depts. of Biochemistry and Botany, Molecular Genetics Program, University of Kansas, Lawrence: tRNA maturation in chloroplasts in vitro.

de Mars, M., Cizdziel, P., Murphy, E.C., Dept. of Tumor Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Intron structure governs MSV RNA splicing.

Denome, R.M., Cole, C.N., Molecular Genetics Center and Dept. of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire: Effect of multiple polyadenylation signals on polyadenylation site selection.

Wine and Cheese Party

Deshler, J.O.,[1,2] Felder, E.H.,[2] Larson, G.P.,[1,2] Rossi, J.J.,[2] [1]Dept. of Microbiology, University of California, Los Angeles; [2]Dept. of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, California: Splicing of the actin pre-mRNA in *K. lactis*.

Thompson-Jäger, S., Domdey, H., Genzentrum der Ludwig-Maximilians-Universität München, Federal Republic of Germany: The intron of the yeast actin gene contains the promoter for an antisense RNA.

Ehrenman, K.,[1,2] Belfort, M.,[1] Povinelli, C.M.,[3] Hall, D.H.,[3] [1]Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany; [2]Dept. of Microbiology and Immunology, Albany Medical College, New York; [3]School of Applied Biology, Georgia Tech, Atlanta: Clustering of mutations in the group I *td* intron of bacteriophage T4.

Shumard, C.M., Eales, S.J., Eichler, D.C., Dept. of Biochemistry, College of Medicine, University of South Florida, Tampa: Mapping of nucleolar RNase II cleavage sites relative to the 5′ end of 18S RNA using run-off transcripts.

Emeson, R.B., Russo, A.F., Rosenfeld, M.G., Howard Huges Medical Institute, University of California, San Diego, La Jolla: Processing of calcitonin/CGRP pre-mRNAs in vitro.

Wiebauer, K., Herrero, J.J., Filipowicz, W., Friedrich Meischer-Institut, Basel, Switzerland: Differences in mRNA splicing specificity between plants and animals.

Freistadt, M.S.,[1] Cross, G.A.M.,[1] Branch, A.D.,[2] Robertson, H.D.,[2] [1]Laboratory of Molecular Parasitology, [2]Laboratory of Genetics, Rockefeller University, New York, New York: The trypanosome "mini-exon" cap contains m^7G and an unusually high number of modified nucleotides.

Fresco, L.D., Keene, J.D., Dept. of Microbiology and immunology, Duke University Medical Center, Durham, North Carolina: Molecular analysis of the U2 snRNP unique protein A′.

Frielle, D.W., Keene, J.D., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: In vitro processing of snRNA precursors isolated from VSV-infected cells.

Petersen-Bjørn, S., Friesen, J.D., Dept. of Medical Genetics, University of Toronto, Canada: Regulation of expression of *RNA4* and cloning of another gene possibly involved in mRNA splicing in yeast.

Fromherz, S.J., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Proteins involved in pre-mRNA splicing.

Fu, X.-Y., Noble, J.C.S., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York: A specific sequence in the SV40 small t intron controls the alternative splicing pattern of SV40 early pre-mRNA in vivo.

Galli, G.,[1] Guise, J.W.,[2] McDevitt, M.A.,[1] Tucker, P.W.,[2] Nevins, J.R.,[1] [1]Howard Hughes Medical Institute, Rockefeller University, New York, New York; [2]Dept. of Microbiology, Southwestern Medical School, University of Texas Health Science Center at Dallas: Relative position and strengths of poly(A) sites as well as transcription termination are critical to membrane vs. secreted μ-chain expression during B-cell development.

Gelfand, R., Smith, L.D., Dept. of Biology, Purdue University, West Lafayette, Indiana: Interspersed RNA in *Xenopus* oocytes.

Goswami, P., Goldenberg, C.J., Dept. of Microbiology and Immunology, University of Miami School of Medicine, Florida: Role of exon sequences in binding hnRNPs in an in vitro splicing reaction.

Gross, R.H., Corell, R.A., Friedlander, L.H., Rice, P.W., Palisi, T., Dept. of Biological Sciences, Dartmouth College, Hanover, New Hampshire: In vivo effects of liposome-delivered antibodies and antisense DNAs on RNA processing.

Habets, W.J., Sillekens, P.T., van Venrooij, W.J., Dept. of Biochemistry, University of Nijmegen, The Netherlands: Molecular cloning of U1 and U2 snRNP-associated proteins.

Hales, K.H., Wilson-Gunn, S.I., Imperiale, M.J., Dept. of Mi-

crobiology and Immunology, University of Michigan Medical School, Ann Arbor: 3′-end formation of adenovirus L1 mRNA.

Hall, K.B.,[1] Green, M.R.,[2] Redfield, A.G.,[1] [1]Dept. of Biochemistry, Brandeis University, Waltham, [2]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Structural analysis of the premRNA branch-point/3′-splice-site region.

Hebbar, S.K., Perlman, P.S., Molecular, Cellular and Developmental Biology Program and Dept. of Genetics, Ohio State University, Columbus: A maturase encoding mitochondrial group II intron of yeast self-splices in vitro.

Hecker, R., Steger, G., Riesner, D., Institut für Physikalische Biologie, Universität Düsseldorf, Federal Republic of Germany: Viroid processing—Structure and structure formation of synthetic and natural replication intermediates.

Hopper, A.K., Traglia, H.M., Wang, S.S., Atkinson, N.S., Hurt, D., Dept. of Biological Chemistry, Hershey Medical Center, Pennsylvania: Progress in characterizing yeast genes involved in tRNA splicing.

Jacob, M., Fuchs, J.P., Lutz, Y., Sittler, A., Gallinaro, H., Institut de Chimie Biologique, Strasbourg, France: hnRNPs are dynamic structures.

Jakowlew, S.B., Kondaiah, P., Dillard, P.J., Roberts, A.B., Sporn, M.B., National Institutes of Health, Bethesda, Maryland: A novel RNA related to transforming growth factor-α.

SESSION 3 mRNA SPLICING I: INTRON RECOGNITION AND SPLICEOSOME ASSEMBLY

Chairman: J. Abelson, California Institute of Technology

Siliciano, P.,[1] Simmons, T.,[1] Parker, R.,[2] Guthrie, C.,[1] University of California, [1]San Francisco; [2]San Diego: Recognition of the UACUAAC box in yeast introns requires base pairing with the yeast U2 analog.

Yuo, C.-Y., Weiner, A.M., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Mutant and antisense U1 RNAs.

Ohno, M., Shimura, Y., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Effect of the 5′ cap structure on in vitro splicing of pre-mRNA containing two introns and a nuclear factor responsible for cap recognition.

Newman, A., MRC Laboratory of Molecular Biology, Cambridge, England: Nonconserved sequences in the *S. cerevisiae CYH2* gene intron play important roles in the splicing of the pre-mRNA.

Hornig, H., Aebi, M., Weissmann, C., Institut für Molekularbiologie I, Universität Zürich, Switzerland: Selection of 5′-cleavage site in eukaryotic pre-mRNA splicing is determined by the overall 5′-splice region rather than by the conserved 5′GU.

Hartmuth, K., Barta, A., Institut für Biochemie, Universität Wien, Austria: Unusual branch-point selection in hGH pre-mRNA—Use of cytosine and uridine residues as natural branch acceptors.

Noble, J.C.S., Pan, Z.-Q., Fu, X.-Y., Prives, C., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: A role for multiple lariat branch points in selection of alternative 5′-splice sites.

Krämer, A., Frendewey, D., Lahr, G., Utans, U., Frick, M., Keller, W., Division of Molecular Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Factors involved in spliceosome assembly and in the processing of nuclear mRNA precursors in vitro.

Bindereif, A., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: An ordered pathway of snRNP binding during mammalian pre-mRNA splicing complex assembly.

Konarska, M.M., Sharp, P.A., Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Interactions between snRNP particles in the formation of spliceosomes.

O'Brien, J.P., Freyer, G.A., Hurwitz, J., Memorial Sloan-Kettering Cancer Center, New York, New York: Complex formation during pre-mRNA splicing using mutated transcripts.

Cheng, S.-C., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: Assembly of the spliceosome and the involvement of snRNAs in yeast.

Rymond, B.C., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: In vitro characterization of pre-mRNA/spliceosome contacts.

SESSION 4 mRNA SPLICING II: *TRANS*-ACTING FACTORS AND *TRANS*-SPLICING

Chairman: W. Keller, German Cancer Research Center, Heidelberg

Bektesh, S.,[1] Krause, M.,[2] Van Doren, K.,[1] Hirsh, D.,[1] [1]Laboratory of Developmental Biology, Synergen, Inc., Boulder, Colorado; [2]Division of Genetics, Fred Hutchinson Cancer Research Center, Seattle, Washington: RNA processing in *C. elegans* involves *trans*-splicing of some mRNAs.

Jacquier, A., Michel, F., Centre de Génétique Moléculaire du CNRS, France: Multiple exon-binding sites in class II introns.

Shinozaki, K., Zaita, N., Torazawa, K., Sugiura, M., Center for Gene Research, Nagoya University, Japan: *trans*-Splicing in vivo—Joining of transcripts from the "divided" gene for chloroplast ribosomal protein S12.

Solnick, D., Memorial Sloan-Kettering Cancer Center, New York, New York: Intron tracking is not required to bring pre-mRNA splice sites together.

Beyer, A., Osheim, Y., Dept. of Microbiology, University of Virginia, Charlottesville: Ultrastructural analysis of splicing in vivo.

Swanson, M.S., Roma, S.P., Nakagawa, T.Y., Dreyfuss, G., Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois: The

hnRNP C proteins contain a putative NTP binding fold and an RNP consensus sequence that is conserved in hnRNA-, mRNA-, snRNA-, and pre-rRNA-binding proteins.

Mayrand, S.H., Pederson, T., Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: hnRNP protein binding to pre-mRNAs is enhanced by the presence of an intron.

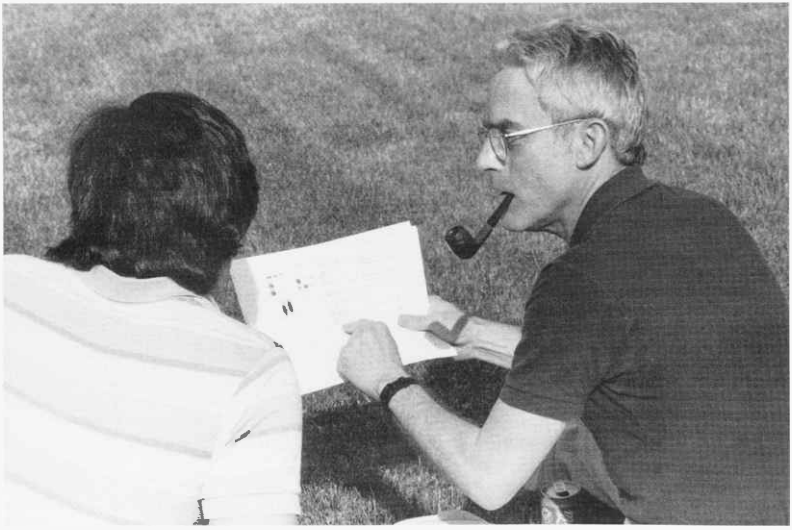
Arad-Dann, H.,[1] Spann, P.,[1] Offen, D.,[2] Sperling, J.,[2] Sperling, R.,[1] [1]Dept. of Genetics, Hebrew University of Jerusalem, [2]Dept. of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel: A role in RNA splicing for a newly identified protein associated with large hnRNP particles.

Goswami, P.,[1] Szeberenyi, J.,[1] Wollenzien, P.L.,[2] Goldenberg, C.J.,[1] [1]Dept. of Microbiology and Immunology, University of Miami School of Medicine, Florida; [2]Dept. of Biochemistry, St. Louis University Medical School, Missouri: Unwinding of the pre-mRNA secondary structure probed with psoralen is necessary for in vitro splicing.

Kole, R., Furdon, P.J., Zwierzynski, T.A., Dept. of Pharmacology and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill: Effects of pre-mRNA structure and conditions of HeLa cell growth on pre-mRNA splicing in vitro.

Chang, T.-H., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: The *RNA*11 gene product is in a pre-formed 30S complex.

Couto, J.,[1] Tamm, J.,[1] Parker, R.,[2] Guthrie, C.,[1] [1]Dept. of Biochemistry and Biophysics, University of California, San Francisco; [2]Dept. of Biology, University of California, San Diego: A *trans*-acting factor suppresses the splicing defect caused by a branch-point mutation in a yeast intron.



W. Keller

Posters:

Jarrell, K.A.,[1] Peebles, C.L.,[2] Dietrich, R.C.,[1] Benatan, E.J.,[2] Perlman, P.S.,[1] [1]Dept. of Genetics, Ohio State University, Columbus; [2]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Reactions of a group II intron to high salt.

Jones, M.H., Riedel, N., Guthrie, C., Dept. of Biochemistry, University of California, San Francisco: A subset of yeast snRNAs having a binding site for the highly conserved Sm protein.

Joyce, G.F., Inoue, T., Nucleotide Chemistry Laboratory, Salk Institute for Biological Studies, La Jolla, California: Cyclization activity in truncated forms of the *Tetrahymena* IVS.

Kay, P.S., Inoue, T., Salk Institute for Biological Studies, La Jolla, California: "Guanosine exchange" reaction of the *Tetrahymena* IVS RNA.

Kedes, D.H., Steitz, J.A., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecti-

cut: 5′-splice site selection in the mouse immunoglobulin κ-light-chain transcript.

Kleinschmidt, A.M., Pederson, T., Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Efficient and accurate processing of U2 snRNA precursors in vitro.

Schuldt, C., Kloetzel, P., Bautz, E.K.F., Institute of Molecular Genetics/ZMBH, University of Heidelberg, Federal Republic of Germany: Identification of heat-shock puff-specific RNA complexes in *Drosophila* – puff-93D-specific repetitive RNA transcripts are organized in small RNP complexes.

Haass, C., Falkenburg, P.E., Hössl, P., Kloetzel, P., Molecular Genetics/ZMBH University of Heidelberg, Federal Republic of Germany: The 19S ring-type scRNPs in *Drosophila* – Their developmental dynamics, their subpopulations, and their potential role in tRNA metabolism.

Konings, D.A.M., EMBL, Heidelberg, Federal Republic of Germany: Secondary structures of both U2 and U3 snRNAs of yeast and metazoans are surprisingly conserved.

Krupp, G., Albrecht, W., Institute for General Microbiology, Kiel, Federal Republic of Germany: A new approach for the in vitro synthesis of defined RNA molecules – Structural requirements for RNase P substrates.

Kumar, A., Sierakowska, H., Szer, W., Dept. of Biochemistry, New York University School of Medicine, New York, New York: RNA binding and unwinding properties of a C-type hnRNP from HeLa.

van der Veen, R.,[1] Kwakman, J.H.J.M.,[1] Arnberg, A.C.,[2] Grivell, L.A.,[1] [1]Laboratory of Biochemistry, University of Amsterdam, [2]Laboratory of Biochemistry, University of Groningen, The Netherlands: Self-splicing of a group II intron from yeast mitochondria.

Labouesse, M., Herbert, C.J., Dujardin, G., Slonimski, P.P., Centre de Génétique Moléculaire du CNRS, France: Mutational and immunological studies on the *NAM2* protein involved in mitochondrial RNA splicing.

Lahiri, D.K., Thomas, J.O., Dept. of Biochemistry, New York University School of Medicine, New York, New York: snRNAs exist as large RNA-protein complexes during mitosis.

Lamond, A.I., Grabowski, P.J., Konarska, M.M., Sharp, P.A., Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Structure and function of mammalian spliceosomes.

Latham, J.A., Cech, T.R., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Small molecule probes of the three-dimensional structure of the *Tetrahymena* ribozyme.

Levine, B.J.,[1] Marzluff, W.,[2] Skoultchi, A.,[1] [1]Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: [2]Dept. of Chemistry, Florida State University, Tallahassee: Differences in expression of individual members of the histone multigene family.

Partono, S., Lewin, A., Dept. of Chemistry, Indiana University, Bloomington: Autocatalytic processing of intron 5 of the *cob* gene of yeast mitochondria.

Li, R., Thomas, J.O., Dept. of Biochemistry, New York University School of Medicine, New York, New York: Nuclear targeting of snRNP and hnRNP following mitosis.

Lin, R.-J.,[1,2] Abelson, J.,[2] [1]Dept. of Microbiology, University of Texas at Austin; [2]Division of Biology, California Institute of Technology, Pasadena: Characterization of a heat-stable factor required for complementing the *rna2Δ* spliceosome.

Lobo, S.M., Marzluff, W.F., Dept. of Chemistry, Florida State University, Tallahassee: Synthesis of U1 RNA in isolated mouse myeloma nuclei.

Lossky, M., Anderson, G.J., King, D.S., Beggs, J.D., Dept. of Molecular Biology, King's Buildings, Edinburgh, Scotland: Is *RNA8* in a splicing complex?

Lumelsky, N., Altman, S., Dept. of Biology, Yale University, New Haven, Connecticut: Random chemical mutagenesis of the gene coding for M1 RNA – Selection and structural and functional analyses of isolated mutants.

Maddock, J.R., Anthony, J.G., Lunz, R.L., Woodford, J.L., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Interacting gene products required for processing of yeast pre-mRNA.

Daar, I.O., Maquat, L.E., Dept. of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York: Premature translation termination results in increased mRNA lability.

Maser, R.L., Calvet, J.P., Dept. of Biochemistry, University of Kansas Medical Center, Kansas City: U3 snRNA psoralen cross-links to RNA hydrid selected by ETS/18S rRNA sequences.

McLauchlan, J., McWilliam, R., Simpson, S., Clements, J.B., MCR Virology Unit, Institute of Virology, Glasgow, Scotland: In vitro cleavage/polyadenylation – Sequence requirements and complex formation.

Mertins, P., Gallwitz, D., Dept. of Molecular Genetics, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Federal Republic of Germany: Stringent requirement for nuclear pre-mRNA splicing of an intron-contained, conserved sequence element in the fission yeast *S. pombe*.

Gewirth, D.T.,[1] Moore, P.B.,[1,2] Depts. of [1]Molecular Biophysics and Biochemistry, [2]Chemistry, Yale University, New Haven, Connecticut: Site-directed mutagenesis as an aid for studying RNAs by NMR.

Mowry, K.L., Steitz, J.A., Howard Hughes Medical Institute, Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Two different complexes are detected on mammalian histone pre-mRNAs during 3′-end formation in vitro.

Müller, M.W.,[1] Schmelzer, C.,[1] Schweyen, R.J.,[2] [1]Institut für Genetik und Mikrobiologie, Universität München, Federal Republic of Germany; [2]Institut für Mikrobiologie und Genetik, Universität Wien, Austria: Group II introns act as endonuclease and ligase in *cis* and in *trans*.

Murphy, F.L., Cech, T.R., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Synthesis of ribozymes with altered specificity for cleavage in *trans*.

Murray, E.E., Buchholz, W., DeBoer, D.L., Firoozabady, E., Frantz, T., Merlo, D.J., Rocheleau, T., Staffeld, G., Stock, C., Adang, M.J., Agrigenetics Advanced Science Company, Madison, Wisconsin: Transcription of a *B. thuringiensis* insecticidal protein gene in plants.

Nelson, K., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Analysis of factor binding and splice-site usage in

P. Moore, C. Guthrie, T. Cech

synthetic splice-site insertion mutants.

Nicholson, A.W.,[1] McOsker, P.L.,[2] Niebling, K.R.,[2] Robertson, H.D.,[2] [1]Dept. of Biological Sciences, Wayne State University, Detroit, Michigan; [2]Rockefeller University, New York, New York: Specific cleavage by RNase III of phosphorothioate RNA from bacteriophage T7.

Maroney, P.A., Hannon, G.J., Nilsen, T.W., Dept. of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio: Processing of eukaryotic rRNA in nucleolar extract.

Harvazinski, D., Westhafer, M.A., Kessler, M.M., Nordstrom, J.L., Dept. of Biochemistry and Biophysics, Texas A & M University, College Station: Implication of inverted repeats in the function of the mouse β-globin and other poly(A) signals.

Sawa, H., Ohno, M., Shimura, Y., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Effect of intron length on the efficiency of the splicing reaction of pre-mRNA in vitro.

Pan, Z.Q., Fu, X.-Y., Gui, H., Manley, J.L., Prives, C., Dept. of Biological Sciences, Columbia University, New York, New York: Role of U1 and U2 snRNPs in SV40 early pre-mRNA splicing.

Pandey, N.B., Marzluff, W.F., Dept. of Chemistry, Florida State University, Tallahassee: Processing and stability of transcripts from chimeric histone-globin genes.

Patton, J.R., Patterson, R.J., Pederson, T., Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: In vitro assembly of U1 snRNP.

Patzelt, E., Thalmann, E., Hartmuth, K., Barta, A., Blaas, D., Kuechler, E., Institute of Biochemistry, University of Vienna, Austria: Assembly of pre-mRNA splicing complex is cap-dependent.

Pedersen-Lane, J., Belfort, M., Wadsworth Center for Laboratories and Research, New York Dept. of Health, Albany: Variable occurrence of the *nrd*B intron in the T-even phages suggests intron mobility.

Peltz, S., Ross, J., McArdle Laboratory for Cancer Research, Madison, Wisconsin: Autoregulation of histone mRNA decay by histone proteins in a cell-free system.

Pruzan, R., Furneaux, H., Belgado, N., Hurwitz, J., Memorial Sloan-Kettering Cancer Center, New York, New York: Fractionation of activities involved in the splicing of pre-mRNA.

Raziuddin, Little, R.D., Schlessinger, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Transient transcription and processing of mammalian rRNA.

Reich, C., Olsen, G.J., Pace, B., Pace, N.R., Dept. of Biology, Indiana University, Bloomington: *B. subtilis* RNase P—Kinetic analysis of the RNA alone and holoenzyme reactions.

Riedel, N.,[1] Goelz, S.,[2] Abelson, J.,[2] Guthrie, C.,[1] [1]Dept. of Biochemistry and Biophysics, University of California, San Francisco; [2]Division of Biology, California Institute of Technology, Pasadena: Yeast spliceosomes contain essential snRNAs.

Ruby, S.W., Abelson, J.N., Division of Biology, California Institute of Technology, Pasadena: Identification of snRNAs in yeast splicing complexes.

Russo, A.F., Crenshaw, B., Rosenfeld, M.G., University of California, San Diego, La Jolla: Neuron-specific alternative RNA processing in transgenic mice expressing a metallothionein-calcitonin fusion gene.

SESSION 5 snRNPs

Chairman: J. Steitz, Yale University School of Medicine

Ares, M., Jr., Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Structure and function of the conserved U2 RNA domain.

Patterson, B., Wrischnik, L.A., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: An snRNA with homology to U5 is required for splicing in yeast.

Lossky, M., Anderson, G.J., Jackson, S.P., Beggs, J.D., Dept. of Molecular Biology, King's Buildings, Edinburgh, Scotland: *RNA8* protein is an snRNP component required for pre-mRNA splicing in *S. cerevisiae*.

Greider, C.W., Blackburn, E.H., Dept. of Molecular Biology, University of California, Berkeley: RNA is an essential component of the telomere terminal transferase of *Tetrahymena*.

Ruskin, B., Pikielny, C., Zamore, P., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: A non-snRNP factor, U2BF, is required for the binding of U2 snRNP to the branch point of a mammalian pre-mRNA.

Query, C.C., Keene, J.D., Dept. of Microbiology and Immunology and Medicine, Duke University Medical Center, Durham, North Carolina: U1 RNA-specific 70K protein—RNA-binding studies and autoimmune cross-reactivity with type-C retroviruses.

Neuman de Vegvar, H.E., Dahlberg, J.E., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Requirements for snRNA 3′-end formation and nucleocytoplasmic transport.

Southgate, C., Busslinger, M., Institute for Molecular Biology, Zürich, Switzerland: Sea urchin U7 RNA gene expression in vivo and in vitro.

Weller, P., Bark, C., Janson, L., Zabielski, J., Pettersson, U., Dept. of Medical Genetics, Biomedical Center, Uppsala, Sweden: Transcription analysis of a human U4 snRNA gene and comparison of enhancer-binding transcription factors in U2 and U4 genes.

Kunkel, G., Pederson, T., Cell Biology Group, Worcester Foundation for Experiment Biology, Shrewsbury, Massachusetts: 5′-flanking sequence elements required for polymerase III transcription of a human U6 RNA gene resemble those used by polymerase II on U1 and U2 RNA genes.

Reddy, R., Henning, D., Das, G., Dept. of Pharmacology, Baylor College of Medicine, Houston, Texas: Factors distinct from transcription factors IIIA, IIIB, and IIIC are required for the transcription of a mouse U6 snRNA gene by RNA polymerase III.

SESSION 6 CATALYTIC RNA

Chairman: O. Uhlenbeck, University of Colorado

Burke, J., Williamson, C., Kerker, B., Tierney, W., Dept. of Chemistry, Williams College, Williamstown, Massachusetts: Mutations in core sequence elements of self-splicing *Tetrahymena* pre-rRNA.

Chandry, P.S.,[1,2] Pedersen-Lane, J.,[1] Belfort, M.,[1] [1]Wadsworth Center for Laboratories and Research, New York State Dept. of Health, [2]Dept. of Microbiology and Immunology, Albany Medical College, New York: Genetic probing of functional elements of the group I phage T4 *td* intron.

Zaug, A.J., Been, M.D., Grosshans, C.A., Cech, T.R., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Sequence-specific endonuclease activity of the *Tetrahymena* ribozyme.

Inoue, T., Kay, P.S., Salk Institute, La Jolla, California: Minimum substrate requirement for group I self-splicing.

Tabak, H.F.,[1] Van der Horst, G.,[1] Winter, A.J.,[1] Arnberg, A.C.,[2] Laboratory of Biochemistry, [1]University of Amsterdam, [2]University of Groningen, The Netherlands: Yeast mitochondrial group I intron RNA-mediated reactions.

Akins, R.A., Cherniack, A., Lambowitz, A.M., Depts. of Genetics and Biochemistry, Ohio State University, Columbus: A protein component required for splicing group I introns in *Neurospora* mitochondria is mitochondrial tyrosyl-tRNA synthetase or some derivative thereof.

Schmelzer, C., Müller, M.W., Institut für Genetik und Mikrobiologie, Universität München, Federal Republic of Germany: Catalytic activity of group II introns in vitro—Effects of branch-point mutations on lariat formation and exon ligation.

Jarrell, K.A.,[1] Dietrich, R.C.,[1] Peebles, C.L.,[2] Uhl, T.M.,[1] Perlman, P.S.,[1] [1]Dept. of Genetics, Ohio State University, Columbus; [2]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: *cis* and *trans* reactions of mutant and wild-type forms of a group II intron.

Sullivan, F.X., Uhlenbeck, O.C., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Design and characterization of a small catalytic RNA.

Hannon, G.J., Maroney, P.A., Nilsen, T.W., Dept. of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio: Autocatalytic processing of eukaryotic rRNA.

Chang, D.D., Topper, J.N., Clayton, D.A., Dept. of Pathology, Stanford University School of Medicine, California: Sequence analysis of a nuclear gene that encodes the RNA component of a mitochondrial RNA-processing enzyme.

Posters:

Raymond, V., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Deletion of an mRNA destabilizing element is responsible for the increased oncogenicity of proto-oncogene *fos*.

Sachs, A.B.,[1] Davis, R.W.,[2] Kornberg, R.D.,[1] Depts. of [1]Cell Biology, [2]Biochemistry, Stanford University School of Medicine, California: A single domain of yeast polyadenylate-binding protein is necessary and sufficient for RNA binding and cell viability.

Sasmor, H.M., deLannoy, P., Dellinger, D.J., Roman, S., Caruthers, M.H., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: 2′–5′ Phosphodiester linkages in mRNA processing and debranching.

Melchers, K., te Heesen, S., Schäfer, K.P., Universität Bochum, Konstanz, Federal Republic of Germany: Structure and expression of hnRNP core protein A1 genes in mammalian cells.

Spencer, D.F., Collings, J.C., Schnare, M.N., Gray, M.W., Dept. of Biochemistry, Dalhousie University, Halifax, Canada: Multiple spacer sequences in the nuclear large subunit rRNA gene in *C. fasciculata*.

Schöneich, J.T., Thomas, J.O., Dept. of Biochemistry, New York University School of Medicine, New York, New York: Interactions of hnRNP A1 protein with single-stranded nucleic acids.

Schuler, M.A., Hanley, B.A., University of Illinois, Urbana: Plant RNA processing—Soybean pre-mRNA in a pea cell-free extract.

Kreike, J.,[1,2] Schmidt, N.,[1] Söllner, T.,[1] Schweyen, R.J.,[2] [1]Institut für Genetik und Mikrobiologie, Universität München, Federal Republic of Germany; [2]Institut für Mikrobiologie und Genetik, Universität Wien, Austria: Involvement of nuclear genes in mitochondrial RNA splicing in yeast.

Sheets, M., Wickens, M., Dept. of Biochemistry, University of Wisconsin, Madison: Polyadenylation of SV40 late mRNA precursors in vitro.

Shiraishi, H., Shimura, Y., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Mutations of the RNA component of RNase P.

Shub, D.A.,[1] Gott, J.M.,[1] Lang, B.F.,[2] [1]Dept. of Biological Sciences, State University of New York, Albany; [2]Dept. of Biochemistry, University of Montreal, Canada: Structure and regulation of expression of the *nrd*B intron of bacteriophage T4.

Siliciano, P., Roiha, H., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: An essential yeast snRNA has properties predicted for U4.

Kiss, T.,[1] Antal, M.,[1] Hadlaczky, G.,[2] Hegyi, H.,[1] Praxnovszky, T.,[2] Yang, L.-J.,[3] Solymosy, F.,[1] Institutes of [1]Plant Physiology, [2]Genetics, Biology Research Center, Hungarian Academy of Science, Szeged, Hungary; [3]Shanghai Institute of Plant Physiology, Chinese Academy of Sciences, China: Plant snRNAs and snRNPs—Structural features and their possible functional implications.

Spann, P.,[1] Cahana, N.,[1] Feinerman, M.,[1] Sperling, R.,[1] Sperling, J.,[2] [1]Dept. of Genetics, Hebrew University of Jerusalem, [2]Dept. of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel: Large nuclear RNP particles.

Stefano, J.E., Adams, D.E., National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado: Identification of a heparin-resistant complex involved in polyadenylation.

Stévenin, J., Schmitt, P., Gattoni, R., Institut de Chimie Biologique, Strasbourg, France: Branching occurs at an abnormally long distance from the 3′ splice site for a minor splicing reaction of adenovirus E1A pre-mRNA.

Stiege, W., Kosack, M., Stade, K., Brimacombe, R., Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Berlin-Dahlem, Federal Republic of Germany: Structure and function of *E. coli* ribosomes as studied by intraRNA cross-linking.

Surowy, C.S.,[1] Spritz, R.A.,[1] Hoch, S.O.,[2] [1]Laboratory of Genetics, University of Wisconsin, Madison; [2]Agouron Institute, La Jolla, California: RNA-binding properties of the human 68-kD U1 snRNP protein.

Tanner, N.K., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: Cross-linking of bromouridine-incorporated precursor phenylalanine tRNA to yeast tRNA ligase.

Tobian, J.A., Zasloff, M., National Institutes of Health, Bethesda, Maryland: Effects of point mutations in the human tRNAmet gene on processing by eukaryotic 5′ and 3′ pre-tRNases.

Tsagris, M., Hillman, M., Linker, W., Tabler, M., Sänger, H.L., Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Processing of viroids of the "PSTV-group" in extracts of host and nonhost cells.

Vankan, P., Filipowicz, W., Friedrich Miescher-Institut, Basel, Switzerland: U2 snRNA gene family of *A. thaliana*.

Vijayraghavan, U., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: Temperature-sensitive splicing mutants in yeast.

Virtanen, A.,[1] Moore, C.L.,[2] Sharp, P.A.,[1] [1]Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Molecular Biology and Microbiology, Tufts University Medical School, Boston, Massachusetts: Alternative RNA processing in vitro—Polyadenylation of murine IgM transcripts.

von Gabain, A., Nilsson, G., Lundberg, U., Mellefors, O., Dept. of Bacteriology, Karolinska Institute, Stockholm, Sweden: Site-specific endonucleolytic cleavages regulate the stability of the *E. coli ompA* mRNA.

West, D., Maley, G., Maley, F., Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany: Expression of the T4 phage *td* open reading frame as a protein product.

Brennwald, P., Porter, G., Wise, J.A., Dept. of Biochemistry, University of Illinois, Urbana: Analysis of snRNAs from *S. pombe*.

Bhat, B., Wold, W., Institute for Molecular Virology, St. Louis University School of Medicine, St. Louis: A small deletion distant from a splice or polyadenylation site dramatically alters pre-mRNA processing.

Xu, Q., Clark, M.W., Green, P., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: Activity domains of yeast tRNA ligase.

Xu, M.-Q.,[1] Belfort, M.,[2] Shub, D.A.,[1] [1]Dept. of Biological Sciences, State University of New York, Albany; [2]New York State Health Department, Albany: A new group I intron in bacteriophage T4.

Young, B., Cech, T.R., Dept. of Molecular, Cellular and De-

velopmental Biology and Chemistry and Biochemistry, University of Colorado, Boulder: A multicomponent ribozyme – Methodology facilitating rapid selective alterations in substrate specificity.

Zhang, J., Deutscher, M.P., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: Cloning and physiological consequences of overexpression of the *E. coli rnd* gene encoding RNase D.

Zhu, L., Deutscher, M.P., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: tRNA nucleotidyltransferase is not essential for the viability of *E. coli*.

Zieve, G.W., Feeney, R.P., Sauterer, R.A., Dept. of Anatomical Sciences and Program in Cellular and Developmental Biology, State University of New York, Stony Brook: Abundances and half-lives of snRNA and snRNP protein precursors in the cytoplasm.

H. Robertson

SESSION 7 tRNA PROCESSING

Chairman: M. Yarus, University of Colorado

Cudny, H., Deutscher, M.P., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: Processing of the 3′ terminus of tRNA in multiple RNase-deficient *E. coli*.

Pace, N.R., James, B.D., Waugh, D.S., Olsen, G.J., Reich, C., Liu, J., Pace, B., Aprison, E.Z., Dept. of Biology, Indiana University, Bloomington: Structure and catalytic function in *B. subtilis* RNase P RNA.

Altman, S., Guerrier-Takada, C., Lawrence, N., Vioque, A., Dept. of Biology, Yale University, New Haven, Connecticut: RNA-RNA and RNA-protein interactions during catalysis by RNase P from *E. coli*.

Burkard, U., Orellana, O., Söll, D., Yale University, New Haven, Connecticut: Processing of histidine tRNA in *E. coli*.

Green, C., Vold, B., Dept. of Molecular Biology, SRI International, Menlo Park, California: Structural requirements for processing of *B. subtilis* precursor tRNAs by catalytic RNA.

Hollingsworth, M.J., Martin, N.C., Dept. of Biochemistry, University of Texas Health Science Center at Dallas: Alteration of a mitochondrial tRNA precursor 5′ leader abolishes its cleavage by yeast mitochondrial RNase P.

Reyes, V., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: Yeast tRNA splicing in vitro.

Szekely, E., Belford, H.G., Greer, C.L., Dept. of Biological Chemistry, College of Medicine, University of California, Irvine: Substrate recognition and splice-site identification by the tRNA splicing enzymes from *S. cerevisiae*.

O'Connor, J.P., Peebles, C.L., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Unexpected diversity in the tRNA processing pathway of yeast.

SESSION 8 REGULATION BY ALTERNATIVE mRNA PROCESSING AND TURNOVER

Chairman: M. Green, Harvard University

Andreadis, A., Breithbart, R.E., Endo, T., Gallego, M.E., Smith, C.W.J., Nadal-Ginard, B., Howard Hughes Medical Institute, Dept. of Cardiology, Children's Hospital, Dept. of Pediatrics, Harvard Medical School, Boston, Massachusetts: Regulatory elements in alternative splicing of contractile protein pre-mRNAs.

Caffarelli, E.,[1] Fragapane, P.,[1] Bozzoni, I.,[2] [1]Centro Acidi Nucleici, CNR, Rome, [2]Universita di Roma, Italy: Regulatory role of splicing in the expression of the L1 ribosomal protein gene in *X. laevis*.

Köhrer, K., Thompson-Jäger, S., Domdey, H., Genzentrum der Ludwig-Maximilians-Universität München, Federal Republic of Germany: To be or not to be a functional yeast intron depends on a minimum distance from the 5′-splice site to the internal branch-acceptor site.

Brady, H., Wold, W., Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Identification of a novel sequence that governs both polyadenylation and alternate splicing.

Powdrill, T.F., Slater, H.S., Nordstrom, J.L., Dept. of Biochemistry and Biophysics, Texas A & M University, College Station: Dominance of splicing over polyadenylation for RNAs processed in transfected COS cells.

Tsurushita, N., Korn, L.J., Dept. of Genetics, Stanford University School of Medicine, California: Regulation of differential processing of mouse immunoglobulin Mu heavy-chain mRNA.

Peterson, M.L., Perry, R.P., Institute for Cancer Research, Philadelphia, Pennsylvania: On the developmentally regulated processing of immunoglobulin mRNA.

de la Peña, P., Zasloff, M., National Institutes of Health, Bethesda, Maryland: Enhancement of mRNA nuclear transport by promoter elements.

Krinke, L., Wulff, D.L., Dept. of Biological Sciences, State University of New York, Albany: OOP RNA negatively regulates λcII gene expression through an RNase-III-dependent mechanism.

Brewer, G., Ross, J., McArdle Laboratory for Cancer Research and Dept. of Pathology, University of Wisconsin, Madison: 3′ and 5′ decay of human c-*myc* mRNA in cell-free extracts.

Shaw, G., Kamen, R., Genetics Institute, Inc., Cambridge, Massachusetts: Characterization of AU sequences functioning as mRNA destabilizers.

RNA Tumor Viruses

May 19—May 24

ARRANGED BY

Harriet L. Robinson, Worcester Foundation for Experimental Biology
Charles J. Sherr, St. Jude Children's Research Hospital

304 participants

The study of RNA tumor viruses continues to provide a focal interest for researchers working on retroviral replication and integration, retroviral vectors, mechanisms of viral pathogenesis, and the activities of transduced viral oncogenes. The field has already had a major impact on diverse arenas of investigation and, in particular, has fostered a rapid understanding of human immunodeficiency viruses, cellular proto-oncogenes, mechanisms of insertional mutagenesis, and the function of endogenous viral elements as model retrotransposons. The 1987 conference brought together more than 400 scientists interested in the most recent developments in the field.

Studies of retroviral replication stressed the development of the first in vitro systems to assay proviral integration, the activity of virus-coded endonucleases, and the fidelity of reverse transcription. Progress in elucidating detailed requirements for ribosomal frameshifting during viral protein synthesis and for virion assembly was also reported. Although the role of viral oncogenes and their cellular proto-oncogene progenitors in cell transformation has become a subject of major conferences elsewhere, several sessions remained dedicated to work in this area. Highlights included functional studies of chimeric insulin/*ros* receptor molecules, the demonstration of autocrine transformation in the c-*fms*/CSF-1 and *erbB*/EGF systems, insertional mutagenesis of the CSF-1 receptor locus in Friend-virus-induced myelogenous leukemia, activation of the transforming potential of the c-*fes* gene product, and the description of revertants of v-*fos*-transformed cells refractory to transformation by other oncogenes.

Another theme centered on the tissue-specific transcription of retroviruses, the role of *trans*-activating factors in regulating viral gene expression, and the importance of viral transcriptional control elements in pathogenesis. Continued progress in the elucidation of the functions of the human immunodeficiency virus *tat* and *art* gene products in regulating viral RNA expression, including the elucidation of virus-coded positive and negative regulatory proteins and LTR target sequences, was reported. A final session concerned the role of other HIV gene products (*sor*) in viral replication, the mechanism of HIV cytopathicity, identification of new HIV isolates, and the relationship of HIV to other recently characterized primate retroviridae.

SESSION 1 INTEGRATION AND REVERSE TRANSCRIPTION

Chairmen: **H.M. Temin,** University of Wisconsin
H.E. Varmus, University of California, San Francisco

Brown, P.O.,[1,3] Bowerman, B.,[2] Varmus, H.E.,[1,2] Bishop, J.M.,[1,2,3] Depts. of [1]Microbiology and Immunology, [2]Biochemistry and Biophysics, [3]G.W. Hooper Foundation, University of California, San Francisco: Correct integration of retroviral DNA in vitro.

Lee, Y.M., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: In vitro integration of avian retroviral DNA.

Bowerman, B.,[1] Brown, P.O.,[2,3] Bishop, J.M.,[1,2,3] Varmus, H.E.,[1,2] Depts. of [1]Biochemistry and Biophysics, [2]Immunology and Microbiology, [3]G.W. Hooper Foundation, University of California, San Francisco: A subviral

nucleoprotein complex participates in the retrovirus integration reaction.

Shih, C.C., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Specificity of retroviral integration in vivo.

Tanese, N., Goff, S.P., Dept. of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York: Fine-structure mutational analysis of the reverse transcriptase domain of Mo-MLV.

Roth, M., Tanese, N., Schwartzberg, P., Goff, S.P., Dept. of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York: Expression and characterization of the integrase protein encoded by the 3′-terminal part of the *pol* gene of Mo-MLV.

Quinn, T.P., Grandgenett, D., St. Louis University, Institute for Molecular Virology, Missouri: Construction of viable point mutations in the RSV pp32 integrase.

Skalka, A.M.,[1] Terry, R.,[1] Soltis, D.,[1] Katzman, M.,[1] Cobrinik, D.,[2] Leis, J.,[2] [1]Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey; [2]Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio: Activity of ASLV endonuclease produced in *E. coli*.

Roberts, J.D.,[1] Preston, B.D.,[2] Johnston, L.A.,[2] Soni, A.,[1] Loeb, L.A.,[2] Kunkel, T.A.,[1] [1]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [2]Dept. of Pathology, University of Washington, Seattle: Genetic variation in RNA tumor viruses based on the infidelity of reverse transcriptase.

Dougherty, J., Dornburg, R., Temin, H.M., McArdle Laboratory, University of Wisconsin, Madison: Retroviral mutation rates and the formation of retrosequences.

SESSION 2 PROTEIN SYNTHESIS, PROCESSING, AND VIRAL ASSEMBLY

Chairmen: **S.P. Goff,** Columbia University
V.M. Vogt, Cornell University

Jacks, T.,[1] Masiartz, F.,[2] Power, M.,[2] Varmus, H.E.,[1] [1]Depts. of Biochemistry and Biophysics and Microbiology, University of California, San Francisco; [2]Chiron Corporation, Emeryville, California: Detailed analysis of the sites of ribosomal frameshifting in RSV and other retroviruses.

Madhani, H.D., Jacks, T., Varmus, H.E., Depts. of Biochemistry and Biophysics and Microbiology, University of California, San Francisco: Secondary-structure requirements for the expression of the *pol* gene of RSV by ribosomal frameshifting.

Felsenstein, K.M., Goff, S.P., Dept. of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York: Site-directed mutagenesis of the *gag* terminator codon and adjacent sequences in Mo-MLV—Effects on virus production.

Vogt, V.M., Schatz, G., Section of Biochemistry, Molecular and Cellular Biology, Cornell University, Ithaca, New York: Site-directed mutagenesis of p15^{gag} protease in ASLVs.

Farmerie, W.,[1] Loeb, D.,[2] Casavant, N.,[2] Hutchison, C. III,[2] Edgell, M.,[2] Swanstrom, R.,[1] Depts. of [1]Biochemistry and Lineberger Cancer Center, [2]Microbiology and Immunology, University of North Carolina, Chapel Hill: Expression and processing of the HIV *pol* gene product in *E. coli*.

Loeb, D.,[1] Farmerie, W.,[2] Swanstrom, R.,[2] Hutchison, C. III,[1] Depts. of [1]Microbiology and Immunology, [2]Biochemistry and Lineberger Cancer Center, University of North Carolina, Chapel Hill: Analysis of the HIV *pol* gene protease domain by site-directed mutagenesis.

Rhee, S.S., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Mutagenic analysis of the membrane-binding protein, p10, of MPMV.

Fu, X.-D.,[1] Katz, R.,[2] Tuazon, P.,[3] Traugh, J.,[3] Skalka, A.,[2] Leis, J.,[1] [1]Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]Roche Institute of Molecular Biology, Nutley, New Jersey; [3]University of California, Riverside: Site-directed mutagenesis of the avian retroviral nucleocapsid protein pp12.

Darlix, J.L., Gabus, C., Prats, A.C., CNRS, Toulouse, France: Dimer structure of the retroviral genome.

Méric, C., Gouilloud, E., Spahr, P.-F., Dept. of Molecular Biology, University of Geneva, Switzerland: Mutations in RSV nucleic-acid-binding protein p12—Deletion of the Cys-His boxes.

Murphy, J.E., Kalnick, S.T., Lobel, L.I., Goff, S.P., Dept. of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York: Mutations near the 5′ end of the genome of Mo-MLV—Effects on packaging of viral RNA and integration of viral DNA.

Miller, D.C., Davis, G.L., Perez, L., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Effects of viral infectivity of mutations in the carboxy-terminal region of the RSV *env* gene.

A. Skalka

Basu, S., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: Characterization of viral functions involved in Mo-MLV integration.

Quinn, T.,[1] Horton, B.,[1] Mumm, S.,[1] Grandgenett, D.,[1] Walsh, M.,[2] [1]St. Louis University, Missouri; [2]Boston University, Massachusetts: Structural and functional studies of the avian retroviral pp32 DNA endonuclease.

Cobrinik, D.,[1] Soskey, L.,[1] Ahuja, D.,[1] Skalka, A.M.,[2] Leis, J.,[1] [1]Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: A *cis*-acting function of U5 required for efficient viral growth.

Kenny, S., Cleavinger, P., Kim, Y.Q., Guntaka, R.V., Dept. of Microbiology, University of Missouri, Columbia: Sequences in the U5 region of RSV LTRs are required for transcription termination and polyadenylation.

Katz, R.A., Kotler, M., Skalka, A.M., Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey: Biochemical analyses of an ASLV splicing mutant and pseudorevertants.

Levin, J.G.,[1] Crouch, R.,[1] Hu, S.,[1] McKelvin, D.,[1] Zweig, M.,[2] Court, D.,[1] Gerwin, B.I.,[1] [1]NCI, National Institutes of Health, Bethesda, [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Bacterially expressed MLV reverse transcriptase having a high ratio of polymerase to RNase H.

Halbreich, A., Heyman, T., Institut Curie, Centre Universitaire, Orsay, France: Homologies between reverse transcriptases from the yeast *S. cerevisiae* and those of several mammalian retroviruses.

Prats, A.C.,[1] Capdevielle, J.,[2] Ferrara, P.,[2] Darlix, J.L.,[1] [1]CNRS, [2]Sanofi-ELFBR, Toulouse, France: Translation initiation of AUG and GUG and multiple ribosomal frameshifts account for the synthesis of MLV glycosylated cell-surface *gag* polyprotein.

Moustakas, A., Petersen, R.B., Hensel, C.H., Hackett, P.B., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Inactivation of an open reading frame of the RSV leader RNA sequence affects transformation of CEFs.

Hizi, A., Hughes, S.H., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Introducing initiation and termination codons at the cleavage sites permits the precise expression of MLV and HIV enzymes.

Gorelick, R.J., Henderson, L.E., Rein, A.R., Oroszlan, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Site-directed mutagenesis of highly conserved amino acid sequences found in all retroviral nucleic-acid-binding proteins.

Kotler, M., Katz, R.A., Skalka, A.M., Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, New Jersey: Mutations that affect proteolytic processing of ASLV precursor proteins.

Lilleĥoj, E., Mervis, R.J., Ahmad, N., Venketesan, S., NCI, National Institutes of Health, Bethesda, Maryland: Identification and characterization of HIV *gag-pol* protease.

Schultz, A., Rein, A., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Exclusion of unmyristylated MLV Pr65^{gag} from viral particles formed in dually infected cells.

Putterman, D.M., Vogt, V.M., Section of Biochemistry, Molecular and Cellular Biology, Cornell University, Ithaca, New York: Ubiquitin in retroviruses.

Wills, J.W., Weldon, R.A., Bangs, L.A., Denman, S.E., Dept. of Microbiology, Oklahoma State University, Stillwater: Localization of a *cis*-acting element of the RSV *gag* gene that blocks the propagation of recombinant SV40 DNA.

Pruett, W., Stoltzfus, M., Dept. of Microbiology, University of Iowa, Iowa City: Role of the RSV *env* protein precursor leader peptide in viral metabolism.

Freed, E.O., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Effects of an envelope-glycoprotein-processing mutation on retroviral infection.

Brody, B.A., Henderson, E.K., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Mutation and expression of the MPMV envelope glycoprotein gene.

Villar, C., Kozak, C.A., NCI, National Institutes of Health, Bethesda, Maryland: Expression of xenotropic and MCF-related *env* sequences in wild mice.

Buller, R.,[1] Sitbon, M.,[2] Portis, J.,[1] [1]National Institutes of Health, NIAID, Rocky Mountain Laboratories, Hamilton, Montana; [2]Hopital Cochin, Paris, France: Expression of an endogenous MCF gp70 by DBA/2 mice imparts partial resistance to Fr-MLV-induced erythroleukemia.

Evans, L., Malik, F., National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Class II polytropic MLVs of AKR/J mice—Possible role in the generation of class I polytropic MLVs.

Trauger, R., Culp, P., Talbot, R., Elder, J., Scripps Clinic and Research Foundation, La Jolla, California: Characterization of proteins immunologically related to recombinant virus gp70S.

Elder, J.,[1] Nicolaisen-Strouss, K.,[1] Kumar, H.,[1] Fitting, T.,[1] Grant, C.,[2] [1]Scripps Clinic and Research Foundation, La Jolla, California; [2]Pacific Northwest Research Foundation, Seattle, Washington: Natural FeLV escapes neutralization by a monoclonal antibody via an amino acid change outside the antibody-binding epitope.

Tsai, W.-P., Briggs, C., Copeland, T.D., Oroszlan, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Cytotoxic antibody directed against the carboxy-terminal segment of gp90 of REV-A.

Query, C.C., Keene, J.D., Depts. of Microbiology and Immunology and Medicine, Duke University Medical Center, Durham, North Carolina: The U1 RNA-specific 70K protein—RNA-binding studies and autoimmune cross-reactivity with type-C retroviruses.

Olsen, J.C., Osheroff, W.P., Swanstrom, R., Dept. of Biochemistry and Lineberger Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Detection of a host determinant in the packaging of large retroviral genomes.

Stoker, A.W., Bissell, M.J., Laboratory of Cell Biology, University of California, Berkeley: Deletion analysis of ALV helper virus and use in the avian-vector-packaging cell system.

D. Boettiger, H. Robinson

Bosselman, R.A., Hsu, R.-Y., Bruszewski, J., Hu, S., Nicolson, M., Amgen Inc., Thousand Oaks, California: Replication-defective chimeric helper proviruses and factors affecting the generation of competent virus – Expression of Mo-MLV structural genes via the metallothionein promoter.

Hunt, L.A., Robinson, H.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Expression of an avian influenza virus hemagglutinin gene with an ALV vector.

Evrard, C., Schremmet, B., Galiana, E., Rouget, P., Institut Jacques Monod, Universités Paris, France: Gene transfer and immortalization of murine brain cells with recombinant retroviral vectors.

Patel, G., Winberg, G., Klein, G., Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Establishment of stable cell lines expressing the EBV-EBNA-1 gene using a retroviral vector.

Paludan, K., Jørgensen, P., Kjeldgaard, N.O., Pedersen, F.S., Dept. of Molecular Biology, University of Aarhus, Denmark: An Akv-neo transmission vector confers no marker gene expression upon lymphoid cell clones, unless selected for G418 resistance.

Hippenmeyer, P., Highkin, M., Dept. of Biological Sciences, Monsanto Company, St. Louis, Missouri: Transfer and expression of the bacterial NPT-II gene in chick embryos using a retroviral vector.

Salmons, B., Günzberg, W.H., Garcha, I., Legrand, S., Sarkar, N.H., Dept. of Cell and Molecular Biology, Medical College of Georgia, Augusta: MMTV-based vector system – Introduction of oncogenes into mammary epithelial cells.

Winberg, G., Fuerstenberg, S., Klein, G., Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden: A versatile retrovirus vector allowing enhancer replacement and multiple gene cloning.

Connors, R., Yokoyama, S., Deen, K., Rosenberg, M., Sweet, R., Smith Kline and French Laboratories, Swedeland, Pennsylvania: Selection of overexpression of transfected genes.

Bonnerot, C., Briand, P., Nicolas, J.F., Unité de Génétique cellulaire du Collège de France et de l'Institut Pasteur, Paris: Recombinant retroviruses encoding a nuclear-targeted *E. coli* β-galactosidase – A marker system for studies on control of gene expression on preimplantation mouse embryos.

SESSION 4 ONCOGENES: *src*, *abl*, *int*-1, *sis*, *ras*, *raf*

Chairmen: **H. Hanafusa,** Rockefeller University
J.T. Parsons, University of Virginia

Tanaka, A., Fujita, D.J., University of Western Ontario, Canada: Chain termination, internal deletion, and point mutation events involved in activated human c-*src* genes.

Potts, W., Reynolds, A., Lansing, T., Parsons, J.T., Dept. of Microbiology, University of Virginia, Charlottesville: Fine-structure analysis of domains of $p60^{src}$ involved in oncogenic transformation using oligonucleotide-directed mutagenesis.

Fox, L., Frost, K., Brugge, J., Dept. of Microbiology, State University of New York, Stony Brook: Generation of a new class of $pp60^{c\text{-}src}$ mutants that display an intermediate level of $pp60^{c\text{-}src}$ activation.

Sugano, S., Stoeckle, M.Y., Hanafusa, H., Rockefeller University, New York, New York: Transformation by RSV induces a novel gene with homology to a mitogenic platelet protein.

Menko, A.S., Boettiger, D., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Integrin serves as a critical target for phosphorylation by v-*src* in the suppression of myogenic differentiation.

Engelman, A., Rosenberg, N., Dept. of Molecular Biology and Microbiology, Tufts University Medical School, Boston, Massachusetts: Construction of conditional Abelson virus mutants by site-directed mutagenesis.

Brown, A.M.C., Papkoff, J., Varmus, H.E., Dept. of Microbiology and Immunology, University of California Medical Center, San Francisco: Studies of the mechanism of action of the mammary oncogene *int*-1.

Sauer, M.K.,[1] Donoghue, D.J.,[2] Depts. of [1]Biology, [2]Chemistry, University of California, San Diego, La Jolla:

Oligonucleotide site-directed mutagenesis of the eight cysteine residues in the minimal transforming region of v-*sis*.

Lee, B.A.,[1] Maher, D.W.,[2] Hannink, M.,[2] Donoghue, D.J.,[2] Depts. of [1]Biology, [2]Chemistry, University of California, San Diego, La Jolla: The v-*sis* protein contains a nuclear targeting signal.

Shih, T.Y., Utsh, L.S., Huang, K.-P., NCI, National Institutes of Health, Bethesda, Maryland: Phosphorylation of p21 products of *ras* oncogenes by protein kinase C.

Tsai, M.-H., Yu, C.-L., Stacey, D.W., Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: c-*ras* proto-oncogene does not function to control phospholipase-A_2 or phospholipase-C activity.

Heidecker, G., Beck, T.W., Cleveland, J.L., Huleihel, M., Rapp, U.R., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Activation of the *raf* family proto-oncogenes.

SESSION 5 ONCOGENES: *ros*, *fms*, *met*, *erb*B, *erb*A, *fps/fes*

Chairmen: **G.S. Martin,** University of California, Berkeley
L.-H. Wang, Rockefeller University

Wang, L.-H.,[1] Lin, B.,[1] Jong, S.-M.,[1] Dixon, D.,[1] Ellis, L.,[2] Rutter, W.J.,[2] Roth, R.,[3] [1]Rockefeller University, New York, New York; [2]University of California, San Francisco, [3]Stanford University, Palo Alto, California: Activation of the transforming potential of human insulin receptor gene.

Wheeler, E.F.,[1] Askew, D.,[2] Ihle, J.,[2] Rettenmier, C.W.,[1] Sherr, C.J.,[1] [1]Dept. of Tumor Cell Biology, St. Jude Children's Hospital, Memphis, Tennessee; [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: The v-*fms* oncogene induces factor-independence and tumorigenicity in murine hematopoietic cell lines of the myeloid lineage.

Heard, J.M.,[1] Roussel, M.F.,[1] Rettenmier, C.W.,[1] Ralph, P.,[2] Sherr, C.J.,[1] [1]Dept. of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee; [2]Cetus Corporation, Emeryville, California: Cell transformation induced by retroviral vectors expressing the human CSF-1 and c-*fms* (CSF-1 receptor) genes.

Rogers, A.,[1,2] Bishop, J.M.,[1,3] [1]Hooper Foundation, and Depts. of [2]Biochemistry and Biophysics, [3]Microbiology, University of California, San Francisco: Mutational analysis of c-*fms* transforming potential.

Bodescot, M., Park, M., Gonzatti-Haces, M., Dean, M., Vande Woude, G., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Sequence of *met* proto-oncogene cDNA reveals features characteristic of the tyrosine kinase family of growth-factor receptors.

Velu, T.J.,[1] Beguinot, L.,[2] Willingham, M.,[2] Merlino, G.T.,[2] Pastan, I.,[2] Vass, W.C.,[1] Lowy, D.R.,[1] Laboratories of [1]Cellular Oncology, [2]Molecular Biology, NCI, Bethesda, Maryland: EGF-dependent transformation by the human EGF receptor proto-oncogene transmitted as a high-titer retrovirus.

Bassiri, M.,[1] Boucher, P.,[2] Koning, A.,[1] Judelson, C.,[1] Privalsky, M.L.,[1] Depts. of [1]Bacteriology, [2]Biochemistry, University of California, Davis: Genetic and biochemical analysis of the v-*erb* A and v-*erb* B oncogenes of AEV.

Zenke, M.,[1] Sap, J.,[1] Munoz, A.,[1] Leutz, A.,[2] Kahn, P.,[1] Choi, H.-R.,[3] Engel, D.,[3] Vennström, B.,[1] Beug, H.,[1] [1]EMBL, Heidelberg, Federal Republic of Germany; [2]Dept. of Microbiology, State University of New York, Stony Brook; [3]Dept. of Biochemistry, Northwestern University, Evanston, Illinois: Effects of the v-*erb*A oncogene on erythroid-specific gene expression.

Feldman, R.A., Vass, W.C., Tambourin, P.E., NCI, National Institutes of Health, Bethesda, Maryland: Human c-*fps-fes* cDNA rescued via the retroviral shuttle vector encodes myeloid cell NCP92 and has transforming potential.

DeClue, J.,[1] Sadowski, I.,[2] Martin, G.S.,[1] Pawson, T.,[2] [1]Dept. of Zoology, University of California, Berkeley; [2]Mount Sinai Hospital Research Institute, Toronto, Canada: A site-directed mutant of the v-*fps* oncogene with host-dependent transforming activity.

SESSION 6 POSTER SESSION: ONCOGENES

Iyer, A., Sutrave, P., Park, M., Dean, M., Gonda, M., Vande Woude, G.F., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Chromosomal location, tissue expression, and characterization of cDNA of the mouse *met* locus.

McMahon, M., Bruskin, A.M., Bishop, J.M., G.W. Hooper Foundation, University of California, San Francisco: Analysis of transformation of established rodent fibroblasts by recombinant murine retroviruses containing the v-*erbB* oncogene.

Kaplan, J.M.,[1] Mardon, G.,[2] Bishop, J.M.,[3] Varmus, H.E.,[2] Depts. of [1]Biochemistry and Biophysics, [2]Microbiology and Immunology, [3]G.W. Hooper Foundation, University of California, San Francisco: The myristylation signal of pp60$^{v\,src}$ is in the first seven amino acids, and codon 7 can be either lysine or arginine.

Ferrell, J.E., Jr., Martin, G.S., Dept. of Zoology, University of California, Berkeley: Protein-tyrosine phosphorylation in intact human platelets.

Howlett, A.R., Cullen, B., Hertle, M., Bissell, M.J., Laboratory of Cell Biology, University of California, Berkeley: Temporal and tissue-specific expression of RSV in microinjected embryonic avian limb.

Sugano, S., Stoeckle, M.Y., Hanafusa, H., Rockefeller University, New York, New York: The 78-kD glucose-regulated protein mRNA is elevated in RSV-transformed cells.

Simon, M., Arrigo, A.-P., Spahr, P.-F., Dept. of Molecular Biology, University of Geneva, Switzerland: Is the 34-kD target of RSV tyrosine kinase associated with a ribonucleoprotein particle?

P. Tsichlis, P. Jolicoeur, N. Teich

Hopper, P., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Effect of antisense *src* RNA on RSV replication and transformation.

Hevezi, P., Rees-Jones, R., Depts. of Microbiology and Medicine, Columbia University College of Physicians and Surgeons, New York, New York: A prominent phosphorylated glycoprotein present at high levels in cells transformed by v-*src* and v-*abl* oncogenes.

Gebhardt, A.I.,[1] Colledge, W.H.,[1] Fry, M.J.,[1] Foulkes, J.G.,[2] [1]National Institute for Medical Research, London, England; [2]Oncogene Science, Inc., Mineola, New York: Characterization of nontumorigenic v-*abl*-transformed fibroblast lines.

Singh, B., Arlinghaus, R., Dept. of Molecular Pathology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Posttranslational modification of vimentin in *ts*110 Mo-MSV-transformed cells.

Ratner, L.,[1] Thielan, B.J.,[1] Collins, T.,[2] [1]Depts. of Medicine and Microbiology and Immunology, Washington University, St. Louis, Missouri; [2]Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Characterization of the c-*sis* transcriptional promoter and regulation of expression of the c-*sis* protein product.

Cleveland, J.,[1] Dean, M.,[2] Ihle, J.,[2] Rapp, U.,[1] [1]BRI-Basic Research Program, [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Role of *myc* in growth-factor abrogation.

Bhat, G., Steffen, D.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Effect of proviral insertion of c-*myc* expression in MLV-induced thymomas.

Steffen, D.L., Donahue, J.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Effect of proviral insertion on methylation of the c-*myc* gene in MLV-induced lymphomas.

Taparowsky, E.,[1] Farina, S.,[2] Parsons, J.T.,[2] [1]Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana; [2]Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Cooperative transformation of C3H/10T1/2 cells by the activated c-Ha-*ras* and MC29 *gag-myc* oncogenes—Identification of structural mutations in *myc* that affect focus formation.

Konieczny, S., Taparowsky, E., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Effect of normal and aberrant oncogene expression on the development of muscle and adipocyte cell lineages in culture.

Bonham, L., Lobelle-Rich, P., Henderson, L.A., Levy, L.S., Dept. of Microbiology and Immunology, Tulane Medical School, New Orleans, Louisiana: Biological activity of *myc*-FeLV in early-passage feline cells in vitro.

Lamph, W.W., Raymond, V., Schweighofer, K., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Human c-*fos* cDNA—Analysis of its transforming potential or transformation by human c-*fos* cDNA.

Teich, N.,M., Harrison, M.A., Rowe, J., Charles, G.S., Imperial Cancer Research Fund, London, England: An oncogene interruption in a nonviral murine osteosarcoma.

Boettiger, D., Olsen, M., University of Pennsylvania, Philadelphia: Differentiation-dependent transformation of cells in the monocyte lineage by v-*myb*.

Klempnauer, K.-H., Biedenkapp, H., Sippel, A.E., ZMBH, University of Heidelberg, Federal Republic of Germany: Specific DNA binding by the protein encoded by the retroviral oncogene v-*myb*.

Peters, C.W.B., Sippel, A.E., Vingron, M., Klempnauer, K.-H., ZMBH, University of Heidelberg, Federal Republic of Germany: *Drosophila* and vertebrate *myb* proteins share two conserved regions, one of which binds DNA.

Ibanez, C.E.,[1] Lipsick, J.S.,[1,2] [1]Dept. of Pathology, University of California School of Medicine, [2]Veteran's Administration Medical Center, San Diego: Nuclear transport of wild-type and mutant v-*myb* oncogene products.

Bordereaux, D., Fichelson, S., Sola, B., Tambourin, P., Gisselbrecht, S., INSERM, Hôpital Cochin, Paris, France: *fim*-3—A third integration region of Fr-MLV in myeloblastic leukemias.

Tainsky, M.A., Chiao, P., Yim, S.O., Dept. of Tumor Biology, M.D. Anderson Hospital and Tumor Institute, University of Texas System Cancer Center, Houston: A human cell system for functional analysis of human oncogenes.

Niman, H.L.,[1] Liu, C.-H.,[2] Ludwig, D.,[2] Stuenkel, C.A.,[2] Houghten, R.A.,[1] Yen, S.S.C.,[2] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, [2]Dept. of Reproductive Medicine, University of California, San Diego: Sex-associated oncogene- and growth-factor-related proteins in newborn and maternal urine.

Niman, H.L., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Expression of oncogene-related proteins in human tumor extracts.

SESSION 9 POSTER SESSION: *TRANS*-ACTIVATION, IMMUNODEFICIENCY VIRUSES

Chairmen: **W.S. Hayward,** Memorial Sloan-Kettering Cancer Center
C. Dickson, Imperial Cancer Research Fund

Zarbl, H.,[1] Latreille, J.,[2] Jolicoeur, P.,[1] [1]Clinical Research Institute of Montreal, [2]Hotel Dieu Hospital, Quebec, Canada: Isolation of revertants from v-*fos*-transformed fibroblasts.

Dang, C.,[1] van Dam, H.,[2] Lee, W.,[1] [1]Cancer Research Institute, Dept. of Medicine, University of California, San Francisco; [2]Sylvius Laboratory, University of Leiden, The Netherlands: Regions of human c-*myc* protein specifying its nuclear location, DNA binding, and transformation ability.

Farina, S.F., Heaney, M.L., Huff, J.L., Parsons, J.T., Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Analysis of structurally altered *gag-myc* proteins.

Cleveland, J.,[1] Huleihel, M.,[1] Bressler, P.,[2] Siebenlist, U.,[2] Rapp, U.,[1] National Institutes of Health, [1]LVC, NCI, [2]LIR, Bethesda, Maryland: Role of *myc* in the expression regulation of *myc* family proto-oncogenes.

Bender, T.P.,[1] Thompson, C.B.,[2] Kuehl, W.M.,[1] [1]NCI, Bethesda, Maryland; [2]Howard Hughes Institute, University of Michigan, Ann Arbor: Differential expression of c-*myb* mRNA is regulated by a block to transcription elongation and is associated with different patterns of chromatin DNase hypersensitivity in murine B lymphoid tumors.

Kanter, M.R.,[1] Smith, R.E.,[2] Hayward, W.S.,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Colorado State University, Fort Collins: Rapid tumor induction by integration of EU-8 virus into the chicken c-*myb* locus.

Fulton, R.,[1] Onions, D.,[2] McFarlane, R.,[1] Tzavaris, T.,[1] Stewart, M.,[1] Neil, J.C.,[1] [1]Beatson Institute for Cancer Research, Bearsden, [2]Dept. of Veterinary Pathology, University of Glasgow, Scotland: Retroviral transduction of T-cell antigen receptor β-chain and *myc* genes.

Gisselbrecht, S., Fichelson, S., Sola, B., Bordereaux, D., Tambourin, P., INSERM, Hôpital Cochin, Paris, France: Transcriptional activation of the *fim*-2 gene by retroviral integration in murine myeloblastic leukemias.

Dickson, C., Smith, R., Moore, R., Thurlow, J., Peters, G., Imperial Cancer Research Fund, London, England: Transcriptional complexity and differential expression of the *int*-2 gene in embryonal carcinoma cells and MMTV-induced mammary tumors.

Isfort, R., Lunsford, D., Schultz, A., Blair, D., Ihle, J.N., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Chromosomal rearrangement and Mo-MLV insertional activation of the c-*ras*H locus in a T-cell lymphoma.

SESSION 8 *TRANS*-ACTIVATION

Chairmen: **I.S.Y. Chen,** University of California, Los Angeles
C. Rosen, Dana-Farber Cancer Institute

Yoshida, M., Inoue, J., Fujisawa, J., Seiki, M., Cancer Institute, Tokyo, Japan: pX sequences of HTLV-I confer transcriptional and posttranscriptional regulations for the viral gene expression.

Cann, A.J., Wachsman, W., Chen, I.S.Y., Division of Hematology-Oncology, University of California School of Medicine, Los Angeles: Mechanism of *trans*-activation by the HTLV *x* protein.

Rosenblatt, J.D., Cann, A.J., Shah, N.P., Wachsman, W., Smalberg, I., Chen, I.S.Y., Division of Hematology-Oncology, University of California School of Medicine, Los Angeles: The alternate open reading frame of HTLV-II pX gene mRNA encodes an inhibitor of *trans*-activation.

Shimotohno, K., Takano, M., Ohta, M., Shima, H., Akagi, T., Nyunoya, H., Okamoto, T., Sugimura, R., National Cancer Center Research Institute, Tokyo, Japan: Structure and function of the *x* gene products of HTLV.

Wachsman, W.,[1] Nyborg, J.K.,[2] Chen, I.S.Y.,[1] Dynan, W.S.,[2] [1]Division of Hematology-Oncology, University of California School of Medicine, Los Angeles; [2]Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Specific binding of nuclear factors to the HTLV-I LTR – Implications for proviral *trans*-activation.

Boros, I., Jeang, K.-T., Giam, C.Z., Brady, J., Khoury, G., Laboratory of Molecular Virology, NCI, NIH, Bethesda, Maryland: Identification of cellular proteins interacting with specific regions of the HTLV-I LTR.

Yoshinaka, Y.,[1] Katoh, I.,[2] Ikawa, Y.,[2] [1]Japan Immunoresearch Laboratories Co., Ltd., Takasaki, [2]Laboratory of Molecular Oncology, Tsukuba Life Science Center, Japan: *trans*-Activator and LTR-binding proteins of BLV-infected cells.

Okamoto, T.,[1] Akagi, T.,[1] Shima, H.,[1] Josephs, S.F.,[2] Arya, S.K.,[2] Benter, T.,[2] Shimotohno, K.,[1] Miwa, M.,[1] Wong-Staal, F.,[2] Sugimura, T.,[1] [1]National Cancer Center Research Institute, Tokyo, Japan; [2]NCI, Bethesda, Maryland: Super-*trans*-activation of HTLV-III expression by a transcription factor induced in HTLV-I-transformed cells.

Cullen, B.R., Hauber, J., Dept. of Molecular Genetics, Hoffmann-La Roche, Nutley, New Jersey: Mechanism of action of the HIV *trans*-acting gene product *tat*.

Rosen, C.,[1] Terwilliger, E.,[1] Sodroski, J.,[1] Haseltine, W.,[2] [1]Laboratory of Biochemical Pharmacology, Dana-Farber Cancer Institute, [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Selective regulation of HIV/HTLV-III gene expression by the *art* gene product is dependent on positive and negative intragenic elements.

Wright, C.M., Felber, B.K., Tse, A., Pavlakis, G.N., NCI-Frederick Cancer Research Facility, Frederick, Maryland: *cis*- and *trans*-acting sequences involved in the *trans*-activation of HIV.

Viglianti, G.A., Mullins, J.I., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Differential requirements for *trans*-activation by STLV-III/HTLV-IV and HIV-I.

J. Coffin

SESSION 9 POSTER SESSION: *Trans*-activation, Immunodeficiency Viruses

Rosen, C.,[1] Park, R.,[1] Sodroski, J.,[1] Haseltine, W.,[2] [1]Dana-Farber Cancer Institute, Dept. of Pathology, Harvard Medical School, [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Nuclear factors bind to HTLV-I LTR sequences responsive to the HTLV-I *tat* gene product.

Giam, C.Z., NCI, National Institutes of Health, Bethesda, Maryland: Purification and characterization of the HTLV-I p40x protein overproduced in *E. coli*.

Derse, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Regulation of BLV gene expression by p38(X-BL) and p18(X-BL).

Ferguson, B., Strehl, L., Bacheler, L., Hendrickson, E., Rayner, M., Colberg-Poley, A., Whealy, M., Ruger, R., Enquist, L., Petteway, S., E.I. du Pont de Nemours & Co., Wilmington, Delaware: HIV *tat*/LTR-mediated control of gene expression.

Felber, B.K., Cladaras, C., Cladaras, M.H., Pavlakis, G.N., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Splicing regulation of HIV.

Terwilliger, E.,[1] Campbell, K.,[1] Sodroski, J.,[1] Haseltine, W.,[2] Rosen, C.,[1] [1]Dana-Farber Cancer Institute, Dept. of Pathology, Harvard Medical School, [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Replication and cytopathic potential of HIV proviruses with *art* gene mutations.

Bohan, C., York, D., Srinivasan, A., Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia: Activation of HIV-LTR-directed expression by tumor promoter, corticosteroids, and sodium butyrate.

Bohan, C.,[1] Luciw, P.,[2] Srinivasan, A.,[1] [1]Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia; [2]University of California, Davis: *trans*-Activation of HIV by herpesviruses—Mutational approach to identify the target sequences.

Hess, J.L., Small, J.A., Clements, J.E., Depts. of Neurology and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Mutational analysis of sequences controlling visna virus transcription and response to viral *trans*-activation.

Mazarin, B., Gourdou, I., Querat, G., Sauze, N., Vigne, R., Faculte de Medicine, Marseille, France: Transcription of visna virus during its lytic cycle—Evidence for a sequential early and late gene expression.

Dorn, P.L., Casey, J.W., Derse, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Identification of EIAV LTR sequences that mediate virus-specific *trans*-activation.

Thielan, B.J.,[1] Hunter, E.,[2] Desrosiers, R.C.,[3] Ratner, L.,[1] [1]Depts. of Medicine and Microbiology and Immunology, Washington University, St. Louis, Missouri; [2]Dept. of Microbiology, University of Alabama, Birmingham; [3]Dept. of Microbiology, New England Primate Research Center, Southborough, Massachusetts: *trans*-Activation of LTR-sequence-mediated gene expression is not a property of type-D retroviral replication.

Albert, J.,[1] Bredberg, U.,[2] Chiodi, F.,[1] Böttiger, B.,[2] Fenyö, E.M.,[1] Ljunggren, K.,[1] Norrby, E.,[1] Biberfeld, G.,[2] [1]Dept. of Biology, Karolinska Institute, [2]Dept. of Immunology, National Bacteriological Laboratory, Stockholm, Sweden: Immunological and biological characteristics of four West African HIV isolates.

Jendis, J.B.,[1] Bächi, T.,[2] Schüpbach, J.,[1] [1]Swiss Retrovirus Reference Laboratory, [2]Institute of Immunology and Virology, University of Zurich, Switzerland: Characterization of a novel human retrovirus isolated from a healthy Swiss blood donor.

Chiodi, F., Fenyö, E.M., Albert, J., Asjö, B., Dept. of Virology, Karolinska Institute, Stockholm, Sweden: HIV isolates differ in replication potential in vitro.

Clouse, K.A.,[1] Justement, J.,[2] Weck, K.,[2] Rabson, A.,[2] Linette, J.,[1] Fauci, A.S.,[2] Folks, T.M.,[2] [1]Georgetown University, Washington, D.C.; [2]NCI, National Institutes of Health, Bethesda, Maryland: Cytokine regulation of HIV expression in a chronically infected promonocyte clone.

Rasheed, S.,[1] Norman, G.L.,[1] Su, S.,[1] Gill, P.S.,[2] Levine, A.,[2] Depts. of [1]Pathology, [2]Internal Medicine, University of Southern California School of Medicine, Los Angeles: High expression of HIV-p24 protein and lack of anti-p24 antibodies as predictors for the development of AIDS.

Pavlakis, G.N., Felber, B.K., NCI-Frederick Cancer Research Facility, Frederick, Maryland: A simple quantitative assay for the detection of infectious HIV.

Jacquemin, P., Dept. of Virology, Institut Pasteur du Brabant, Brussels, Belgium: Enhanced membrane expression of an endogenous glycoprotein with reverse-transcriptase-related antigenic determinants in HTLV- and HIV-infected cells.

Hirsch, V., Riedel, N., Kornfeld, H., Mullins, J.I., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Genome organization of STLV-III_{AGM}.

Overbaugh, J.M.,[1] Hoover, E.A.,[2] Quackenbush, S.L.,[2] Donahue, P.R.,[1] Mullins, J.I.,[1] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; [2]Dept. of Pathology, Colorado State University, Fort Collins: Feline AIDS viruses exhibit a spectrum of pathogenic effects in vitro and in vivo.

Hoover, E.A.,[1] Mullins, J.I.,[2] Quackenbush, S.L.,[1] Overbaugh, J.M.,[2] Donahue, P.R.,[2] [1]Dept. of Pathology, Colorado State University, Fort Collins; [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Molecularly cloned FeLVs induce the fatal immunodeficiency syndrome and are T-cell-cytopathic in vitro.

Poss, M.L.,[1] Hoover, E.A.,[1] Mullins, J.I.,[2] [1]Dept. of Pathology, Colorado State University, Fort Collins; [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Analysis of feline AIDS virus glycoproteins.

Bilello, J.A., Tracey, E., Benjers, B., Hoffman, P.M., VA Medical Center and University of Maryland, Baltimore: Effects of AZT on the replication of Cas-Br-M MLV in vitro and in vivo.

Goudsmit, J.,[1] Smit, L.,[1] Gibbs, C.J., Jr.,[2] Asher, D.M.,[2] Gajdusek, D.C.,[2] [1]Academic Medical Center, Amsterdam, The Netherlands; [2]NINCDS, National Institutes of Health, Bethesda, Maryland: HIV induces antibodies inhibiting CD4-dependent cell fusion by HIV glycoprotein in experimentally infected chimpanzees.

Löwer, J., Löwer, R., Kurth, R., Paul-Ehrlich-Institut, Frankfurt, Federal Republic of Germany: Correlation between expression of HTDV and state of differentiation in cell lines established from human germ-cell tumors.

SESSION 10 TISSUE-SPECIFIC EXPRESSION

Chairmen: **A.M. Skalka,** Roche Institute of Molecular Biology
J.M. Coffin, Tufts University School of Medicine

Embretson, J.E., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The 5′ LTR of SNV-derived vectors is not transcribed in mouse cells when a 3′ SNV is present.

Arrigo, S., Carlberg, K., Ryden, T., Beemon, K., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Regulatory element(s) within the ASV genome.

S. Hughes, M. Federspiel

Lenz, J., Okenquist, S., Short, M., Boral, A., Cupelli, L., Morrison, H., Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Cell-type-specific transcription from MLV LTRs in leukemogenesis.

Golemis, E.,[1] Li, Y.,[1] Hartley, J.W.,[2] Hopkins, N.,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]National Institutes of Health, Bethesda, Maryland: Disease specificity of MLVs.

Gorska-Flipot, I.,[1] Rassart, E.,[2] Jolicoeur, P.,[1] [1]Clinical Research Institute of Montreal, [2]Université du Quebec, Canada: Nuclear factors interacting with the LTR of lymphotropic and fibrotropic MLV.

Buetti, E., Kühnel, B., Sossi, T., Diggelmann, H., Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Regulation of transcription in MMTV and functional relationships between control elements.

Ball, J.K.,[1] Diggelmann, H.,[2] Grossi, G.F.,[2] [1]Dept. of Biochemistry, University of Western Ontario, London, Canada; [2]Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: Type-B retroviruses associated with nonmammary gland tumors have alterations in the U3 region of the LTR.

Hsu, C.-L.L., Fabritius, C., Dudley, J., Dept. of Microbiology, University of Texas, Austin: Structure and function of MMTV LTR variants in T-cell lymphomas.

Grez, M., Hilberg, F., Ostertag, W., Heinrich-Pette-Institut, Abteilung Zellbiologie, Hamburg, Federal Republic of

Germany: Retroviral gene expression in embryonal carcinoma cells.

Valerio, D., Li, C.L., Wamsley, P.M., Verma, I.M., Salk Institute, San Diego, California: Retroviral vectors with recombinant LTRs.

Anklesaria, P., Sakakeeny, M., Greenberger, J., Holland, C., Dept. of Radiation Oncology, University of Massachusetts Medical School, Worcester: Enhancer sequences of a retroviral vector augment gene transfer to multipotent hematopoietic progenitors.

SESSION 11 PATHOGENESIS

Chairmen: **S. Ruscetti,** National Cancer Institute
P. Jolicoeur, Clinical Research Institute of Montreal

Crittenden, L.B., Salter, D.W., USDA-ARS Regional Poultry Research Laboratory, East Lansing, Michigan: Artificial insertion of avian leukosis proviruses into the chicken germ line.

Brown, D.W., Robinson, H.L., Worcestor Foundation for Experimental Biology, Shrewsbury, Massachusetts: Analysis of recombinants of RAV-0 and RAV-1 for associated pathology and tissue tropism.

Brightman, B.K.,[1] Chandy, K.G.,[2] Gupta, S.,[2] Pattengale, P.K.,[3] Fan, H.,[1] Depts. of [1]Molecular Biology and Biochemistry, [2]Medicine, University of California School of Medicine, Irvine; [3]Dept. of Pathology, University of Southern California School of Medicine, Los Angeles: Characterization of lymphoid tumors induced by a recombinant murine retrovirus carrying the avian v-*myc* oncogene – Identification of a novel lymphoid cell type.

Green, P.L., Kaehler, D.A., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Cell transformation and tumor induction by helper-virus-free A-MuLV.

Porrier, Y., Savard, P., Jolicoeur, P., Clinical Research Institute, Montreal, Canada: Abelson lymphomas – Role of the helper virus and identification of a common proviral integration site in these tumors.

Chung, S.-W., Wong, P.M.C., Ruscetti, S., NCI, National Institutes of Health, Bethesda, Maryland: Murine embryonic multipotent hematopoietic progenitors are targets for Abelson virus.

Wolff, L., Ruscetti, S., NCI, National Institutes of Health, Bethesda, Maryland: The envelope gene of the SFFV, when introduced into mice in the absence of other SFFV genes, induces an acute erythroleukemia.

Sitbon, M.,[1] d'Auriol, L.,[2] Perryman, S.,[3] Nishio, J.,[3] Wehrly, K.,[3] Galibert, F.,[2] Chesebro, B.,[3] [1]INSERM, Hôpital Cochin, [2]Centre Hayem, Hôpital Saint-Louis, Paris, France; [3]National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: A single base-pair change in the leader sequence of the *env* gene is responsible for the lower level of hemolytic anemia induced by some strains of Fr-MLV.

Bovenhoff, M.E., de Groene, E.M., Visser, T.P., Dorssers, L. Wagemaker, G., Radiobiological Institute TNO, Rijswijk, The Netherlands: Effect of replication-defective Friend virus on hematopoietic cell differentiation in genetically defective W/W^v mice.

Holland, C.A.,[1] Chattopadhyah, S.,[2] Thomas, C.,[3] Koehne, C.,[3] O'Donnell, P.V.,[4] [1]Dept. of Radiation Oncology, University of Massachusetts Medical School, Worcester; [2]NIAID, National Institutes of Health, Bethesda, Maryland; [3]University of Virginia, Charlottesville; [4]Memorial Sloan-Kettering Cancer Center, New York, New York: Localization of the lesion that blocks the acceleration of leukemia in AKR mice by an MCF virus.

Fan, H., Davis, B.R., Brightman, B.K., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Evidence for two infection events in leukemogenesis by Mo-MLV.

Tsichlis, P.N., Shinton, S., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Provirus integration and clonal cell growth in the early and late states of Mo-MLV-induced lymphomagenesis in the rat.

SESSION 12 POSTER SESSION: TISSUE-SPECIFIC EXPRESSION, PATHOGENESIS

Manley, N.,[1] Mbangkollo, D.,[1] Hartley, J.W.,[2] Hopkins, N.,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]NCI, National Institutes of Health, Bethesda, Maryland: Disease specificity of MLVs – Differences in cellular protein-binding sites in the region responsible for targeting.

Runnels, J., Rosenberg, N., Dept. of Pathology, Tufts University Medical School, Boston, Massachusetts: M-MuLV immortalizes B cells in vitro.

Rassart, E.,[1] Bergeron, R.,[1] Paquette, Y.,[2] Jolicoeur, P.,[2] [1]Dept. Sciences Biologiques, Université du Québec, [2]Institut de Recherches Cliniques de Montréal, Canada: Mapping the viral determinant blocking the replication of the BL/VL$_3$ RadLV on fibroblasts.

White, J.H., McCune, J.M., Weissman, I.L., Kaneshima, H., Sen Majumdar, A., Lieberman, M., Stanford University School of Medicine, California: Tissue-specific expression driven by the RadLV LTR.

Sen Majumdar, A., Kaneshima, H., White, J.H., Weissman, I.L., Leiberman, M., Stanford University School of Medicine, California: Monoclonal antibodies against radiation-induced lymphomas of C57BL/Ka mice.

Kaneshima, H., Sen Majumdar, A., Heimfeld, S., Okada, C.Y., Weissman, I.L., Leiberman, M., Stanford University School of Medicine, California: Establishment of culture conditions to study preleukemic thymocytes from irradiated C57BL/Ka mice.

King, S.R.,[1] Berson, B.J.,[2] Risser, R.,[2] [1]Dept. of Immunology and Microbiology, Wayne State University, Detroit, Michigan; [2]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Functional analysis of the germ-line ecotropic murine leukemia provirus of C57BL/6 mice.

Chung, S.-W., Wolff, L., Ruscetti, S., NCI, National Institutes of Health, Bethesda, Maryland: Sequences responsible for the altered Epo responsiveness of SFFVp-infected cells are localized to a 120-bp transmembrane portion at the carboxyl terminus of the envelope gene.

Nagasu, N., Amanuma, H., Ikawa, Y., Institute of Physical and Chemical Research, Ibaraki, Japan: Reconstructed, nonleukemogenic Fr-SFFV recovers its leukemogenicity in 2 months when injected into newborn mice.

Pinter, A., Honnen, W., Sarafoglue, V., Laboratory of Retroviral Biology, Public Health Research Institute, New York, New York: Characterization of secreted gene products of Fr-SFFV involved in leukemogenesis.

Friedrich, R.W., Koch, W., von Maydell, U., Schrewe, H., Institute of Medical Virology, University of Giessen, and Institute of Immunobiology, University of Freiburg, Federal Republic of Germany: Nucleotide sequence of the Fr-MLV genome.

Geib, R.W., Seaward, M.B., Stevens, M.L., Dept. of Microbiology, Terre Haute Center for Medical Education, Indiana University School of Medicine: Studies on EY10/B6—A strain of Friend virus adapted to replicate in resistant mice.

Stoye, J.P., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Genetics of endogenous MLVs.

Morrey, J.D., Evans, L.H., National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Analysis of different populations of polytropic viruses induced by Friend and Moloney MLVs.

Kahn, A.S., Theodore, T.S., NIAID, National Institutes of Health, Bethesda, Maryland: The mouse genome contains at least two distinct LTR progenitors to MCF MLVs.

Ch'ang, L.-Y.,[1] Myer, F.E.,[2] Yang, D.M.,[2] Koh, C.K.,[2] Yang, W.K.,[2] [1]Dept. of Microbiology, University of Tennessee, Knoxville; [2]Biology Division, Oak Ridge National Laboratory, Tennessee: Promoter and enhancer elements in the LTRs of endogenous MLV-related proviral genes.

Nikbakht, K.N., Yang, D.M., Ch'ang, L.-Y., Yang, W.K., Biology Division, Oak Ridge National Laboratories, Dept. of Microbiology, University of Tennessee, Knoxville: Functional characterization of a mouse endogenous MLV-related proviral DNA clone stably transfected into a human fibrosarcoma cell line.

Policastro, P.F., Fredholm, M., Wilson, M.C., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Structure and dispersion of replication-defective, but transcribed, endogenous retroviral loci.

Kiessling, A.A.,[1] Crowell, R.C.,[1] Connell, R.S.,[2] [1]Dept. of Obstetrics and Gynecology, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Anatomy, Oregon Health Sciences University, Portland: Retroviral particles in the male reproductive tract of mice are sperm-associated.

Voytek, P., Kozak, C., NCI, National Institutes of Health, Bethesda, Maryland: Isolation and partial characterization of a novel MLV from the wild-mouse species *M. hortulanus*.

Riedel, N.,[1] Hoover, E.A.,[2] Dornsife, R.E.,[2] Overbaugh, J.M.,[1] Mullings, J.I.,[1] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; [2]Dept. of Pathology, Colorado State University, Fort Collins: Pathogenic and host-range determinants of the feline aplastic anemia retrovirus.

Spodick, D.A.,[1] Ghosh, A.K.,[2] Parimoo, S.,[2] Roy-Burman, P.,[1,2] Depts. of [1]Biochemistry, [2]Pathology, University of Southern California School of Medicine, Los Angeles: Variability within U3 sequences of endogenous feline RD114 LTRs.

Berry, B.T.,[1] Spodick, D.A.,[2] Roy-Burman, P.,[1,2] Depts. of [1]Pathology, [2]Biochemistry, University of Southern California School of Medicine, Los Angeles: Structural and functional characterization of endogenous FeLV LTRs.

Rhoads, D.,[1,2] Kiessling, A.,[2] Kirsch, K.,[1,2] Isselbacher, K.J.,[1,2] Gattoni-Celli, S.,[1,2] [1]Harvard Medical School, [2]GI Unit, Massachusetts General Hospital, Boston: Human endogenous retroviral sequences—Expression in human colon tumors and characterization of a cDNA clone.

Boyce-Jacino, M., Resnick, R., Faras, A., Institute of Human Genetics and Dept. of Microbiology, University of Minnesota, Minneapolis: Analysis of the LTRs and other regulatory elements of the avian endogenous retrovirus family identified in EV-O line chickens.

Vaidya, A.B., Morrisey, J.M., Connors, R.W., Long, C.A., Hahnemann University, Philadelphia, Pennsylvania: Negative regulation of MMTV LTR-linked genes in a mammary epithelial cell line from the C57BL strain.

Heeney, J.L.,[1] Valli, V.E.O.,[2] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Pathology, University of Guelph, Canada: Genotypic and phenotypic characteristics of BLV lymphomas reflect alterations in humoral immunity.

Lagarias, D., Grossman, D., Adamson, L., Radke, K., Dept. of Avian Sciences, University of California, Davis: Early responses of sheep to infection by BLV.

Steiner, B., Johnson, K., Aronoff, R., Linial, M., Fred Hutchinson Cancer Center, Seattle, Washington: Characterization of retrovirus-generated processed pseudogenes.

C. Holland, R. Risser

SESSION 13 IMMUNODEFICIENCY VIRUSES

Chairmen: **J.I. Mullins,** Harvard School of Public Health
P.J. Kanki, Harvard School of Public Health

Strebel, K.,[1] Daugherty, D.F.,[2] Folks, T.M.,[3] Clouse, K.A.,[4] Martin, M.A.,[1] Laboratories of [1]Molecular Microbiology, [2]Immunoregulation, NIAID, National Institutes of Health, Bethesda, Maryland; [3]University of Michigan, Ann Arbor; [4]Georgetown University, Washington, D.C.: Functional analysis of the HIV A (sor) gene product.

Dayton, A.I.,[1] Potz, J.,[1] Dorfman, T.,[1] Walker, B.,[2] Haseltine, W.A.,[1] [1]Dana-Farber Cancer Institute, [2]Massachusetts General Hospital, Boston: HIV requires the integrase gene for normal viral replication.

Kowalski, M.,[1] Potz, J.,[1] Rosen, C.,[1] Terwilliger, E.,[1] Basiripour, L.,[1] Goh, W.C.,[1] Dayton, A.,[1] Haseltine, W.,[2] Sodroski, J.,[1] [1]Dept. of Pathology, Dana-Farber Cancer Institute, Harvard Medical School, [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Structural and functional studies of the HIV envelope glycoprotein.

Somasundaran, M., Robinson, H.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Cytopathic effects of HIV do not correlate necessarily with cell fusion.

Koyanagi, Y.,[1] Miles, S.,[1] Mitsuyasu, R.T.,[1] Merrill, J.E.,[2] Vinters, H.V.,[3] Chen, I.S.Y.,[1] Depts. of [1]Medicine, [2]Neurology, [3]Neuropathology, University of California School of Medicine, Los Angeles: Dual infection of the CNS by HIV with distinct cellular tropisms.

Emerman, M., Guyader, M., Sonigo, P., Clavel, F., Montagnier, L., Alizon, M., Institut Pasteur, Paris, France: Analysis of HIV-2.

Kanki, P.J.,[1] M'Boup, S.,[2] Barin, F.,[3] Denis, F.,[4] Essex, M.,[1] [1]Harvard School of Public Health, Boston, Massachusetts; [2]University of Dakar, Senegal; [3]University of Tours, [4]University of Limoges, France: HTLV-IV and HTLV-III/HIV in West Africa.

Kong, L.,[1] Lee, S.-W.,[1] Hahn, B.H.,[1] Kanki, P.J.,[2] Essex, M.,[2] Shaw, G.M.,[1] [1]University of Alabama, Birmingham; [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Isolation and molecular characterization of a new human retrovirus from West Africa.

Kornfeld, H., Riedel, N., Viglianti, G., Hirsch, V., Mullins, J.I., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: HTLV-IV and its relationship to STLVs and HIVs.

Kanki, P.J.,[1] Eichberg, J.,[2] Dreesman, G.,[2] Essex, M.,[1] [1]Harvard School of Public Health, Boston, Massachusetts; [2]Southwest Foundation of Biomedical Research, San Antonio, Texas: STLV-III_{AGM}-induced immunosuppression in macaques and baboons.

Donahue, P.R.,[1] Hoover, E.A.,[2] Overbaugh, J.M.,[1] Quackenbush, S.L.,[2] de Noronha, C.M.C.,[1] Mullins, J.I.,[1] [1]Harvard School of Public Health, Boston, Massachusetts; [2]Colorado State University, Fort Collins: Pathogenic determinants of the feline AIDS virus.

Yeast Cell Biology

August 11 — August 16

ARRANGED BY

Amar Klar, Cold Spring Harbor Laboratory
Paul Nurse, Imperial Cancer Research Fund
Randy W. Schekman, University of California, Berkeley

268 participants

The main meeting of Yeast Genetics and Molecular Biology was previously held every other year at Cold Spring Harbor Laboratory. The success of these meetings contributed to the speed with which this field has progressed. However, there was a price to pay for this success. The Cold Spring Harbor facilities could no longer accommodate all of the applicants who wished to attend the proceedings, and so the main meeting will now be held elsewhere. Nevertheless, it was decided that a meeting on a yeast subtopic would be appropriate for the laboratory to continue with in the future in alternate years. This year's topic was Yeast Cell Biology. The areas of cell-cycle controls, developmental choices, cytoskeleton and cell structure, protein secretion and targeting were emphasized. The meeting was very successful; it was a universal consensus of the participants that such a meeting should be continued at Cold Spring Harbor Laboratory.

This meeting was supported in part by the Anheuser-Busch Companies; Gist-Brocades NV; Labatt Brewing Company Ltd.; Miller Brewing Company; Stroh Brewery Company; and ZymoGenetics, Inc.

SESSION 1 CYTOSKELETON

Chairman: J. Pringle, University of Michigan

Adams, A., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic analysis of the actin-based cytoskeleton of yeast.

Drubin, D., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Identification of yeast actin-binding proteins—Evidence for in vivo interactions.

Liu, H., Bretscher, A., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Purification and characterization of a tropomyosin-like protein from *S. cerevisiae* and cloning of its gene.

Wertman, K.F., Drubin, D., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Myosin-like molecules in *S. cerevisiae*.

Katz, W., Solomon, F., Massachusetts Institute of Technology, Cambridge: Molecular analysis of tubulin function.

Schroeder-Lorenz, A.,[1] Marks, J.,[2] Hyams, J.,[2] Fantes, P.,[1] [1]Dept. of Zoology, University of Edinburgh, Scotland; [2]Dept. of Botany and Microbiology, University College, London, England: *Ben4* mutations of *S. pombe* interact with the actin and β-tubulin genes.

Uno, I., Ishikawa, T., Institute of Applied Microbiology, University of Tokyo, Japan: Regulation of cytoskeletal structure by cAMP cascade.

Schmitt, H.D., Wagner, P., Haubruck, H., Gallwitz, D., Dept. of Molecular Genetics, Max-Planck Institute for Biophysical Chemistry, Gottingen, Federal Republic of Germany: The *ras*-related YPT protein in yeast—Mutations affect the regulation of intracellular Ca^{++} and cause cytoskeletal lesions.

SESSION 2 ORGANELLE STRUCTURE, INTERACTIONS, AND DYNAMICS

Chairman: E. Jones, Carnegie Mellon University

Shio, H., Small, G.M., Lazarow, P.B., Rockefeller University, New York, New York: Immunofluorescence detection of one or a few tiny peroxisomes in all glucose-repressed *Candida tropicalis* cells.

Wright, R.M., Poyton, R.O., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Intergenomic signaling in yeast.

Segev, N., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The yeast *RAS*-related gene, *YPT1*, is involved in bud growth and secretion.

Franzusoff, A.J., Schekman, R., Dept. of Biochemistry, University of California, Berkeley: Phosphorylation of an essential component of the yeast secretory pathway.

Chappell, T.G., Warren, G., Dept. of Biochemistry, University of Dundee, Scotland: Biogenesis and partitioning of the Golgi apparatus in *S. pombe*.

Preston, R.A., Kirkpatrick, D., Jones, E.W., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Characterization of *PEP3*, an essential gene required for vacuole biogenesis.

Weisman, L.S., Bacallao, R., Guthrie, B., Wickner, W., Molecular Biology Institute, University of California, Los Angeles: Vacuole dynamics in the yeast *S. cerevisiae*.

Novick, P., Salminen, A., Goud, B., Walworth, N., Yale University School of Medicine, New Haven, Connecticut: A *ras*-like GTP-binding protein is required for a post-Golgi event of the yeast secretory pathway.

Kambouris, N.G.,[1] Martin, W.H.,[1] Fromer, E.S.,[1] Snyder, S.,[1] Hamman, H.C.,[1] Creutz, C.E.,[1] Smith, M.M.,[2] Depts. of [1]Pharmacology, [2]Microbiology, University of Virginia, Charlottesville: Ca^{++}-dependent membrane-binding proteins from yeast—Possible mediators of exocytosis.

SESSION 3 POSTER SESSION

Cytoskeleton

Huffaker, T., Thomas, J., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Role of microtubules in yeast.

Kim, J.,[1] Fink, G.,[1,2] [1]Dept. of Biology, Massachusetts Institute of Technology, [2]Whitehead Institute, Cambridge: Kar^--enhancing mutations (*kem*) in yeast.

Lillie, S.H., Brown, S.S., Dept. of Anatomy and Cell Biology, University of Michigan, Ann Arbor: Characterization of putative actin-binding proteins from the yeast *S. cerevisiae*.

Stearns, T., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Visualizing yeast mitochondria in vivo—The role of actin and tubulin in organization and movement.

Mating

Chant, J., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Mutants with altered spatial control of bud initiation.

Cross, F., Fred Hutchinson Cancer Research Center, Seattle, Washington: Dominant α-factor resistance mutations.

Jahng, K.-Y., Ferguson, J., Reed, S., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Isolation and characterization of mutations capable of allowing an *ste2*-deletion mutant strain to conjugate.

Morgan, B.A., Mittman, B.A., Smith, M.M., Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Identification of a histone H4 mutation that affects mating-type expression.

Whiteway, M., Hougan, L., Thomas, D.Y., NRC Biotechnology Research Institute, Montreal, Canada: Expression of MFα1 in MAT**a** cells supersensitive to α-factor.

Courchesne, W.E., Kunisawa, R., Thorner, J., Dept. of Biochemistry, University of California, Berkeley: Two genes (*SST2* and *SSS1*) involved in recovery from pheromone-induced growth arrest in *S. cerevisiae*.

Cell Cycle

Singer, R.A., Hanic-Joyce, P.J., Johnston, G.C., Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada: Distinct effects of *prt1* mutations on translation and on control of cell proliferation.

Levin, L., Kuret, J., Powers, S., Michaeli, T., Cameron, S., Wigler, M., Zoller, M., Cold Spring Harbor Laboratory, New York: Suppression of *ras*^{2} by disruption of subunit interactions of cAMP-dependent protein kinase.

Lisziewicz, J.,[1] Agoston, D.,[2] Förster, H.H.,[1] Küntzel, H.,[1] [1]Abteilung Chemie, Max-Planck-Institut für experimentelle Medizin, [2]Abteilung Neurochemie, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, German Federated Republic: Overexpression of cAMP-dependent protein kinase in a *cdc25* mutant containing a plasmid-amplified suppressor gene.

Powers, S., Wigler, M., Cold Spring Harbor Laboratory, New York: Dominant conditionally lethal *RAS2* alleles that interfere with the function of *RAS* and *CDC25* genes.

Kim, W.-J., Jong, A.Y.-S., Dept. of Pediatrics and Microbiology, University of Southern California, Davis: Yeast *SOC8-1* gene that suppresses *cdc8* mutation possesses deoxypyrimidine monosphosphate kinase activity.

Baum, P., Goetsch, L., Byers, B., Dept. of Genetics, University of Washington, Seattle: Integration of spindle-pole-body duplication with cell division by ESP1.

Crouzet, M., Dulau, L., Aigle, M., Laboratoire de Génétique, Université de Bordeaux, Talence, France: Yeast mutants impaired in stationary-phase entry.

Brizuela, L., Draetta, G., Beach, D., Cold Spring Harbor Laboratory, New York: Physical association between the *cdc2*$^+$ and the *suc1*$^+$ gene products in fission yeast.

Colasanti, J., Bruschi, C.V., Dept. of Microbiology, East Carolina University, Greenville, North Carolina: Molecular analysis of *cdc6* gene function in *S. cerevisiae*.

Draetta, G., Brizuela, L., Beach, D., Cold Spring Harbor Laboratory, New York: Human p34-p13 complex—Structural homology with p34^{cdc2} and p13^{suc1} cell-cycle regulators of yeast.

Drebot, M.A., Veinot-Drebot, L.M., Johnston, G.C., Singer, R.A., Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada: Stationary phase is a distinct developmental state.

Hagan, I.,[1] Riddle, P.,[2] Nurse, P.,[2] Hyams, J.S.,[1] University College London, [2]Imperial Cancer Research Fund, London, England: The mitotic period of the fission yeast *S. pombe* is extended in cells dividing at an increased size but maintained in cells dividing at a reduced size.

Högel, A., Müller, I., Institut für Entwicklungsphysiologie, Köln, Federal Republic of Germany: Inheritance of life span during the vegetative reproduction of *S. cerevisiae*.

Martegani, E., Baroni, M.D., Vanoni, M., Frascotti, G., Monti, P., Alberghina, L., Dipt. di Fisiologie e Biochimica Generali, Universitá di Milano, Italy: Characterization of the *CDC25* start gene of budding yeast—Functional interrelationship with the *RAS2* gene and modulation of the cell size.

Booher, R., Beach, D., Cold Spring Harbor Laboratory, New York: Isolation of a temperature-sensitive *cdc13* mutation that supresses a cold-sensitive allele of the *cdc2* protein kinase in *S. pombe*.

Prendergast, J.A., Murray, L.E., Rowley, A., Carruthers, D.R., Singer, R.A., Johnston, G.C., Faculty of Medicine, Dalhousie University, Nova Scotia, Canada: New mutations show that many genes are involved in the regulation of yeast cell proliferation.

Shalitin, C., Levy, M., Kenoshi, E., Dept. of Biology, Technion-Israel Institute of Technology, Haifa: Stable binding of yeast *ras*-related proteins to DNA as revealed by immunological cross-reactivities.

Smith, S., Rosamond, J., Dept. of Biochemistry and Molecular Biology, University of Manchester, England: Characterization of a gene whose product is required at two stages of the yeast cell cycle.

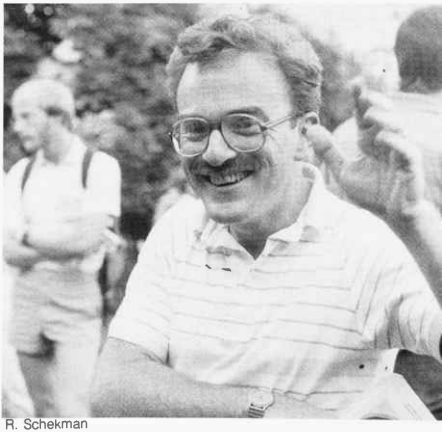
Sporulation and Life Cycle

Fukui, Y., Miyake, S., Yamamoto, M., Institute of Medical Science, University of Tokyo, Japan: Genes closely related to *ras1* either functionally or structurally in *S. pombe*.

Nadin-Davis, S.A., Nasim, A., Division of Biological Sciences, National Research Council, Ontario, Canada: A putative protein kinase encoded by the *Byr1*$^+$ gene bypasses the sporulation defect of *ras1*$^-$ fission yeast.

Jakubowski, H., Dept. of Microbiology, UMDNJ-New Jersey Medical School, Newark: Cell-density-dependent interactions in yeast sporulation.

Watanabe, Y., Iino, Y., Furuhata, K., Yamamoto, M., Institute of Medical Science, University of Tokyo, Japan: *mei2* gene expression and control of meiosis in *S. pombe*.



R. Schekman

J. Thorner, B. Stillman, J.D. Watson

Secretion and Localization

Jigami, Y.,[1] Iwakura, M.,[2] Muraki, M.,[1] Kioke, T.,[3] [1]National Chemical Laboratory for Industry, Tsukuba, [2]Research Institute for Polymers and Textiles, Tsukuba, [3]Rohto Pharmaceutical Co., Ltd., Osaka, Japan: Participation of the vacuole-associated process in the secretion of human lysozyme and bacterial DHFR fusion protein in yeast.

Tipper, D.J., Kane, J., Zhu, Y.S., Zang, X.Y., Kang, Y.S., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Use of β-lactamase fusions to study processing of killer preprotoxin in *S. cerevisiae*.

Silver, P., Chiang, A., Yang, X., Salder, I., Dept. of Biology, Princeton University, New Jersey: Mutations affecting GAL4 nuclear localization.

Silveira, L.A., Schekman, R., Dept. of Biochemistry, University of California, Berkeley: Molecular analysis of clathrin light chain from *S. cerevisiae*.

Nero, D., Farrell, L.B., Gearing, D.P., Braidotti, G., Meltzer, S., Devenish, R.J., Nagley, P., Dept. of Biochemistry, Monash University, Clayton, Victoria, Australia: An imported version of a hydrophobic protein normally made inside mitochondria correctly assembles into the mitochondrial ATPase complex.

Sutherland, M., Bozzato, R., Allelix, Inc, Biochemicals Division, Ontario, Canada: Effects of signal-sequence alterations on the level of protein secretion in yeast.

Sidhu, R.S., Bollon, A.P., Wadley Institutes of Molecular Medicine, Dallas, Texas: *GAL1-PHO5* gene fusions—An internal signal sequence can be functional for protein secretion.

Cohen, G., Hartig, A., Ruis, H., Institut für Allgemeine Biochemie der Universität Wien and Ludwig Boltzmann-Forschungsstelle für Biochemie, Vienna, Austria: Comparison of structures of peroxisomal and nonperoxisomal catalases of *S. cerevisiae*.

Matoba, S., Fukayama, J., Ogrydziak, D., Institute of Marine Resources, University of California, Davis: Precursors and processing of the alkaline extracellular protease of *Yarrowia lipolytica*.

Preuss, D., Botstein, D., Massachusetts Institute of Technology, Cambridge: Suppression of mutations in the invertase signal sequence.

Newman, A., Ferro-Novick, S., Dept. of Cell Biology, Yale University, New Haven, Connecticut: Characterization of new mutants in the early part of the yeast secretory pathway isolated by a [^{3}H]mannose suicide selection.

Li, Y.Y., Qin, N., Zhao, Z.G., Institute of Genetics, Fudan University, Shanghai, People's Republic of China: Expression of heterologous genes directed by *SUC2* promoter-signal sequence in yeast.

Lehman, D.J., Das, R.C., Kaumeyer, J.F., Biotechnology Group R&D, Miles Laboratories, Inc., Elkhart, Indiana: Expression and secretion of human inter-α-trypsin inhibitor-related polypeptides in *S. cerevisiae*.

Legrain, M., Portetelle, D., Dumont, J., Burny, A., Hilger, F., Chaire de Microbiologie, Faculté des Sciences Agronomiques de l'Etat, Gembloux, Belgium: Expression of the gene coding for the BLV envelope glycoprotein gp51 in *S. cerevisiae*.

Bielefeld, M., Reipen, G., Hollenberg, C.P., Institut für Mikrobiologie, Universität Düsseldorf, Federal Republic of Germany: Analysis of the secretory pathway in *S. cerevisiae* by *SUC2* mutations and fusions.

Dihanich, M.,[1] Benz, R.,[2] Suda, K.,[1] Oppliger, W.,[1] [1]Biocenter, University of Basel, Switzerland; [2]University of Würzburg, Federal Republic of Germany: A yeast mutant lacking mitochondrial porin accumulates an 86-kD protein.

Processing

Atkinson, P.H.,[1] Trimble, R.B.,[2] [1]Albert Einstein College of Medicine, Bronx, [2]Wadsworth Center, New York State Department of Health, Albany: Structure of yeast N-linked oligosaccharide processing intermediates by high-field one- and two-dimensional ^{1}H NMR spectroscopy.

Bourbonnais, Y., Shields, D., Depts. of Anatomy and Structural Biology and Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Pro α-factor–peptide hormone chimeras are proteolytically cleaved by the enzymes encoded by the *STE13* and *KEX2* genes.

Butt, T.R., Khan, M.I., Marsh, J., Ecker, D.J., Crooke, S.T., Smith Kline and French Laboratories, Dept. of Molecular Pharmacology, King of Prussia, Pennsylvania: Ubiquitin-mettallothionein gene fusion—A genetic system for protein-turnover studies.

Chen, J.L., Padmanabha, R., Glover, C.V.C., Dept. of Biochemistry, University of Georgia, Athens: Isolation and sequencing of genomic clones encoding the α-subunit of yeast casein kinase II.

Davis, T.N., Thorner, J., Dept. of Biochemistry, University of California, Berkeley: Vertebrate calmodulin can substitute for yeast calmodulin in vivo.

Alberghina, L., Vai, M., Grandori, R., Popolo, L., Dipt. de Fisiologia e Biochimica Generali, Universita di Milani, Italy: Detection of phosphotyrosine-containing protein modulated by growth conditions in *S. cerevisiae*.

Haselbeck, A., Tanner, W., Institut für Botanik, Universität Regensbur, Federal Republic of Germany: Purification of a GDP-Man—Dol-P mannosyltranferase from yeast and cloning of the corresponding gene.

Jackson, B.J.,[1] Warren, C.D.,[2] Bugge, B.,[2] Robbins, P.W.,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]Laboratory for Carbohydrate Research, Massachusetts General Hospital and Harvard Medical School, Boston: Synthesis of lipid-linked oligosaccharides in *S. cerevisiae*—Man(1,3)ManGlcNAc$_2$ and Man$_1$GlcNAc$_2$ are transferred to protein.

Orlean, P., Robbins, P.W., Massachusetts Institute of Technology, Cambridge: Yeast dol-P-man synthase—Selection of mutants defective in this enzyme and cloning of the gene.

Roitsch, T., Lehle, L., Faculty for Biology and Pre-clinical Medicine, University of Regensburg, Federal Republic of Germany: Post-transcriptional translocation of truncated yeast invertase across the endoplasmic reticulum membrane.

SESSION 4 CELL SURFACE AND MORPHOGENESIS

Chairman: K. Nasmyth, MRC, England

Ford, S.K., Ketcham, S., Haarer, B., Ashcroft, D., Pringle, J.R., Dept. of Biology, University of Michigan, Ann Arbor: On the roles of *CDC3, CDC10, CDC11,* and *CDC12* gene products in cellular morphogenesis during the *S. cerevisiae* cell cycle.

Johnson, D.I., Pringle, J.R., Dept. of Biology, University of Michigan, Ann Arbor: Molecular characterization of the *S. cerevisiae CDC42* gene and its product.

Conklin, D.S., Gustin, M.C., Culbertson, M.R., Kung, C., Laboratory of Molecular Biology, University of Wisconsin, Madison: Toward a mutational study of ion channels in yeast.

Gustin, M.C., Zhou, X.L., Martinac, B., Culbertson, M.R., Kung, C., Laboratory of Molecular Biology, University of Wisconsin, Madison: Ion channels opened by membrane stretch in the yeast *S. cerevisiae*.

Shih, C.-K., Wagner, R., Feinstein, S., Ennulat, C., Neff, N., Memorial Sloan-Kettering Cancer Center, New York, New York: Dominant trifluoperazine-resistant gene *TFP-1* has homology with F^1F^0 ATP synthetase.

Perlin, D.S.,[1] McCusker, J.H.,[2] Haber, J.E.,[2] [1]Dept. of Biochemistry, Public Health Research Institute, New York, New York; [2]Dept. of Biology, Brandeis University, Waltham, Massachusetts: Mutations affecting the plasma membrane-ATPase from *S. cerevisiae*.

Teem, J., Fink, G., Whitehead Institute, Cambridge, Massachusetts: Mutations in the yeast plasma membrane ATPase.

Chvatchko, Y., van Tuinen, E., Riezman, H., Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Pheromone endocytosis and action in yeast.

SESSION 5 MATING

Chairman: A. Klar, Cold Spring Harbor Laboratory

Berlin, V.,[1] Fink, G.R.,[1,2] [1]Whitehead Institute, [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A genetic selection for mutants defective in zygote formation in *S. cerevisiae*.

Ner, S.S., Johnson, J.A., Smith, M., Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: Mutational analysis of operator sequences that bind *MAT* regulatory protein a1 and α2.

Blinder, D., Bouvier, S., Jenness, D., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Mutant *a* cells that show pheromone-independent arrest of cell division.

Klar, A., Cafferkey, R., Flynn, K., Cold Spring Harbor Laboratory, New York: Determination of the yeast cell lineages.

Nasmyth, K., Seddon, A., MRC Laboratory of Molecular Biology, Cambridge, England: Mother-cell-specific *HO* expression.

Jarvis, E., Hagen, D., Bender, A., Sprague, G., Jr., Institute of Molecular Biology, University of Oregon, Eugene: α-specific gene expression—Binding of a *MAT*α1 to upstream activation site elements.

Blumer, K.J., Reneke, J.E., Thorner, J., Dept. of Biochemistry, University of California, Berkeley: Chemical evidence that the *STE2* gene encodes the pheromone-binding component of the α-factor receptor of *S. cerevisiae*.

Chaleff, D.T., Focht, R.J., Rizzo, C.J., E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware: The *STE7* gene encodes a protein kinase.

SESSION 6 CELL CYCLE AND SPORULATION

Chairman: P. Nurse, Imperial Cancer Research Fund

Broek, D., Lee, M., Nurse, P., Imperial Cancer Research Fund, London, England: Role of fission yeast *cdc2* in cell cycle control.

Mendenhall, M.D.[1] Jones, C.A.,[2] Reed, S.I.[1] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California; [2]Leicester Biocentre, University of Leicester, England: Dual regulation of the yeast *CDC28* protein kinase: Cell cycle, pheromone, and nutrient limitation effects.

Booher, R., Molz, L., Beach, D., Cold Spring Harbor Labo-

ratory, New York: Characterization of *mcs* mutants, extragenic suppressors of "mitotic catastrophe" in fission yeast.

Nash, R., Tokiwa, G., Futcher, B., McMaster University, Hamilton, Canada: Characterization of a new Whi mutant of *S. cerevisiae*.

Bahman, M., Rosamond, J., Dept. of Biochemistry and Molecular Biology, University of Manchester, England: Characterization of the *CDC7* gene product as a protein kinase.

Goebl, M.G., Byers, B., Dept. of Genetics, University of Washington, Seattle: The gene products of *CDC4* and *RAD6* are interrelated.

Icho, T., Wickner, R.B., NIDDK, National Institutes of Health, Bethesda, Maryland: The *CDC16* protein has three potential metal-binding nucleic-acid-binding domains.

McLeod, M., Beach, D., Cold Spring Harbor Laboratory, New York: Control of meiosis in fission yeast by the interaction of $p21^{mei3}$ with the $p52^{ran1}$ protein kinase.

SESSION 7A GROWTH CONTROL

Chairman: P. Nurse, Imperial Cancer Research Fund

Cameron, S., Toda, T., Levin, L., Zoller, M., Wigler, M., Cold Spring Harbor Laboratory, New York: Characterization of the cAMP effector system of *S. cerevisiae*.

Field, J.,[1] Nikawa, J.-I.,[1] Broek, D.,[1] Rodgers, L.,[1] MacDonald, B.,[1] Kataoka, T.,[1] Wilson, I.A.,[2] Lerner, R.A.,[2] Wigler, M.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Research Institute of Scripps Clinic, La Jolla, California: Interactions between yeast *RAS* proteins and adenylate cyclase.

Jacquet, M., Boy-Marcotte, E., Camonis, J., Damak, F., Verdier, J.M., Garreau, H., Groupe Information Génétique et Développement, Université Paris, France: Suppressors of the *CDC25* mutation in *S. cerevisiae*—Cloning of a new gene *SCD25* and isolation of a new mutation *ICY1*.

Fields, F.O., Flanagan, C.A., Gimble, F.S., Thorner, J., Dept. of Biochemistry, University of California, Berkeley: Biochemical evidence for components of the phosphatidylinositol pathway in *S. cerevisiae*.

SESSION 7B POSTTRANSCRIPTIONAL TRANSPORT

Chairman: R. Schekman, University of California, Berkeley

Herman, P.K., Emr, S.C., Division of Biology, California Institute of Technology, Pasadena: Localization of the *KEX2* protein.

Raymond, C.K., Rothman, J.H., Stevens, T.H., Institute of Molecular Biology, University of Oregon, Eugene: Molecular analysis of yeast vacuolar protein localization genes.

Dulić, V., Riezman, H., Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Characterization of two genes important in endocytosis by budding yeast.

Sturley, S.L., Palmer, J.D., Bostian, K.A., Division of Biomedicine, Brown University, Providence, Rhode Island: Mutagenic analysis of trans-acting factors that determine the expression of specific immunity to the type-1 killer toxin of *S. cerevisiae*.

SESSION 8 PROTEIN LOCALIZATION

Chairman: R. Schekman, University of California, Berkeley

Li, J.-M.[1] Ellis, S.R.,[1] Hooper, A.K.,[2] Martin, N.C.,[1] [1]Dept. of Biochemistry, University of Texas Health Science Center, Dallas; [2]Dept. of Biological Chemistry, M.S. Hershey Medical Center, Hershey, Pennsylvania: Characterization of mitochondrial and nuclear targeting signals of the *TRM1* gene product.

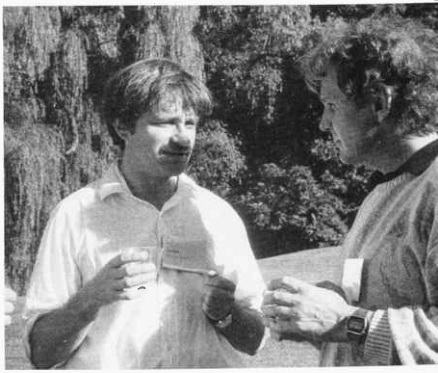
Haldi, M., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Molecular genetic analysis of mitochondrial protein import.

Small, G.M., Szabo, L.J., Lazarow, P.B., Rockefeller University, New York, New York: Targeting of acyl-CoA oxidase to peroxisomes in *Candida tropicalis*.

Hansen, H., Roggenkamp, R., Institut für Mikrobiologie, Universität Düsseldorf, Federal Republic of Germany: Heterologous gene expression in yeast peroxisomes.

Kaiser, C., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Amino-terminal peptide sequences with low information content can direct cytoplasmic proteins to the ER.

Brennwald, P., Liao, X., Holm, K., Porter, G., Wise, J.A., Dept. of Biochemistry, University of Illinois, Urbana: Identification of a *S. pombe* RNA homologous to the 7SL



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component of signal-recognition particle.

Deshaies, R., Schekman R., Dept. of Biochemistry, University of California, Berkeley: Characterization of *S. cerevisiae* mutants defective in protein translocation.

Ferro-Novick, S., Ruohola, H., Bangs, J., Dept. of Cell Biology, Yale University, New Haven, Connecticut: A protein on the cytoplasmic surface of the ER membrane functions at a late stage of protein transport into the ER lumen.

SESSION 9 POSTER SESSION

Proteins

Das, G.,[1] Mann, K.,[1] Fingar, S.,[1] Sherman, F.,[2] Depts. of [1]Biochemistry, [2]Biophysics, University of Rochester Medical Center, New York: Structure and function of yeast iso-1-cytochrome c studied using alterations in the structural gene *CYC1*.

Karwan, R., Wintersberger, U., Institute for Tumorbiology and Cancer Research, University of Vienna, Austria: In addition to RNase H (70), two other proteins of *S. cerevisiae* exhibit ribonuclease-H activity.

Moore, C.,[1,2] Jones, C.,[1] Wall, L.,[1] [1]University of Rochester, [2]City University of New York Medical Schools, New York: Deoxyribonuclease-like activities of structurally related, low-molecular-weight (M^r = ~1500) glycopeptides exhibited preferential internucleosomal chromatin cleavage.

Marguet, D., Lauquin, G.J.-M., Labortorie Physiologie Cellulaire Faculté des Sciences de Luminy, Université Aix-Marseille, France: The *SRP* gene—Cloning and characterization of a new family of repetitive elements homologous to the *SRP* gene.

Karwan, R., Wintersberger, U., Institute for Tumorbiology and Cancer Research, University of Vienna, Austria: In addition to RNAse H(70) two other proteins of *S. cerevisiae* exhibit ribonuclease H Activity.

Gralla, E.B., Bermingham-McDonogh, O., Valentine, J.S., Dept. of Chemistry and Biochemistry, University of California, Los Angeles: The Cu, Zn superoxide dismutase gene of *S. cerevisiae*—Cloning, sequencing, and initial characterization.

Fraundorfer, F.-J., Kuehne, C., Wintersberger, U., Institute of Tumorbiology-Cancer Research, Dept. of Molecular Biology, Vienna, Austria: Cloning of a single gene coding for both ribonuclease H 55 and ribonuclease H 42 in *S. cerevisiae* (RNH 55).

Minasi, L.-A.E., Willsky, G.R., Dept. of Biochemistry, State University of New York, Buffalo: Vanadate-stimulated NADH oxidation activity associated with *S. cerevisiae* plasma membranes.

Mortitz, M., Rotenberg, M., Woolford, J., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Formation of "halfmer" polyribosomes upon depletion of ribosomal protein 39.

Chromosomes

Haggren, W., Burgum, A., Kolodrubetz, D., Dept. of Microbiology, University of Texas Health Science Center, San Antonio: A gene encoding an HMG1-like protein has an essential function in yeast.

Thoma, F., Institut für Zellbiologie, ETH-Hönggerberg, Zürich, Switzerland: Regulation of nucleosome positioning in yeast plasmid chromatin.

Cottarel, G., Funk, M., Jiang, W., Wenick, P., Jager, D., Gegemann, J.H., Philippsen, P., Institut fur Mikrobiologie und Molekularbiologie, Giessen, Federal Republic of Germany: Functional analysis of mutated centromeres.

Allshire, R.,[1] Fantes, P.,[2] [1]MRC CAPCU, Western General Hospital, [2]Dept. of Zoology, Edinburgh, Scotland: Maintenance of a fission yeast chromosome in mouse cells.

Resnick, M.,[1] Westmoreland, J.,[1] Harwell, E.,[2] Bloom, K.,[2] [1]Cellular and Genetic Toxicology Branch, National Institutes of Environmental Health Science, Research Triangle Park, [2]Dept. of Biology, University of North Carolina, Chapel Hill: Heterogeneity in copy number of centromere plasmids in yeast.

Bonaduce, M.J., Mittman, B.A., Dept. of Biology, University of Richmond, Virginia: Analysis of a sodium-bisulfite-generated mutation of histone H4 in yeast.

Chan, C., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: New screens for genes and mutations that affect chromosome segregation in yeast.

Hadfield, C., Leicester Biocentre, University of Leicester, England: Probes for determining chromosome ploidy in yeast.

Pifer, M.L., Karpov, V.L., Institute of Molecular Biology, USSR Academy Sciences, Moscow: Investigation of *S. cerevisiae* centromere structure by protein-DNA cross-linking.

Steensma, H.Y.,[1] de Jonge, P.,[1] Linnekamp, M.,[1] Kaback, D.B.,[2] Dept. of Microbiology and Enzymology, Delft University of Technology, The Netherlands; [2]Dept. of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark: Molecular organization of chromosome I from *S. cerevisiae*—Physical map of the DNA molecule.

Villadsen, I.S., Riis, P., Dept. of Microbiology, Technical University of Denmark: Characterization and cloning of a new nuclear division cycle mutant of the yeast *S. cerevisiae*.

Wakem, L.P.,[1] Bulgac, E.,[2] Hicks, J.,[2] Sherman, F.,[1] [1]University of Rochester, New York; [2]Scripps Clinic, La Jolla, California: A new method for the chromosomal assignment of mutations in yeast.

Gene Structure and Expression

Ye, Z.H., Bhattacharjee, J.K., Dept. of Microbiology, Miami University, Oxford, Ohio: Gene, enzyme relationship, and heterologous expression of lysine genes in *S. pombe*.

Messenguy, F., Dubois, E., Research Institute of the CERIA, Brussels, Belgium: The yeast ARGRII regulatory protein has homology with various RNases and DNA-binding proteins.

Freeman, K., Karns, L., Smith, M.M., Dept. of Microbiology, University of Virginia Medical School, Charlottesville: Transcriptional regulation of the cell-cycle-expressed histone genes, H3 and H4.

Goyer, C., Altmann, M., Sonenberg, N., Dept. of Biochemistry, McGill University, Quebec, Canada: Characterization of *S. cerevisiae* cap-binding proteins.

Schultz, J., Carlson, M., Columbia University College of Physicians & Surgeons, New York, New York: *SSN6*, a gene functionally related to the *SNF1* protein kinase.

Herrick, D., Jacobson, A., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Structural determinants of mRNA stability in *S. cerevisiae*.

Jacobs, E.,[1] Laloux, I.,[2] Dubois, E.,[3] [1]Smith Kline-RIT, Dept. of Molecular and Celluar Biology, Rixensart, [2]University Libre de Bruxelles, Laboratory de Microbiologie, [3]Research Institute CERIA, Bruxelles, Belgium: Molecular cloning and characterization of the *S. cerevisiae ROC1* gene.

Jaehning, J.A., Wilcoxen, S.E., Peterson, C.R., Schultz, P.W., Marczynski, G.T., Dept. of Biology, Indiana University, Bloomington: Transcription by the yeast mitochondrial RNA polymerase.

Khan, N., Dept. of Biology, Brooklyn College, New York: The *gal3* mutation and maltose utilization in yeast.

Mehta, K.D., Smith, M., Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: Identification of *cis*-acting DNA sequence(s) involved in the negative regulation of *ANB1* gene by oxygen in yeast by in vitro mutagenesis using a synthetic oligonucleotide with a low level of misincorporated nucleotides.

Macreadie, I.G., Vaughan, P.R., Jagadish, M.N., CSIRO, Division of Protein Chemistry, Victoria, Australia: Controlled, heterologous gene expression in yeast – Use of a highly versatile, modified *CUP1* gene.

Osley, M.A., Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York: *Trans*-acting mutations that abolish periodic transcription of the histone genes.

Witte, M.M., Dickson, R.C., Dept. of Biochemistry, University of Kentucky, Lexington: Characterization of the DNA-binding domain of a eukaryotic-positive regulatory gene.

Thompson, J.R., Tsay, Y.F., Woolford, J.L., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Posttranscriptional regulation of ribosomal protein synthesis.

Trueblood, C.E., Poyton, R.O., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Differential regulation of *COX5a* and *COX5b* by *HAP1*, *HAP2*, and a newly identified gene, *REO1*.

Uemura, H., Wickner, R.B., NIDDKD, National Institutes of Health, Bethesda, Maryland: Cytoplasmic suppression of some *mak* mutations is a property of a variant of L-A.

Camilloni, G., Caserta, M., Della Seta, F., Di Mauro, E., Ficca, A.C., Negri, R., Venditti, S., Dipt. di Genetica e Biologia Molecolare, Università "La Sapienza," Roma, Italy: *S. cerevisiae* RNA polymerase II promoters – Intrinsic topological information and interaction with purified RNA pol II.

Morgan, B.A., Mittman, B.A., Smith, M.M., Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Identification of a histone H4 mutation that affects mating-type expression.

de Larminat, M.A., Lee, G.S.-F., von Borstel, R.C., Dept. of Genetics, University of Alberta, Edmonton, Canada: An A-T transversion in the initiation codon results in a leaky phenotype.

Crabeel, M., Seneca, S., Devos, K., Heimberg, H., Glansdorff, N., Dept. of Microbiology, University of Brussels, Belgium: Role of conserved sequences in the control region of the arginine regulon of *S. cerevisiae*.

Linder, P., Slonimski, P.P., Centre de Genetique Moleculaire du CNRS, Gif-sur-Yvette, France: Cloning and characterization of two iso-functional nuclear genes essential for growth and involved in nucleo-mitochondrial interactions.

Capsey, L.,[1] Yotsuyanagi, Y.,[2] Banks, G.,[1] Williamson, D.H.,[1] [1]National Institute for Medical Research, London, England; [2]Centre Genetique Moleculaire due CNRS, France: Frequent virus-like particles in the *S. uvarum* strain NCYC74 may be intermediates in Ty element transposition.

Casey, G.P.,[1] Xiao, W.,[2] Rank, G.H.,[2] Depts of [1]Applied Microbiology and Food Science, [2]Biology, University of Saskatchewan, Saskatoon, Canada: *SMR1-410*-encoded resistance to the herbicide sulfometuron methyl as a dominant selection marker in industrial strains of *Saccharomyces* yeast.

Fahrig, R., Fraunhofer-Institut für Toxikologie und Aerosolforschung, Dept. of Genetics, Hannover, Federal Republic of Germany: Adaptive response of *S. cerevisiae* to chronic treatment with alkylating and nonalkylating mutagens.

Hill, J.E., Henry, S.A., Depts of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: *S. pombe* mutants blocked in phosphatidylcholine biosynthesis.

Gangloff, S., Marguet, D., Lauquin, G.J.-M., Laboratory of Physiologie Cellulaire, Université d'Aix-Marseille, France: Aconitase – Cloning, disruption, and regulation of the *ACO* gene.

Hoffmann, A.,[1,2] Swida, U.,[1] Käufer, N.F.,[2] [1]Institut für Biochemie und Molekularbiologie, Freie Universität Berlin, Federal Republic of Germany; [2]Dept. of Bioscience and Biotechnology, Drexel University, Philadelphia, Pennsylvania: Studies with artificial introns in the *ura4* gene of *S. pombe*.

Ming, M., Thomas, W., Holm, C., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Fine-structure mapping of the structural gene for DNA topoisomerase II.

Balzi, E., Weining, C., Ulaszewski, S., Capieaux, E., Goffeau, A., Laboratoire d'Enzymologie, Université de Louvain, Belgium: A regulatory gene for yeast multiple-drug resistance.

Köhrer, K., Domdey, H., Genzentrum der Ludwig-Maximilians-Universität München, Martinsried, Federal Republic of Germany: The *S. cerevisiae MATa1* transcript is differentially spliced in vitro and in vivo.

Kuang, D.R., Xuan, J.W., Fan, J.B., Jiang, W.D., Zhus, Y.W., Zhang, X.G., Shanghai Institute of Cell Biology, Academia Sinica, People's Republic of China: Studies on yeast

genes encoding proline-synthesizing enzymes.

Zang, G., Kuang, D.-R., Shanghai Institute of Cell Biology, Academia Sinica, People's Republic of China: Sequence analysis of the *pro3* gene and the revolutionary significance of *pro3* and *proC* proteins.

Hanes, S.D., Bostian, K.A., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Genetic mapping of *ESS1* and use of a regulated promoter to investigate its function.

Lemmon, S., Freund, C., Conley, K., Jones, E., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Mutants bearing a deletion of the clathrin heavy-chain gene (*CHC1*) become polyploid at high frequency.

Porter, G., Brennwald, P., Jermone, J., Wise, J.A., Dept. of Biochemistry, University of Illinois, Urbana: *S. pombe* analogs of mammalian snRNAs.

Roof, L.L., Klein, R.D., Molecular Biology Research, Upjohn Company, Kalamazoo, Michigan: Cloning and characterization of the gene encoding orotidine 5′-phosphate decarboxylase of *Schwanniomyces occidentalis*.

Wilkinson, B.M., Walmsley, R.M., Dept. of Biochemistry and Applied Molecular Biology, UMIST, Manchester, England: Deletion of potential Z DNA sequences from the yeast genome.

Cafferkey, R., Klar, A., Cold Spring Harbor Laboratory, New York: Cloning of the *swi3*$^+$ gene of *S. pombe*.

Racher, A.J.,[1] Kinghorn, J.R.,[1] Wright, D.M.,[2] [1]Molecular Genetics Unit, University of St. Andrews, [2]Research and Development Dept., Distillers Company (Yeast) Ltd., Clackmannanshire, Scotland: Isolation and characterization of glutamate synthase mutants of *S. cerevisiae*.

Barbhaiya, H., Dept. of Microbiology, University of Baroda, India: Functional qualities of yeast.

SESSION 10 COVALENT MODIFICATION

Chairman: J. Thorner, University of California, Berkeley

Ennulat, C., Neff, N., Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of sodium-orthovanadate-resistant mutants of *S. cerevisiae* and comparison with cells expressing v-*src*.

Levin, D.E., Bishop, J.M., G.W. Hooper Foundation and Dept. of Microbiology and Immunology, University of California, San Francisco: Mutants in the *KIN* gene of *S. pombe* display multiple phenotypic defects.

Deschenes, R.J., Broach, J.R., Dept. of Molecular Biology, Princeton University, New Jersey: Characterization of *RAS2* fatty acylation mutations and second-site suppressors that allow growth of mutant strains.

Bohni, P.C., Schekman, R.W., Dept. of Biochemistry, University of California, Berkeley: *SEC11* is required for signal peptide processing and yeast cell growth.

Fuller, R.S.,[1] Brake, A.,[2] Thorner, J.,[3] [1]Dept. of Biochemistry, Stanford University School of Medicine, [2]Chiron Corporation, Emeryville, [3]Dept. of Biochemistry, University of California, Berkeley: *KEX2* endopeptidase, despite its sensitivity to thiol reagents, is a serine protease with homology to the bacterial subtilisins.

Sturley, S.L.,[1] LeVitre, J.,[1] Tipper, D.J.,[2] Bostian, K.A.,[1] [1]Division of Biomedicine, Brown University, Providence, Rhode Island; [2]Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Processing of the *S. cerevisiae* type-1 killer toxin precursor and its relationship to the expression of immunity.

Bachmair, A., Finley, D., Wunning, I., Varshavsky, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Amino-terminal targeting in selective protein degradation.

Jentsch, S., McGrath, J.P., Varshavsky, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Ubiquitin-ligation system of *S. cerevisiae*.

A. Klar

SESSION 11 CHROMOSOMES

Chairman: B.K. Tye, Cornell University

Hoyt, M.A., Stearns, T., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic analysis of chromosome segregation in *S. cerevisiae*.

Surosky, R.T., Tye, B.K., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca: Homologous pairing of the chromosome arms, but not the centromeres, determines the orientation of kinetochores in the meiotic disjunction of homologs.

Niwa, O., Matsumoto, T., Chikashige, Y., Nakaseko, Y., Yanagida, M., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Construction of minichromosomes in *S. pombe*—Relation of structure to meiotic behavior.

Kenna, M., Amaya, E., Bloom, K., Dept. of Biology, University of North Carolina, Chapel Hill: Isolation of the centromere chromatin complex from yeast.

Rose, D., Thomas, W., Holm, C., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: DNA topoisomerase II is required for nuclear division in meiosis.

Jazwinski, S.M., Cosgrove, K.L., Dept. of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans: Protein kinase activity in preparations of yeast replicative complex is *CDC7*-dependent.

Diffley, J.F.X., Stillman, B., Cold Spring Harbor Laboratory, New York: DNA-protein interactions at the chromosomal replicator, ARS1, and their effect on DNA bending.

Gasser, S., Swiss Institute for Experimental Cancer Research (SREC), Epalinges, Switzerland: Specific association of yeast sequences with a yeast nuclear scaffold.

Lowary, P.T., Widom, J., Depts. of Chemistry and Biochemistry, University of Illinois, Urbana: Structure of yeast chromosomes.

SESSION 12 NUCLEAR STRUCTURE AND TRANSPORT

Chairman: M. Douglas, University of Texas Health Science Center

Allen, J., Douglas, M., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Nuclear pore complex.

Wright, R., Rine, J., Dept. of Biochemistry, University of California, Berkeley: Karmellae formation—Overproduction of HMG-CoA reductase induces nuclear-associated membrane proliferation.

Armstrong, K.,[1] Talty, J.,[2] Broach, J.,[1] [1]Dept. of Molecular Biology, Princeton University, New Jersey; [2]Dept. of Biochemistry, University College, Cork, Ireland: Interaction of the *REP1* and *REP2* partitioning proteins encoded by the 2-μm circle plasmid.

Rose, M.D., Roberts, T., Lister, K., Princeton University, New Jersey: *KAR1-lacZ* gene fusions localize β-galactosidase to the vicinity of the spindle plaque.

Clark, M.W., Jong, A., Abelson, J., Campbell, J.L., Divisions of Chemistry and Biology, California Institute of Technology, Pasadena: Use of SSB-1, a nucleolar protein, and *rna1* mutants to probe nuclear structure. The *rna1* mutation disrupts the location of proteins found in the yeast nucleolus.

Sadler, I., Goodson, H., Silver, P., Dept. of Biology, Princeton University, New Jersey: Genetic analysis of nuclear protein localization.

Benton, B.,[1] Eng, W.-K.,[2] Fisher, P.A.,[1] Studier, F.W.,[3] Dunn, J.,[3] Sternglanz, R.,[2] Depts. of [1]Pharmacology, [2]Biochemistry, State University of New York, Stony Brook, [3]Biology Division, Brookhaven National Laboratory, Upton, New York: Regulated import of bacteriophage T7 RNA polymerase into the yeast nucleus and transcription of target genes.

McLaughlin, C.,[1] Garrels, J.,[2] Johnson, S.P.,[3] Warner, J.R.[3] [1]Dept. of Biological Chemistry, University of California, Irvine; [2]Cold Spring Harbor Laboratory, New York; [3]Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: A database for the proteins of *S. cerevisiae*.

Racher, A.J.,[1] Kinghorn, J.R.,[1] Wright, D.M.,[2] [1]Molecular Genetics Unit, University of St. Andrews; [2]Distillers Co. (Yeast) Ltd., Menstrie, Scotland: Isolation and characterization of glutamate synthase mutants of *S. cerevisiae*.

D. Broek

Molecular Genetics of Bacteria and Phages

August 18—August 23

ARRANGED BY

Gary Gussin, University of Iowa
Gisela Mosig, Vanderbilt University
John Roth, University of Utah

360 participants

The 1987 meeting on Molecular Genetics of Bacteria and Phages was one of the most successful "phage" meetings in the past several years. More than 360 people attended; approximately 120 talks and 135 posters were presented. A major strength of the phage meeting is that it brings together scientists working on virtually every aspect of prokaryotic molecular genetics. This year's topics included DNA replication, recombination, and transposition; transcription initiation and termination; developmental regulation of gene expression; RNA processing and translation; phage morphogenesis; membrane proteins; and recombinant DNA technology.

Among the exciting new results presented, perhaps the most surprising was the finding of Huang and Fang that a region of T4 gene 60 is a functional intron which is not spliced. Apparently, secondary structure of the mRNA permits ribosomes to "jump" from one "exon" to the next without translating the intervening 50 nucleotides. Several other papers documented the unusual transcriptional and translational properties of T4 genes. These include genes with genuine introns, and other genes that code for more than one polypeptide, depending on the choice of transcription and/or translation startpoint at different stages of phage development.

Several groups (Gardella and Susskind; Siegele et al.; Healy et al.) have now isolated mutations in genes encoding sigma subunits of RNA polymerases. These mutations alter the promoter recognition specificity of RNA polymerase; the nature of the amino acid changes strongly suggests that the wild-type amino acids make contacts with specific nucleotides in promoter DNA. These results were part of a common thread: the mechanisms by which specific protein-DNA interactions influence DNA replication, site-specific recombination, transposition, regulation of gene expression, and phage DNA packaging.

The quality of the presentations throughout the meeting was exceptional.

SESSION 1 DNA REPLICATION

Baker, T., Bramhill, D., Kornberg, A., Dept. of Biochemistry, Stanford University School of Medicine, California: Transcriptional activation of initiation of replication at the origin of the *E. coli* chromosome.

Polaczek, P., Wright, A., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Effect of *dnaA* box mutations on *dnaA* gene expression in *E. coli*.

Russell, D.W., Zinder, N.D., Rockefeller University, New York, New York: Hemimethylation prevents DNA replication in *E. coli*.

Traxler, B., Minkley, E.G., Jr., Dept. of Biological Sciences and Mellon Institute, Carnegie Mellon University, Pittsburgh, Pennsylvania: Studies of the *traI* gene and *oriT* nicking of F plasmid.

Greenstein, D., Roth, A., Horiuchi, K., Rockefeller University, New York, New York: Double-strand cleavage and strand joining by the replication initiator protein of bacteriophage f1.

Gennaro, M.L., Novick, R.P., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: Structural and functional analysis of *cmp*, an enhancer of plasmid replication.

Wahle, E., Kornberg, A., Dept. of Biochemistry, Stanford University School of Medicine, California: The *par* sequence of pSC101 is a gyrase-binding site.

Ohmori, H., Murakami, Y., Nagata, T., Institute for Virus Research, Kyoto University, Japan: Nucleotide sequence required for replication of the ColE1-type plasmid in *E. coli* cells with or without RNase H.

Cohen, G., Sternberg, N., E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware: Localization and characterization of the bacteriophage P1 lytic replicon.

Pelletier, A.J., Hill, T.M., Kuempel, P.L., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Identification of the sites that inhibit DNA replication in the terminus region of the *E. coli* chromosome.

Funnell, B.E., Yarmolinsky, M.B., NCI, National Institutes of Health, Bethesda, Maryland: Overproduction of the P1 ParB protein inhibits proper partitioning of P1-derived plasmids.

Davis, M.A., Martin, K.A., Austin, S.J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Partition proteins of the P1 plasmid.

SESSION 2 RECOMBINATION AND REPAIR

Segall, A., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: Half-tetrad analysis in bacteria.

Mosig, G., Powell, D., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Complex regulation of two proteins from bacteriophage T4 gene *49* (recombination endonuclease VII).

Panayotatos, N.,[1] Fontaine, A.,[2] Biogen SA, Geneva, Switzerland, [1]Michigan Biotechnology Institute, Lansing; [2]Institut Pasteur, Paris, France: Native cruciforms in bacteria.

Abremski, K., Frommer, B., Wierzbicki, A., Hoess, R., E.I. du Pont de Nemours and Co., Inc., Central Research and Development Dept., Wilmington, Delaware: Properties of a mutant Cre protein that alters the topological linkage of recombination products.

Glasgow, A.C., Simon, M.I., Dept. of Biology, California Institute of Technology, Pasadena: Interactions between *fis* and the recombinational enhancer of the *hin* inversion system of *Salmonella*.

Heitman, J., Model, P., Rockefeller University, New York, New York: Mutagenesis of the *Eco*RI endonuclease—Alleles with altered or reduced cleavage-site specificity.

Gann, A.A.F., Fuller-Pace, F.V., Murray, N.E., Dept. of Molecular Biology, Edinburgh, Scotland: Reassortment of DNA recognition domains by recombination leads to the generation of new specificities.

Semerjian, A.V., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Bacteriophage P22 p_L operon genes—Roles in growth and recombination and comparison with λ.

Sassanfar, M., Roberts, J., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Effects of the *recF* mutation on SOS induction.

Phillips, G.J., Kushner, S.R., Dept. of Genetics, University of Georgia, Athens: Analysis of the role of exonuclease I in DNA repair, genetic recombination, and the SOS response.

SESSION 3 POSTER SESSION

Abeles, A.L., Austin, S.J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Role of *E. coli dam* methylase in P1 replication.

Andersson, D., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: Transcriptional regulation of cobalamin biosynthetic genes.

Alifano, P., Nappo, A.G., Bruni, C.B., Carlomagno, M.S., Dept. of Molecular and Cellular Pathobiology, University of Naples, Italy: Strong transcriptional polarity in a missense mutant of *S. typhimurium*.

Angov, E., Solomon, K., Brusilow, W., Dept. of Chemistry and Biochemistry, University of Maryland, College Park: Autoregulation of *E. coli* ATPase gene expression.

Armengod, M.E., García-Sogo, M., Lambíes, E., Instituto de Investigaciones Citológicas, Valencia, Spain: The *recF* and *dnaN* genes of *E. coli* constitute an operon.

Arraiano, C.M., Yancey, S.D., Kushner, S.R., Dept. of Genetics, University of Georgia, Athens: Analysis of specfic cleavage sites in mRNA decay in *E. coli*.

Baracchini, E., Bremer, H., Biology Programs, University of Texas of Dallas, Richardson: Control of stable RNA synthesis in bacteria by ppGpp.

Benedik, M., Ball, T., Saurugger, P., Dept. of Biology, Texas A & M University, College Station: Extracellular nuclease of *S. marcescens*.

Berg, C.M.,[1] Liu, L.,[1] Whalen, W.A.,[2] Das, A.,[2] [1]Dept. of Molecular and Cell Biology, University of Connecticut, Storrs; [2]Dept. of Microbiology, University of Connecticut Health Center, Farmington: Position-effect mutations induced by γ-δ insertions adjacent to the *E. coli avtA* gene.

Berg, C.M., Liu, L., Wang, B., Dept. of Molecular and Cell Biology, University of Connecticut, Storrs: Rapid identification of lethal genes.

Brissette, J.L., Russel, M., Rockefeller University, New York, New York: Expression of f1 gene IV induces a cellular protein.

Brooks, J.E.,[1] Nwankwo, D.,[2] Sznyter, L.,[1] Jager, T.,[1] Wilson, G.,[1] Heiter, D.,[3] Slatko, B.,[1] Moran, L.,[1] Benner, J.,[1] [1]New England Biolabs, Beverly Massachusetts; [2]Dept. of Biological Sciences, University of Calabar, Nigeria; [3]Dept. of Molecular Biology, Princeton University, New Jersey; Cloning of the *Bam*HI restriction-modification system.

Bukau, B., Walker, G.C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: New phenotypes of a Δ*dnaK* mutant of *E. coli* suggest an essential role for DnaK at low temperature.

Burckhardt, S., Woodgate, R., Scheuermann, R., Echols, H., Dept. of Molecular Biology, University of California, Berkeley: The UmuD mutagenesis protein of *E. coli*—Overproduction, purification, and cleavage by RecA.

Campbell, J.L., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Methylation control of IS*10*'s transposase promoter involves a second competing promoter, pC.

Champness, W.C., Dept. of Microbiology, Michigan State University, East Lansing: New loci required for *S. coelicolor* morphological and physiological differentiation.

Clyman, J., Cunningham, R.P., Dept. of Biological Sciences, State University of New York, Albany: *E. coli* mutants dependent on *recA* function for viability.

Condemine, G., Smith, C., Dept. of Genetics and Development, College of Physicians & Surgeons, Columbia University, New York, New York: Site-specific cleavage of *E. coli* chromosome by DNA gyrase.

Conley, P.B., Lemaux, P.G., Grossman, A., Dept. of Plant Biology, Carnegie Institution of Washington, Stanford, California: Transcriptional regulation by specific wavelengths of light of a photosynthetic antenna complex gene family.

Connell, N., Siekhaus, D., Kolter, R., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Regulation of a stationary-phase-induced promoter.

Costantino, N.,[1] Zuber, M.,[2] Court, D.L.,[3] [1]Program Resources, Inc., Frederick, Maryland; [2]Oregon State University, Corvallis; [3]NCI-Frederick Cancer Research Facility, Frederick, Maryland: The *byp1* mutation of bacteriophage λ.

Cotter, T., McClung, C.R., Chelm, B., DOE-Plant Research Laboratory and Dept. of Microbiology, Michigan State University, East Lansing: Genetic locus essential for formate-dependent growth by *B. japonicum*.

Coulby, J.N., Sternberg, N.L., E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware: Localization of the bacteriophage P1 *dam* gene.

Craig, N.L., Chen, T., Gringuaz, E., McKown, R., Orle, K., Waddell, C., Dept. of Microbiology and Immunology, University of California, San Francisco: *att*-Tn*7*–A specific site for transposon Tn*7* insertion.

Dila, D., Trimarchi, R., Raleigh, E.A., New England Biolabs, Beverly, Massachusetts: Dissection of the *mcrB* region of *E. coli* K12.

Dodd, I.B., Egan, J.B., Dept. of Biochemistry, University of Adelaide, Australia: Control of lysis and lysogeny in coliphage 186.

Dodson, K.W., Berg, D.E., Dept. of Microbiology and Immunology, Washington University, St. Louis, Missouri: Factors controlling the use of IS*50* ends during transposition.

Drivdahl, R., D'Acci, K., Guttman, B., Kutter, E., Evergreen State College, Olympia, Washington: Inhibition of transcription elongation by the bacteriophage T4 alc gene product

Ehrenman, K.,[1,2] Belfort, M.,[1] Povinelli, C.M.,[3] Hall, D.H.,[3] [1]Wadsworth Center for Laboratories and Research, New York State Dept. of Health, [2]Dept. of Microbiology and Immunology, Albany Medical College, New York; [3]School of Applied Biology, Georgia Tech, Atlanta: Clustering of mutations in the group I *td* intron of bacteriophage T4.

Eisenstein, B.I., Sweet, D., Vaugh, V., Friedman, D.I., University of Michigan, Ann Arbor: Integration host factor is required for the DNA inversion controlling phase variation in *E. coli*.

J. Roth

Elliott, T., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: Genetic analysis of heme synthesis in *S. typhimurium*.

Erickson, B.D., Lesley, S.A., Burgess, R.R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Transcription of the *rpsU-dnaG-rpoD* operon of *E. coli* under different growth conditions.

Fane, B., King, J., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Second-site suppressors that correct missense defects in the folding and chain association of the P22 tailspike.

Fornwald, J.,[1] Schimdt, F.,[1,2] Adams, C.,[1] Rosenberg, M.,[1] Brawner, M.,[1] [1]Smith, Kline and French Laboratories, Dept. of Molecular Genetics, King of Prussia, Pennsylvania; [2]Dept. of Biochemistry, University of Missouri, Columbia: Two independently regulated promoters control transcription of the *S. lividans* galactose operon.

Forsman, K., Göransson, M., Uhlin, B.E., Dept. of Microbiology, University of Umea, Sweden: Regulatory genes activating transcription of a pilus operon in *E. coli*.

French, S.,[1] Miller, O.L., Jr.,[1] Gourse, R.,[2] [1]Dept. of Biology, University of Virginia, Charlottesville; [2]Dept. of Genetics, University of Georgia, Athens: Distribution of RNA polymerases along *rrnB* and the regulation of rRNA synthesis in *E. coli*.

Friedman, S.A., Austin, S.J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Regulation of the P1 partition operon.

Gary, T.,[1] Lukas, T.,[2] Trupin, M.,[1] Mosig, G.,[1] [1]Dept. of Molecular Biology, [2]Howard Hughes Medical Institute, Vanderbilt University, Nashville, Tennessee: Bacteriophage T4 dCTPase is one of three proteins encoded in the same reading frame by a gene overlapping an origin of DNA replication.

Gentry, D., Burgess, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Operon structure and mutational analysis of the gene encoding the ω subunit of *E. coli* RNA polymerase.

Glaser, P.,[1] Sezer, O.,[1] Danchin, A.,[1] Ladant, D.,[2] Pichot, F.,[2] Ullmann, A.,[2] [1]Régulation de l'Expression Génétique, [2]Biochimie des Régulations Cellulaires, Institut Pasteur, Paris, France: Cloning of *B. pertussis* adenylate cyclase gene and expression in *E. coli*.

Goliger, J.A., Roberts, J.W., Dept. of Biochemistry, Cornell University, Ithaca, New York: Phage 82 Q-dependent and Q-independent antitermination in vitro.

Göransson, M., Båga, M., Forsman, K., Normark, S., Uhlin, D.E., Dept. of Microbiology, University of Umeå, Sweden: Transcriptional organization of a temperature-regulated pilus gene cluster in *E. coli*.

Greenberg, J.T., Demple, B., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Regulation of resistance to oxidative damage in *E. coli*—Mutations that suppress the *oxyR*$^-$ phenotype.

Griffo, G.,[1] Gottesman, M.E.,[1] Oppenheim, A.,[2] [1]Columbia University, New York, New York; [2]Hebrew University, Jerusalem, Israel: Why λ*cin*-1 mutants fail to grow on an IHF host.

Gustafsson, C.E.D., Lindström, P.H.R., Hagervall, T.G., Björk, G.R., Dept. of Microbiology, University of Umeå, Sweden: The promoter of the gene coding for tRNA (m^5U54)-methyltransferase has a nucleotide sequence similar to that of the P1 promoter of stable RNA.

Guzman-Verduzco, L.M., Kupersztoch, Y.M., Dept. of Microbiology, University of Texas Health Science Center, Dallas: Fusion of the heat-stable and the B subunit of the heat-labile enterotoxins.

SESSION 4 TRANSPOSONS AND MU

Falvey, E., Grindley, N.D.F., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Protein-DNA interactions at the *res* site of γ-δ—Analysis of site mutations.

Prentki, P.,[1] Zerbib, D.,[2] Jakowec, M.,[1] Gamas, P.,[2] Chandler, M.,[2] Galas, D.J.,[1] [1]Dept. of Molecular Biology, University of California, Los Angeles; [2]CNRS, Toulouse, France: Sequence-specific DNA-protein interactions at the ends of IS*1*.

Fuchs, K., Schnetz, K., Rak, B., Institut für Biologie, Universität, Freiburg, Federal Republic of Germany: The mobile DNA element IS*5* of *E. coli* can function as an enhancer activating gene expression.

DeLong, A., Syvanen, M., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Transposon Tn*5* mutants with *trans*-dominant defects in the regulation of transposition.

Makris, J.C., Nordmann, P., Krebs, M., Reznikoff, W.S., Dept. of Biochemistry, University of Wisconsin, Madison: IS*50* outside and inside end mutations.

Kubo, K., Waddell, C., Craig, N.L., Dept. of Microbiology and Immunology and the Hooper Foundation, University

of California, San Francisco: Tn*7* transposition—Choosing the target site.

Darzins, A., Kent, N.E., Buckwalter, M.S., Casadaban, M.J., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Mu *cis*-transposition immunity.

Higgins, P.,[1] McGovern, V.,[1] Hillyard, D.,[2] [1]Dept. of Biochemistry, University of Alabama, Birmingham; [2]Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City: Roles of histone-like DNA-binding proteins in bacteriophage Mu.

Alazard, R.,[1] Bétermier, M.,[1] Chandler, M.,[1] Desmet, L.,[2] Gama, M.J.,[2] Toussaint, A.,[2] [1]Centre de Recherche de Biochimie et Génétique Cellulaire du CNRS, France; [2]Dépt. de Biologie Moléculaire, Université libre de Bruxelles, Belgium: Transposase mutants of bacteriophage Mu.

Craigie, R., Mizuuchi, K., NIDDK, National Institutes of Health, Bethesda, Maryland: Cleavage of the 3′ ends of Mu is an early step in the Mu DNA strand-transfer reaction.

Adzuma, K., Mizuuchi, K., NIDDK, National Institutes of Health, Bethesda, Maryland: Involvement of the Mu B ATPase activity in target immunity in the Mu DNA strand-transfer reaction.

van de Putte, P., Vollering, M., University of Leiden, The Netherlands: Regulation of Mom expression in bacteriophage Mu.

Nagaraja, V., Hattman, S., Dept. of Biology, University of Rochester, New York: Bacteriophage Mu "late" gene transcription activator, C, is a site-specific DNA-binding protein.

SESSION 5 TRANSCRIPTION. I. REGULATION BY REPRESSORS AND ACTIVATORS

Haber, R., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Mechanism of repression of the *gal* operon.

Lee, D.-H., Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Lower limits to the spacing of two operators for repression in the ara BAD promoter.

Vogel, J.,[1] Toussaint, A.,[2] Higgins, P.,[1] [1]Dept. of Biochemistry, University of Alabama, Birmingham; [2]Laboratory of Genetics, Free University of Brussels, Belgium: Changes of the Mu repressor protein near its normal carboxyl terminus can confer a super repressor or a temperature-sensitive repressor phenotype.

Dallmann, G., Papp, P., Orosz, L., Dept. of Genetics, Attila Jozsef University, Szeged, Hungary: Related repressor specificity of unrelated phages and autonomy of the DNA-binding region.

Eliason, J.L., Conley, M.A., Gabriel, G., Sternberg, N., E.I. du Pont de Nemours and Co., Inc., Wilmington, Delaware: Studies on the specific binding of the C1 repressor of bacteriophage P1 to DNA.

Gussin, G.N., Hwang, J.-J., Dept. of Biology, University of Iowa, Iowa City: Interactions between λ repressor and RNA polymerase—Mutations in p_{RM} quantitatively affect repression of p_R.

Kim, J., Garges, S., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Analysis of CRP* mutants—What causes cAMP independence?

Brendler, T., Takeda, Y., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Analysis of the binding of CRP protein to the *lac*-binding site.

Polayes, D.A., Rice, P.W., Dahlberg, J.E., Dept. of Physiological Chemistry, University of Wisconsin, Madison: cAMP-CRP as a repressor of transcription of the *spf* gene of *E. coli*.

Ross, W.E., Park, S.-J., Summers, A.O., Dept. of Microbiology, University of Georgia, Athens: Genetic analysis of transcriptional regulation in the *mer* operon of Tn*21*.

Newlands, J.T., Dickson, R.R., Gourse, R.L., Dept. of Genetics, University of Georgia, Athens: Mechanism of activation of rRNA transcription initiation.

SESSION 6 POSTER SESSION

Halling, C., Calendar, R., Dept. of Molecular Biology, University of California, Berkeley: Activation of P2 and P4 late gene transcription by the P4 *d* gene product.

Hardy, L.W., Rennell, D., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester: Mutational studies of bacteriophage T4 lysozyme structure.

Hayes, S., Hayes, C., Dept. of Microbiology, College of Medicine, University of Saskatchewan, Saskatoon, Canada: Elevated IS*2* transposition in RK* strains with defective λ prophage.

Heitman, J., Model, P., Rockefeller University, New York, New York: Mutagenesis of the *Eco*RI endonuclease—Alleles with altered or reduced cleavage-site specificity.

Highlander, S.K., Engler, M.J., Weinstock, G.M., University of Texas Medical School, Houston: Expression of the cloned *P. haemolytica* leukotoxin in *E. coli*.

Hoess, R., Wierzbicki, A., Abremski, K., E.I. du Pont de Nemours and Co., Inc., Central Research and Development Department, Experimental Station, Wilmington, Delaware: Isolation and characterization of intermediates in the *cre-lox* site-specific recombination system of P1.

Hsu, T., Karam, J., Dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston: Transcription and RNA processing in the control of a T4 DNA replication gene cluster.

Inada, T.,[1] Kawakami, K.,[1] Egawa, K.,[1] Takiff, H.,[2] Court, D.,[2] Nakamura, Y.,[1] [1]Dept. of Tumor Biology, Institute of Medical Science, University of Tokyo, Japan; [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Isolation of temperature-sensitive lethal mutations in the *era*, *rnc*, and *lep* genes of *E. coli*.

Iordanescu, S., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: *pla*C*I*—A chromosomal mutation increases plasmid pT181 copy number by specifically blocking transcription of the regulatory countertranscript.

N. Sternberg, H. Miller

Jensen, K.F., Institute of Biological Chemistry, University of Copenhagen, Denmark: Regulation of *pyr* gene and *lacZ* gene expression in *E. coli* cells with "slow" ribosomes.

Jin, D.-J., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Structural and functional analysis of rifampicin-resistant mutations of *E. coli* RNA polymerase.

Johnson, G., Feiss, M., Xin, W., Dept. of Microbiology, University of Iowa, Iowa City: A mutation in the Shine-Dalgarno ribosome-binding sequence that increases λ *Nu1* translation.

Jurss, L.M., Olson, E.R., Molecular Biology Research, Upjohn Co., Kalamazoo, Michigan: Growth in minimal media induces the high-level expression of a 50-Kd periplasmic protein in certain strains of *E. coli*.

Kano, Y., Wada, M., Kono, K., Goshima, N., Imamoto, F., Tsukuba Life Science Center, Japan: Structure and function of the *hup* genes encoding the HU nucleoid proteins.

Kao, C., Snyder, L., Dept. of Microbiology, Michigan State University, East Lansing: The *lit* protein that blocks bacteriophage T4 late gene expression is a membrane protein encoded by a cryptic DNA element.

Kawakami, K., Nakamura, Y., Dept. of Tumor Biology, Institute of Medical Science, University of Tokyo, Japan: A gene affecting ColE1 DNA replication lies immediately downstream, presumably in the same operon, to the RF2 gene of *E. coli*.

Kent, N.E., Darzins, A., Casadaban, M.J., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: D3112 *P. aeruginosa* transposon bacteriophage—Terminal sequences and derivative elements.

Kiino, D.R.,[1] Rothman-Denes, L.B.,[1,2] Depts. of [1]Molecular Genetics and Cell Biology, [2]Biochemistry and Molecular Biology, University of Chicago, Illinois: Identification of a locus, *nfrA*, involved in coliphage N4 adsorption.

Kraczkiewicz-Dowjat, A., Harris, C.J., Fishel, R.A., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Effect of mismatch nucleotide repair on the recombination of an adjacent gene.

Krishna Rao, A.S.M., Goldberg, E., Dept. of Molecular Biology, Tufts University Medical School, Boston, Massachusetts: Does the product of the bacteriophage T4 gene *2* bind specifically to double-stranded DNA termini?

Kuhstoss, S., Richardson, M.A., Rao, R.N., Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana: Gene transplacement and site-specific integration in *Streptomyces*.

Kunkel, B.N., Sandman, K.M., Losick, R., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Temporal regulation of the *B. subtilis* sporulation gene *spoIVC*.

Lanzer, M., Bujard, H., Zentrum für Molekulare Biologie Heidelberg, Universität Heidelberg, Federal Republic of Germany: Mechanism of repression of the *E. coli lac* operator/repressor system.

Lewis, K., Dept. of Biochemistry, University of Wisconsin, Madison: Participation of the taxis system in controlling the *E. coli* expression of motility.

Liao, S.-M., McClure, W.R., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Structural studies of *sar* RNA in inhibition of P22 antirepressor synthesis.

Linderoth, N.A., Calendar, R., Dept. of Molecular Biology, University of California, Berkeley: Cloning of the P4 *psu* gene and demonstration of gpPsu activity.

Lindsey, D.F., Walker, J.R., Dept. of Microbiology, University of Texas, Austin: Genetic structure of the *E. coli dnaY-dsy* region.

Lopilato, J., Wright, A., Dept. of Molecular Biology and Microbiology, Tufts University Medical School, Boston, Massachusetts: Mutations in *bglR* that activate the *bgl* operon in *E. coli* K12.

Mahan, M.J., Roth, J.R., Dept. of Biology, University of Utah, Salt Lake City: Invertable and noninvertable segments of the *Salmonella* chromosome.

Mandal, N.C., Raha, M., Dept. of Biochemistry, Bose Institute, Calcutta, India: A mutation of the *nutR*-*tR1* region of λ increases expression of genes beyond *tR1* and *tR2* in the absence of *N* gene function.

Martin, K.A., Davis, M.A., Austin, S.J., NCI-Frederick Can-

cer Research Facility, Maryland: Definition of the minimal partition site of the P1 plasmid.

McConnell, M.R., Middleton, P.C. Rydelski, M.J., Gutierrez, H.G., Owens, J.D., Risley, S.L., Sanford, W.W., Depts. of Biology and Chemistry, Point Loma Nazarene College, San Diego, California: Screening of cloned bacteriophage E^{15} DNA fragments for the genes that govern cell-surface conversion upon infection of *S. anatum*.

McCormick, J.R., Zengel, J.M., Lindahl, L., Dept. of Biology, University of Rochester, New York: Translation efficiency and RNA stability in *E. coli*.

McGovern, K., Green, G.N., Beckwith, J., Dept. of Microbiology and Molecular Genetics, Harvard University School of Medicine, Boston, Massachusetts: Study of membrane localization of MalF, an integral membrane protein of *E. coli*.

McKown, R., Arcizewska, L., Waddell, C., Craig, N.L., Dept. of Microgiology and Immunology and the Hooper Foundation, University of California, San Francisco: *cis*-Acting transposition sequences at the ends of Tn7 and a Tn7-dependent end-binding activity.

Metzger, S.,[1] Glaser, G.,[1] Cashel, M.,[2] [1]Dept. of Celular Biochemistry, Hebrew University, Jerusalem, Israel; [2]NICHD, National Institutes of Heath, Bethesda. Maryland: Characterization of *relA* null and *relA*1 mutant alleles in *E. coli* – Strigent factor is not the only enzyme that synthesizes ppGpp.

Misra, R., Benson, S.A., Dept. of Molecular Biology, Princeton University, New Jersey: Structural model for the OmpC porin protein of *E. coli* K12.

Mizuuchi, M., Mizuuchi, K., NIDDK, National Institutes of Health, Bethesda, Maryland: Target-site specificity in bacteriophage Mu transposition.

Mullin, D.A., Newton, A., Dept. of Molecular Biology, Princeton University, New Jersey: Analysis of developmentally regulated flagellar gene promoters in *C. crescentus* using site-directed mutagenesis.

Murphy, K.C., Fenton, A.C., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester: RecBCD-inhibiting activity of bacteriophage P22.

Nash, H.N.,[1] Robertson, C.A.,[1] Flamm, E.L.,[2] Weisberg, R.A.,[2] Miller, H.I.,[3] [1]Laboratory of Molecular Biology, NIMH, [2]Laboratory of Molecular Genetics, NICHD, Bethesda, Maryland; [3]Cell Genetics Dept., Genentech, Inc., South San Francisco, California: Overproduction of *E. coli* integration host factor, a protein with nonidentical subunits.

Nicholson, A.W., Michalewicz, J., Larson, J.J., Dept. of Biological Sciences, Wayne State University, Detroit, Michigan: Characterization of the T-odd coliphage BA14 and the relationship of relation to that of its early region bacteriophages T7 and T3.

Nunes-Duby, S.E., Marsumoto. L., Landy, A., Dept. of Biology and Medicine, Brown University, Providence, Rhode Island: Site-specific recombination intermediates trapped with suicide substrates.

SESSION 7 TRANSCRIPTION. II. INITIATION SPECIFICITY

Gardella, T., Susskind, M.M., Dept. of Biological Sciences, University of Southern California, Los Angeles: Changing the promoter specificity of the *E. coli* σ^{70} subunit of RNA polymerase.

Siegele, D.A., Hu, J.C., Walter, W.A., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Analysis of mutations in *rpoD* (the gene encoding the σ^{70} subunit of *E. coli* RNA polymerase) that affect promoter recognition.

Healy, J., Zuber, P., Carter, H.L., Cutting, S., Moran, C.P., Losick, R., The Biological Laboratories, Harvard University, Cambridge, Massachusetts; Dept. of Microbiology, Emory University, Atlanta, Georgia: Mutation changing the specificity of an RNA polymerase σ factor.

Carter, H.L. III,[1] Dubnau, E.,[2] Weir, J.,[2] Smith, I.,[2] Moran, C.P., Jr.,[1] [1]Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia; [2]Dept. of Microbiology, Public Health Research Institute, New York, New York: A sporulation essential gene (*spo*OH) encodes an RNA polymerase σ factor from *B. subtilis*.

Malik, S., Goldfarb, A., Dept. of Microbiology, Columbia University, New York, New York: Properties of bacteriophage T4 late σ factor.

Straus, D., Walter, W., Gross, C., Dept. of Bacteriology, University of Wisconsin, Madison: The heat-shock response in *E. coli* is regulated by changes in the level of σ^{32}.

Cowing, D.,[1] Fisher, M.,[2] Record, M.T., Jr.,[2] Gross, C.,[1] Depts. of [1]Bacteriology, [2]Chemistry, University of Wisconsin, Madison: Interactions of *E. coli* RNA polymerase containing σ^{32} with heat-shock promoters.

Popham, D.L., Keener, J.W., Kustu, S.G., Dept. of Microbiology and Immunology, University of California, Berkeley: ATP requirement for activation of transcription at nitrogen-regulated promoters in *S. typhimurium*.

Reitzer, L.,[1,2] Movsas, B.,[1] Magasanik, B.,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Biology Programs, University of Texas at Dallas, Richardson: Transcription from the nitrogen-regulated promoter, *glnAp2*, of *E. coli* – Effect of variation of the distance between the two binding sites for the activator, NR_I, and between the binding sites for NR_I and RNA polymerase.

Ronson, C.W.,[1,2,3] Astwood, P.M.,[2] Nixon, B.T.,[3,4] Ausubel, F.M.,[3] [1]Biotechnica International, Cambridge, Massachusetts; [2]Grasslands Division, DSIR, Palmerston North, New Zealand; [3]Dept. of Molecular Biology, Massachusetts General Hospital, Boston; [4]Dept. of Molecular and Cellular Biology, Pennsylvania State University, University Park: C4-dicarboxylate transport and nitrogen regulatory systems are homologous.

Fischer, H.M., Gubler, M., Thöny, B., Hennecke, H., Mikrobiologisches Institute, Eidgenössische Technische Hochschule, ETH-Zentrum, Universitätstrasse, Zürich, Switzerland: Control of symbiotic nitrogen fixation genes in the soybean root-nodule bacterium, *B. japonicum*.

Bujard, H., Brunner, M., Deuschle, U., Kammerer, W., Knaus, R., Lanzer, M., Peschke, U., Schröter, M., Zentrum für Molekulare Biologie Heidelberg, Universität Heidelberg, Federal Republic of Germany: Functional program of promoter sequences from *E. coli*.

SESSION 8 TRANSCRIPTION. III. GENERAL REGULATION

Glucksmann, A.,[1] Rothman-Denes, L.B.,[1,2] Depts. of [1]Molecular Genetics and Cell Biology, [2]Biochemistry and Molecular Biology, University of Chicago, Illinois: N4 virion RNA polymerase–promoter interaction.

Baek, N.Y., Rothman-Denes, L.C., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Control of bacteriophage N4 late transcription.

Sengupta, D., Maitra, U., Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Correlation of efficiency of promoter utilization by T3 RNA polymerase to nucleotide sequence of its promoters.

Grambow, N., Christie, G.E., Dept. of Microbiology and Immunology, Virginia Commonwealth University, Richmond: Upstream sequences are required for expression from a bacteriophage P2 late gene promoter.

Dale, E.C.,[1] Keener, J.,[2] Kustu, S.,[2] Calendar, R.,[1] Depts. of [1]Molecular Biology, [2]Microbiology, University of California, Berkeley: Activation of the P4 late promoter in vitro and partial purification of the P4 *δ* gene product.

Margolin, W., Rao, G., Howe, M.M., Dept. of Microbiology and Immunology, Univeristy of Tennessee, Memphis: Four late promoters of bacteriophage Mu share conserved sequences.

Dibbens, J.A., Egan, J.B., Dept. of Biochemistry, University of Adelaide, Australia: Control of gene expression by replicative increase in activator dosage.

Miller, H.I., Dept. of Cell Genetics, Genentech Inc., South San Francisco, California: Novel mechanism for SOS regulation—LexA protein is an antirepressor for transcription of the *pheST-himA* operon.

Menzel, R., E.I. du Pont de Nemours, Experimental Station, Wilmington, Delaware: DNA supercoiling and transcription.

Jovanovich, S.B., Martinell, M.E., Burgess, R.R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: In vivo effects of supercoiling, anaerobiosis, and osmotic upshift on gene expression of a collection of known operon fusions.

Thomulka, K.W.,[1] Droffner, M.L.,[2] Cambell, P.,[1] Yamamoto, N.,[2] [1]Dept. of Biological Sciences, Philadelphia College of Pharmacy and Science, [2]Dept. of Microbiology and Immunology, Hahnemann University School of Medicine, Philadelphia, Pennsylvania: Aerobic and anaerobic control of gene expression independent of DNA superhelicity.

Arnold, G.F., Tessman, I., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Involvement of the RpoB protein of *E. coli* in the regulation of DNA supercoiling.

SESSION 9 POSTER SESSION

Oeschger, M., Donze, D., Leitz, L., Wust, J., Dept. of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans: Antitermination regulation of RNA polymerase genes in *E. coli*.

Ost, K., Lazinski, D., DeVito, J., Dept. of Microbiology and the Program in Molecular Biology and Biochemistry, University of Connecticut, Farmington: A new *E. coli* gene whose product is involved in transcription elongation and regulation.

Pagratis, N., Revel, H.R., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Biological activity and overexpression of cDNA clones of bacteriophage *ϕ*6.

Palchaudhuri, S.,[1] McFall, E.,[2] [1]Dept. of Microbiology, Wayne State University School of Medicine, Detroit, Michigan; [2]Dept. of Microbiology, New York University School of Medicine, New York, New York: Sequence alterations in *dsdA* promoter constitutive mutations.

Parmley, S.F., Smith, G.P., Division of Biological Sciences, University of Missouri, Columbia: Filamentous phage as an antibody selectable expression vector.

Pereira, R.F., Lawther, R.P., Dept. of Biology, University of South Carolina: Single-copy analysis of the regulatory region of the *ilvGMEDA* operon.

Peruski, L.F., Neidhardt, F.C., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Analysis of *htpI*, a conditionally essential heat-shock gene of *E. coli*.

Rather, P.N., Moran, C.P., Jr., Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia: Compartmentalized expression of *gdh* is controlled by transcription in *B. subtilis*.

Rimphanitchayakit, V., Hatfull, G.F., Grindley, N.D.F., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Recognition sequence of the carboxy-terminal DNA-binding domain of the *γ-δ* resolvase.

Rimsky, S., Spassky, A., Institut Pasteur, Paris, France: In vitro, the L8 mutation, changing at −68 CG to AT, alters the contacts between the protein CRP–RNA polymerase complex and the *E. coli* lactose promoter.

Rojiani, M., Goldman, E., Dept. of Microbiology, New Jersey Medical School, Newark: Evidence for participation of unchanged tRNA during rate-limiting elongation of protein synthesis in bacteriophage MS2-infected *E. coli*.

Schandel, K.,[1] Maneewannakul, S.,[2] Ippen-Ihler, K.,[2] Webster, R.,[1] [1]Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina; [2]Dept. of Medical Microbiology and Immunology, College of Medicine, Texas A & M University, College Station: A *traC* mutant that retains sensitivity to f1 bacteriophage but lacks F pili.

Schmitt, C.K., Molineux, I.J., Dept. of Microbiology. University of Texas, Austin: Effects of the incompatibility between T7 gene *1.2* and the F plasmid.

Schmitt, M.P., Payne, S.M., Dept. of Microbiology, University of Texas, Austin: Cloning and characterization of the

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enterobactin genes in strains of *S. flexneri*.

Slauch, J.M., Garrett, S., Jackson, D.J., Silhavy, T.J., Dept. of Molecular Biology, Princeton University, New Jersey: Regulation of porin gene expression in *E. coli* K12.

Smith, N.H., Lindahl, L., Dept. of Biology, University of Rochester, New York: Cloning the S10 (ribosomal protein) operon from *S. typhimurium* (LT2).

Sparkowski, J., Dept. of Microbiology and the Program in Molecular Biology and Biochemistry, University of Connecticut, Farmington: Identification of a new *E. coli* gene, *rap*, whose product interacts with RNA polymerase.

Spassky, A.,[1] Busby, S.,[2] Buc, H.,[1] Rimsky, S.,[1] [1]Dépt. de Biologie Moléculaire, Institut Pasteur, Paris, France; [2]Dept. of Biochemistry, University of Birmingham, England: Point Pribnow box mutation directly affects the rate of the formation of the transcriptionally active complex by changing the conformational state of the promoter.

Stewart, V., Dept. of Microbiology, Cornell University, Ithaca, New York: Expression of *narL*, a regulatory gene required for induction of respiratory nitrate reductase synthesis in *E. coli*.

Stitt, B., Dept. of Biology, New York University, New York, New York: ATP-binding sites on ρ.

Stoddard, S.F., Howe, M.M., Dept. of Microbiology and Immunology, University of Tennessee, Memphis: *C* gene transcript of bacteriophage Mu.

Sullivan, J., Banerjee, P., Wood, D., Jovanovich, S., Lebowitz, J., University of Alabama, Birmingham: Carbodiimide inactivation of transcription from supercoiled ColE1 DNA modification occurs almost exclusively in key promoters.

Szymkowiak, C., Wagner, R., Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin, Federal Republic of Germany: Influence of structural motives in the spacer region of the *rrnB* operon in *E. coli* on the synthesis of rRNA.

Takeda, Y.,[1] Sarai, A.,[2] NCI, [1]Frederick Cancer Research Facility, Frederick, [2]National Institutes of Health, Bethesda, Maryland: λRepressor recognizes its operator via amino acids of amino-terminal arms, loops, and α3-helices interacting with the exposed parts of base pairs within the major groove.

Tartaglia, L.A., Storz, G., Christman, M.F., Ames, B.N., Dept. of Biochemistry, University of California, Berkeley: Characterization of the bacterial response to oxidative stress.

Thermes, C.,[1] Tuerk, C.,[2] Gauss, P.,[2] Gayle, M.,[2,3] Guild, N.,[2] Stormo, G.,[2] d'Aubenton-Carafa, Y.,[1] Brody, E.N.,[1] Gold, L.,[2] [1]Institut de Biologie Physico-Chimique, Paris, France; [2]Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder; [3]Oncogen, Seattle, Washington: CUUCGG hairpins are special signals in RNA.

Tsui, P., Stevenson, B., Freundlich, M., Biochemistry Dept., State University of New York, Stony Brook: Effects of integration host factor on transcription in the *ilvB* leader.

Uzan, M., Favre, R., Brody, E., Institut de Biologie Physico-Chimique, Paris, France: Sequence and expression of the bacteriophage T4 *motA* regulatory gene.

van Rijn, P.A., Goosen, N., van de Putte, P., Dept. of Molecular Genetics, University of Leiden, The Netherlands: Regulation of Mu transcription by integration host factor.

Wanner, B.L., Wilmes, M.R., Purdue University, West Lafayette, Indiana: Control of bacterial alkaline phosphatase clonal variation in *phoR* mutant *E. coli*.

Wei, R., Liang, Y., Karam, J., Dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston: Autogenous translational repression by the T4 RegA protein—Dependence of repression on initiation context.

Weston-Hafer, K., Berg, D.E., Dept. of Microbiology and Immunology, Washington University, St. Louis, Missouri: Palindromy and the choice of deletion endpoints in *E. coli*.

Wiater, L.A., Grindley, N.D.F., Dept. of Molecelar Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Binding of γ-δ transposase to its inverted repeat.

Wikström, P.M., Byström, A.S., Björk, G.R., Dept. of Microbiology, University of Umeå, Sweden: The *trmD* ribosomal protein operon of *E. coli* is not autogenously regulated.

Winkelman, J.W., Hatfield, G.W., Dept. of Microbiology and Molecular Genetics, University of California, Irvine: Upstream enhancement of transcription initiation of the *ilvGMEDA* in *E. coli*.

Wu, T.-H., Barras, F., Marinus, M.G., Dept. of Pharmacology, University of Massachusetts Medical School, Worcester: Specificity of the *dam*-directed mismatch repair system of *E. coli* K12.

Wu, Y., Weinstock, G., Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Correlation between the amount of RecA protein and its functions in vivo.

Yancey, S.D., Arraiano, C.M., Kushner, S.R., Dept. of Ge-

netics, University of Georgia, Athens: Comparison of the cleavage patterns of poly- and monocistronic mRNAs in *E. coli*.

Yang, X., Roberts, J.W., Dept. of Biochemistry, Cornell University, Ithaca, New York: Point mutational analysis of the λ Q protein recognition site.

Yang, Y., Ames, G.F.-L., Dept. of Biochemistry, University of California, Berkeley: DNA-binding proteins that bind to a conserved repetitive extragenic palindromic sequence.

Zagotta, M.T., Wilson, D.B., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Antibody studies of the localization and oligomerization of the λ S protein.

Zeeh, A., Xu, M.-Q., Shub, D.A., Dept. of Biological Sciences, State University of New York, Albany: A new group I intron in bacteriophage T4.

Zhang, C., Margolin, P., Dept. of Biology, City University of New York, City College, New York: Evidence that abolition of most EMS mutagenesis in *topA* mutant cells is not due to lack of access to DNA sites.

Zheng, L.,[1] Donovan, W.P.,[1] Losick, R.,[1] Fitz-James, P.,[2] [1]The Biological Laboratories, Harvard University, Cambridge, Massachusetts; [2]University of Western Ontario, Canada: Identification of a gene required for spore coat morphogenesis in *B. subtilis*.

SESSION 10 TRANSCRIPTION TERMINATION AND ANTITERMINATION

Franklin, N.C., Doelling, J., Dept. of Biology, University of Utah, Salt Lake City: Hyper-N-icity – Effects of high expression of the antitermination proteins of lambdoid bacteriophages.

Craven, M.,[1] Granston, A.,[1] Carver, D.,[1] Alessi, D.,[1] Schauer, A.,[1] Henner, D.,[2] [1]Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor; [2]Dept. of Cell Genetics, Genentech, Inc., South San Francisco, California: NusA – Structure-function and interactions.

Tsugawa, A.,[1] Saito, M.,[1] Egawa, K.,[1] Fornwald, L.,[2] Showalter, S.D.,[2] Court, D.,[3] Nakamura, Y.,[1] [1]Dept. of Tumor Biology, Institute of Medical Science, University of Tokyo, Japan; [2]Program Resources, Inc., [3]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Sequence, genetic, and monoclonal antibody analyses of *nusA* protein of *E. coli*.

Whalen, W., Ghosh, B., Das, A., Dept. of Microbiology, University of Connecticut, Farmington: Formation of a core transcription antitermination apparatus at bacteriophage λ *nut* sites.

Olson, E.,[1] Craven, M.,[2] Friedman, D.,[2] [1]Molecular Biology Research, Upjohn Co., Kalamazoo; [2]Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: Defining the optimum *boxA* sequence.

Patterson, T., Johnson, K., Wigle, T., Court, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Requirements for antitermination at the λ *t*R1 terminator.

Robledo, R.,[1] Gottesman, M.E.,[1] Weisberg, R.A.,[2] [1]Columbia University, New York, New York: [2]NCI, National Institutes of Health, Bethesda, Maryland: λ *nutR* mutations affecting λ pN and HK022 pNUN activity.

Yang, X., Roberts, J.W., Dept. of Biochemistry, Cornell University, Ithaca, New York: Point mutational analysis of the λ Q protein recognition site.

Mahadevan, S., Houman, F., Pangier, L., Wright, A., Dept. of Molecular Biology and Microbiology, Tufts University Medical School, Boston, Massachusetts: Positive regulation of the *bgl* operon by antitermination.

Schnetz, K., Toloczyki, C., Rak, B., Institut für Biologie, Universität, Freiburg, Federal Republic of Germany: The β-glucoside operon of *E. coli* – Nucleotide, sequence, genetic organization, and a novel mechanism of regulation by inducer-dependent transcriptional antitermination.

Hasan, N. G., Posfai, G., Szybalski, W., McArdle Laboratory, University of Wisconsin, Madison: *N* and *nut*-dependent stimulation of transcription in the absence of antitermination.

SESSION 11 RNA PROCESSING AND TRANSLATION

Chandry, P.S.,[1,2] Salvo, J.G.,[1] Belfort, M.,[1] [1]Wadsworth Center for Laboratories and Research, New York State Dept. of Health, [2]Dept. of Microbiology and Immunology, Albany Medical College, New York: Genetic delineation of functional components of the group I bacteriophage T4 *td* intron.

Gott, J.M., Shub, D.A., Dept. of Biological Sciences, State University of New York, Albany: A family of self-splicing group I introns in bacteriophage T4.

Huang, W.M., Fang, M., Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City: An untranslated sequence within T4 gene *60* may not be spliced.

Krinke, L., Wulff, D.L., Dept. of Biological Sciences, State University of New York, Albany: RNase-III-dependent inhibition of cII gene expression by *oop* RNA.

Susskind, M.M., Dept. of Biological Sciences, University of Southern California, Los Angeles: Mutations affecting ri-

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bosome-binding-site activity and antisense RNA control of the bacteriophage P22 *ant* gene.

Young, R.,[1] Raab, R.,[2] Depts. of [1]Biochemistry and Biophysics, [2]Biology, Texas A & M University, College Station: λ *sdi* mutations—Evidence for RNA structure controlling the choice of initiation codons of the *S* lysis gene.

Olins, P.O., Rangwala, S.H., Monsanto Co., Chesterfield, Missouri: A bacteriophage ribosome-binding site reveals a novel mechanism for translation initiation.

Hattman, S., Ives, J., Wall, L., Maric, S., Dept. of Biology, University of Rochester, New York: The bacteriophage Mu *com* gene appears to specify a translation factor required for *mom* gene expression.

O'Connor, M.,[4] Falahee, B.,[4] Hughes, D.,[2] O'Mahony, D.,[2] Mims, B.,[3] Murgola, E.,[3] Tuohy, T.,[2] Weiss, B.,[1] Gesteland, R.,[1] Thompson, S.,[2] Atkins, J.,[1,4] [1]Howard Hughes Medical Institute and Dept. of Human Genetics, University of Utah, Salt Lake City; [2]Dept. of Genetics, Trinity College, Dublin, Ireland; [3]Dept. of Genetics, University of Texas, M.D. Anderson Hospital, Houston; [4]Dept. of Biochemistry, University College, Cork, Ireland: External suppressors for a −1 frameshift mutant.

Levin, M., Ripepi, L., Hendrix, R., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Translational frameshifting in bacteriophage λ tail genes *G* and *T*.

Ivey, M.R., Steege, D.A., Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Expression of phage f1 gene VII occurs from an inherently inactive initiation site made functional by translational coupling.

Lindahl, L., Zengel, J.M., Dept. of Biology, University of Rochester, New York: Expression of the S10 ribosomal protein operon of *E. coli*.

SESSION 12 PHAGE ASSEMBLY AND DEVELOPMENT

Sternberg, N., Coulby, J., E.I. du Pont de Nemours and Co., Inc., Experimental Station, Wilmington, Delaware: Cleavage of the bacteriophage P1 packaging site (*pac*) is regulated by adenine methylation and DNA replication.

Randall, S., Eppler, K., Casjens, S., Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City: Molecular analysis of mutants that affect the frequency of generalized transduction by bacteriophage P22.

Xin, W., Feiss, M., Dept. of Microbiology and Genetics Ph.D. Program, University of Iowa, Iowa City: Interaction of *E. coli* integration host factor at the bacteriophage λ *cos* site.

Kosturko, L.D., Dept. of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut: Integration host factor binds to λ DNA near *cos*.

Smith, G.P., Bauer, M., Division of Biological Sciences, University of Missouri, Columbia: Morphogenetic signal sequence *mos* and the orientation of DNA in filamentous phage.

Russel, M., Rockefeller University, New York, New York: Filamentous phage assembly.

Gottlieb, P.,[1] Strassman, J.,[1] Bamford, D.,[2] Mindich, L.[1] [1]Public Health Research Institute, New York, New York; [2]University of Helsinki, Finland: Production of polyhedral particles in *E. coli* from a cDNA copy of the large genomic segment of bacteriophage ϕ6.

Villafane, R., King, J., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Sites of temperature-sensitive folding (*tsf*) mutations in the P22 tailspike polypeptide chain.

Cheng, H.,[1] Muhlrad, P.J.,[1] Hoyt, M.A.,[1] Crowl, R.M.,[2]

Echols, H.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Molecular Genetics, Hoffman-La Roche, Inc., Nutley, New Jersey: Characterization of the HflA protease, a key element in the choice of lysis or lysogeny by bacteriophage λ.

Minnich, S.A., Newton, A., Dept. of Molecular Biology, Princeton University, New Jersey: Flagellin gene regulation in *C. crescentus*.

Grossman, A.D., Losick, R., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Mutations affecting extracellular control of sporulation in *B. subtilis*.

Weinberg, R.A., Zusman, D.R., Dept. of Microbiology and Immunology, University of California, Berkeley: Characterization of the frizzy mutants of *M. xanthus*.

Monneron, A.,[1] Ladant, D.,[2] d'Alayer, J.,[1] Ballalou, J.,[2] Bârzu, O.,[2] Ullmann, A.,[2] [1]Dépt. de Biologie Moléculaire, [2]Dépt. de Génétique Moléculaire, Institut Pasteur, Paris: Is *Bordetella pertussis* adenylate cyclase of eukaryotic origin?

SESSION 13 PROTEIN LOCALIZATION AND GENETIC TRICKS

Myers, R., Maloy, S., Dept. of Microbiology, University of Illinois, Urbana: Genetic analysis of protein structure and function—Mutations that alter the active site of proline permease in *S. typhimurium*.

Bergsland, K., Snyder, L., Dept. of Microbiology, Michigan State University, East Lansing: The *gol* site of bacteriophage T4—A new type of signal sequence?

Boyd, D., Manoil, C., Beckwith, J., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Genetic analysis of the topological structure of a complex membrane protein, *E. coli* MalF protein.

Garrido, M.C.,[1] Moreno, F.,[1] Kolter, R.,[2] [1]Hospital Ramon y Cajal, Madrid, Spain; [2]Harvard Medical School, Boston, Massachusetts: Immunity to the DNA replication inhibitor microcin B-17 involves its export from the cell.

Sun, T.-P.,[1] Levengood, S.,[1] Russel, M.,[2] Webster, R.E.,[1] [1]Dept. of Biochemistry, Duke University Medical Center, Durham; [2]Rockefeller University, New York, New York: The products of *tol Q*, *R*, and *A* genes are required for infection of the filamentous phage and the entry of the E colicins into *E. coli*.

Schnaitman, C., Catron, K., Click, E.M., Dept. of Microbiology, University of Virginia Medical School, Charlottesville: Regulation of outer membrane protein expression coupled to protein translocation.

Strauch, K.L., Beckwith, J., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Mutations that reduce proteolysis in the periplasm of *E. coli*.

Groisman, E.A., Pagratis, N., Richard, B.H., Casadaban, M., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: In vivo DNA cloning and protein identification in *enteric* bacteria with mini-Mu transposons.

Hasan, N., Szybalski, W., McArdle Laboratory, University of Wisconsin, Madison: Multistep method utilizing *Mbo*II- and *Fok*I-mediated precise deletions with removal of up to 12 base pairs per step.

Sauer, B., Henderson, N., E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware: Cyclization of linear transforming DNA in *E. coli* by Cre-mediated site-specific recombination.

Sznyter, L.A.,[1] Howard, K.A.,[2] Slatko, B.E.,[1] Moran, L.S.,[1] Brooks, J.E.,[1] [1]New England Biolabs, Beverly, [2]Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Sequence and characterization of the *Dde*I restriction-modification system.

Molecular Biology of Mitochondria and Chloroplasts

August 25—August 30

ARRANGED BY

John E. Boynton, Duke University
Ronald A. Butow, University of Texas Health Sciences Center
David A. Clayton, Stanford University
Nicholas W. Gillham, Duke University

286 participants

The Molecular Biology of Mitochondria and Chloroplasts meeting, held in August, 1987, was the first such international conference on the molecular aspects of the biogenesis of both organelles since a similar meeting was held in Munich, Germany in 1976. Approximately 250 presentations were given, of which about 30% were platform talks and the remaining were poster presentations. The major topics, which covered a wide range of organisms, included the organization, expression, and replication of organelle genomes; the targeting and assembly of nuclear-encoded organelle macromolecules; interactions between organelle and nuclear genomes; and the evolution of organelle genomes. The meeting provided a unique opportunity to compare and contrast the remarkably diverse ways in which chloroplasts and mitochondria solve the fundamental problems of arranging, expressing, and replicating their genomes and assembling their various constitutents encoded both by the organelle and nuclear genomes. A major thrust of current research seeks to understand the controls that operate to coordinate organelle biogenesis with nuclear and organelle gene expression.

This meeting was supported in part by Amoco Corporation; Ciba-Geigy Corporation, Del Monte, Inc.; E.I. du Pont De Nemours & Company; Monsanto Company; Smith Kline & French Laboratories; U.S. Department of Energy; U.S. Department of Agriculture; Zoecon Research Institute/Sandoz Crop Protection Corporation; National Science Foundation; Phillips Petroleum Company; Pioneer Hi-Bred International, Inc.; and the following divisions of the National Institutes of Health: National Institute of Diabetes and Digestive and Kidney Diseases; National Institute of General Medical Sciences.

SESSION 1A OPENING LECTURES

Chairman: D. von Wettstein, Carlsberg Laboratory

P. Slonimski, Centre de Genetique Moleculaire du CNRS: Mitochondria
L. Bogorad, Biological Laboratories Harvard University: Chloroplasts

SESSION 1B REPLICATION OF ORGANELLE GENOMES

Chairman: M. Simpson, State University of New York, Stony Brook

Ryan, K.A., Englund, P.T., Dept. of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland: Replication of kinetoplast DNA in trypanosomes.

Wu, M., Lou, J.K., Nie, Z.Q., Wang, Z.F., Dept. of Biological Sciences, University of Maryland, Baltimore: Initiation of chloroplast DNA replication in C. *reinhardtii*.

Chang, D.D., Topper, J.N., Clayton, D.A., Dept. of Pathology, Stanford University School of Medicine, California: Sequence analysis of a nuclear gene that encodes the RNA component of an mtRNA processing enzyme.

SESSION 2 GENES AND GENOMES

Chairman: E. Stutz, Université de Neuchâtel

Ohyama, K.,[1] Kohchi, T.,[1] Umesono, K.,[2] Ozeki, H.,[2] [1]Research Center for Tissue and Cell Culture, Faculty of Agriculture, [2]Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Entire gene organization of the *M. polymorpha* chloroplast genome and gene expression (*trans*-splicing).

Shinozaki, K., Sugiura, M., Center for Gene Research, Nagoya University, Japan: Organization and expression of tobacco chloroplast genes.

Fromm, H., Galun, E., Edelman, M., Dept. of Plant Genetics, Weizmann Institute of Science, Rehovot, Israel: Molecular genetics of rRNA-dependent spectonomycin resistance in *Nicotiana* chloroplasts.

Clark-Walker, G.D., Research School of Biological Sciences, Australian National University, Canberra, Australia: Creation of rearranged mtDNA in *S. cerevisiae* – Consequences for gene expression and genome evolution.

Wolstenholme, D.R., Okimoto, R., Macfarlane, J.L., Clary, D.O., Garey, J.R., Wahleithner, J.A., Dept. of Biology, University of Utah, Salt Lake City: Mitochondrial genomes of lower invertebrates.

Lonsdale, D.M., Rottmann, W., Dept. of Molecular Genetics, Plant Breeding Institute, Cambridge, England: *T-URF13* – A gene uniquely associated with the mitochondrial genome and male sterility in the T cytoplasm of maize.

Pring, D.R.,[1] Gengenbach, B.G.,[2] Kennell, J.C.,[1] Wise, R.P.,[1] [1]USDA-ARS, Dept. of Plant Pathology, University of Florida, Gainesville; [2]Dept. of Agronomy and Plant Genetics, University of Minnesota, St. Paul: Mitochondrial mutants of T (Texas) cytoplasm maize.

Levings, C.S., Dept. of Genetics, North Carolina State University, Raleigh: Cytoplasmic male sterility in maize.

Hanson, M.R.,[1] Young, E.G.,[3] Rothenberg, M.,[2] Nivison, H.T.,[1] Pruitt, K.D.,[1] Sections of [1]Genetics and Development, [2]Biochemistry, Cellular and Molecular Biology, Cornell University, Ithaca, New York; [3]Dept. of Biology, University of Virginia, Charlottesville: An abnormal fused mitochondrial gene is associated with cytoplasmic male sterility in *Petunia*.

SESSION 3 POSTER SESSION

Replication

Barat-Gueride, M.,[1] Mignotte, B.,[1] Delain, E.,[2] Mounolou, J.C.,[1] [1]Laboratoire de Biologie Générale, Université Paris-Sud, [2]Laboratoire de Microscopie Cellulaire et Moléculaire, Institut Gustave Roussy, France: A mitochondrial protein with a high affinity for supercoiled DNA.

Bendixen, B., Flatmark, T., Dept. of Biochemistry, University of Bergen, Norway: Mitochondrial thymidine kinase, a regulator of mtDNA replication?

Cairns, S.S., Bogenhagen, D.F., Dept. of Pharmacology, State University of New York, Stony Brook: Detection and analysis of DNA-binding activities in lysates of *X. laevis* mitochondria.

de Haas, J.M., Kors, F., Kool, A., Hille, J., Nijkamp, H.J.J., Dept. of Genetics, Free University, Amsterdam, The Netherlands: Analysis of putative *P. hybrida* chloroplast and mitochondrial replication origins.

Dunon-Bluteau, D.,[1] Cordonnier, A.,[1] Brun, G.,[2] [1]Section de Biologie, Institut Curie, Paris, [2]Laboratoire de Biochimie, Ecole Normale Superieure de Saint-Cloud, France: In vitro replication of *Xenopus* mtDNA – Initiation and elongation of the H strand.

Ragnini, A., Fukuhara, H., Institut Curie Section de Biologie, Centre universitaire, Orsay, France: Amplification of mtDNA segments in the petite-negative yeast *K. lactis*.

Kaguni, L.S., Wernette, C.M., Conway, M.C., Dept. of Biochemistry, Michigan State University, East Lansing: Structural and catalytic features of the mtDNA polymerase from *D. melanogaster* embryos.

Cummings, O.W.,[1] King, T.C.,[1] Holden, J.A.,[1] Buzan, J.,[1] Low, R.L.,[1,2] Depts. of [1]Pathology, [2]Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri: The bovine mitochondrial endonuclease prefers a conserved sequence tract in the D-loop region.

Michaels, G.S.,[1] Laipis, P.J.,[2] Hauswirth, W.W.,[3] [1]Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland; Depts. of [2]Biochemistry and Molecular Biology, [3]Immunology and Medical Microbiology, University of Florida, Gainesville: Developmental accumulation of mitochondria and mtDNA in mammalian oocytes.

Solignac, M.,[1] Génermont, J.,[2] Monnerot, M.,[2] Mounolou, J.C.,[2] [1]Laboratoire de Biologie et Génétique Evolutives, CNRS, [2]Université de Paris, Orsay, France: How do *Drosophila* maintain repeated sequences in the noncoding region of their mtDNA?

Nass, M.M.K., Dept. of Radiation Therapy, University of Pennsylvania School of Medicine, Philadelphia: Mammalian mtDNA replication and protein synthesis patterns in response to heat shock.

Nielsen, B., Meeker, R., Tewari, K.K., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: In vitro replication of pea chloroplast DNA recombinants confirms location of the replication origins in pea chloroplast DNA.

Sbisà, E.,[1] Loguercio Polosa, P.,[2] Cantatore, P.,[2] Saccone, C.,[2] [1]CSMME CNR, Bari, [2]Dept. of Biochemistry/Molecular Biology, University of Bari, Italy: Characterization of H-strand transcripts in the replication origin of rat mtDNA.

Silliker, M.E., Cummings, D.J., Dept. of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver: Analysis of reverse transcriptase activity in wild-type strains and a temperature-sensitive mutant of *P. anserina*.

Keilbaugh, S.A.,[1] De Giorgi, C.,[2] Simpson, M.V.,[1] [1]Dept. of Biochemistry, State University of New York, Stony Brook; [2]Dept. of Biochemistry and Molecular Biology,

E. Stutz, R. Hallick, C. Price, D. von Wettstein

University of Bari, Italy: Studies of DNA polymerase γ using a supercoiled template.

Surzycki, S.J., Fong, S., Hong, T.-H., Opperman, T., Dept. of Biology, Indiana University, Bloomington: Identification and analysis of expression of chloroplast genes involved in transcription, DNA replication, and repair in *C. reinhardtii*.

Hoke, G.D., Ledwith, B.J., Van Tuyle, G.C., Dept. of Biochemistry and Molecular Biophysics, Virginia Commonwealth University, Richmond: Binding parameters and functional characteristics of the rat mtDNA-binding protein P16.

Genes and Genomes

Boer, P.H., Gray, M.W., Dept. of Biochemistry, Dalhousie University, Halifax, Canada: Scrambled rRNA gene pieces in algal mitochondria.

Bryant, D.A., Annarella, M.B., Stirewalt, V.L., Dept. of Molecular and Cell Biology, Pennsylvania State University, University Park: The *atpD* gene of *C. paradoxa* is encoded by cyanellar DNA and there is no intron in its *atpF* gene.

Cantatore, P., Roberti, M., Loguercio Polosa, P., Rainaldi, G., Saccone, C., Gadaleta, M.N., Dept. of Biochemistry and Molecular Biology, University of Bari, Italy: Organization and expression of *P. lividus* mtDNA.

Csaikl, F.,[1] Horvath, S.,[1] Csaikl, U.,[1,2] Institutes of [1]Tumorbiology, [2]Applied and Experimental Oncology, Vienna, Austria: Mitochondrial genome of the guinea pig (*C. porcellus*).

Fauron, C., Howard Hughes Medical Institute, University of Utah, Salt Lake City: Maize mtDNA—A comparison of restriction maps in various types.

Howell, N., Nalty, N.S., Gilbert, K., Dept. of Radiation Therapy, University of Texas Medical Branch, Galveston: Mutational analysis of the mitochondrial cytochrome *b* gene.

Jacobs, H.T., Elliott, D.J., Math, V.R., Farquharson, A., Dept. of Genetics, University of Glasgow, Scotland: Sequence and gene organization of sea urchin mtDNA.

Joyce, P.B.M.,[1] Spencer, D.F.,[1] Bonen, L.,[2] Gray, M.W.,[1] [1]Dept. of Biochemistry, Dalhousie University, Halifax, [2]Dept. of Biology, University of Ottawa, Canada: Wheat mitochondrial tRNA genes.

Kipp, D.V., Kuhl, S.A., Birky, C.W., Dept. of Molecular Genetics, Ohio State University, Columbus: Isolation and characterization of leukoplast DNA from *P. uvella*.

Kück, U., Lehrstuhl für Allgemeine Botanik, Ruhr Universität Bochum, Federal Republic of Germany: mtDNA from a chlorella-like alga—isolation and characterization of organelle genes.

Lee, R.W.,[1] Dumas, C.,[2] Lemieux, C.,[2] Turmel, M.,[2] [1]Dept. of Biology, Dalhousie University, Halifax, [2]Dept. de biochimie, Faculté des sciences et génie, Université Laval, Québec, Canada: Physical and gene mapping of the *C. moewusii* mitochondrial genome.

Leon-Mejia, P., Bedinger, P., Walbot, V., Dept. of Biological Sciences, Stanford University, California: Molecular characterization of the 2.3-kb linear plasmid of maize mitochondria.

Löffelhardt, W.,[1] Mucke, H.,[1] Janssen, I.,[1] Pfanzagl, B.,[1] Berenguer, J.,[2] de Pedro, M.A.,[2] Michalowski, C.,[3] Bohnert, H.J.,[3] [1]Institut für Allgemeine Biochemie der Universität Wien und Ludwig Boltzmann-Forschungsstelle für Biochemie, Vienna, Austria; [2]Centro de Biologia Molecular, Universidad Autonoma de Madrid, Spain; [3]Dept. of Biochemistry, University of Arizona, Tucson: Molecular biology of the cyanelles from *C. paradoxa*.

Masters, B.S., Stohl, L.L., Clayton, D.A., Dept. of Pathology, Stanford University School of Medicine, California: Yeast mtRNA polymerase is homologous to those encoded by bacteriophages T3 and T7.

Morin, G.B., Cech, T.R., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: The mitochondrial telomeres of six species of *Tetrahymena* form four groups possessing unusual features.

Okimoto, R., Wostenholme, D.R., Dept. of Biology, University of Utah, Salt Lake City: *C. elegans* mtDNA.

Rasmussen, J.L., Hanson, M.R., Section of Genetics and Development, Cornell University, Ithaca, New York: An ORF in the 3′ flank of a *Petunia* CMS-associated gene.

Robertson, D.,[1] Woessner, J.P.,[1] Gillham, N.W.,[1] Boynton, J.E.,[2] Depts. of [1]Zoology, [2]Botany, Duke University, Durham, North Carolina: Molecular analysis of *C. reinhardtii* point mutants mapping in the chloroplast-encoded *atpB* gene encoding the β-subunit of the ATP synthase.

Cattolico, R.A., Shivji, M.S., Dept. of Botany, University of Washington, Seattle: Chloroplast DNA analysis of chromophytic and rhodophytic algae.

Siculella, L., Palmer, J.D., Dept. of Biology, University of Michigan, Ann Arbor: Physical and gene organization of the sunflower mitochondrial genome.

Suyama, Y., Ziaie, Z., Jenney, F., Dept. of Biology, University of Pennsylvania, Philadelphia: *Tetrahymena* mitochondrial genome—tRNA genes, protein-coding sequences, genome organization, and the genetic code.

Turmel, M., Bergeron, A., Boulanger, J., Lemieux, C., Dépt. de biochimie, Faculté de sciences et génie, Université Laval, Québec, Canada: Sequence analysis of two regions of major deletion/addition differences in the chloroplast genomes of *C. eugametos* and *C. moewusii*.

Wahleithner, J.A., Wolstenholme, D.R., Dept. of Biology, University of Utah, Salt Lake City: Ribosomal protein S14 genes in broad bean mtDNA.

Yamada, T., Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan: Rearrangements of the chloroplast genome—Unusual organization of the *Chlorella* chloroplast rRNA operons.

Transmission, Segregation, and Recombination

Aviv, D., Galun, E., Dept. of Plant Genetics, Weizmann Institute of Science, Rehovot, Israel: Can mitochondrial composition of cybrid plants be controlled?

Chiu, W.-L.,[1] Stubbe, W.,[2] Sears, B.B.,[1] [1]Dept. of Botany and Plant Pathology, Michigan State University, East Lansing: Institute of Botany, University of Dusseldorf, Federal Republic of Germany: The organelle genome determines the extent of biparental plastid inheritance in *Oenothera*.

Foury, F., Genga, A., Lahaye, A., Van Dyck, E., Laboratory of Enzymology, Belgium: Characterization of nuclear genes involved in recombination and/or replication of yeast mtDNA.

Gross, S.R., Mary, A., Levine, P.H., Dept. of Biochemistry, Duke University, Durham, North Carolina: Amplification of short DNA sequences in circular derivatives of the mitochondrial chromosome of *Neurospora*.

Haring, M.A., Hille, J., Nijkamp, H.J.J., Dept. of Genetics, Free University, Amsterdam, The Netherlands: Chloroplast transformation—Development of integrating and replicating vectors.

Harris, E.H., Boynton, J.E., Gillham, N.W., Depts. of Botany and Zoology, Duke University, Durham, North Carolina: Genetic and physical mapping of antibiotic resistance mutations in *C. reinhardtii*.

Hurko, O.,[1] Reynafarje, B.,[2] Kuncl, R.,[1] McKee, L.,[1] Feldman, E.,[2] Depts. of [1]Neurology, [2]Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Defective cytochrome oxidase activity in siblings from a pedigree suggestive of matroclinal inheritance and heteroplasmy.

King, M.P., Attardi, G., Division of Biology, California Institute of Technology, Pasadena: Transmitochondrial human cell lines—A new approach to mitochondrion-mediated transformation.

Kuroiwa, T.,[1] Nakamura, S.,[2] [1]Dept. of Biology, Faculty of Science, University of Tokyo, [2]Dept. of Cell Biology, National Institute for Basic Biology, Okazaki, Japan: Polypeptides responsible for maternal inheritance of chloroplast DNA in *C. reinhardtii*.

Matagne, R.F., Bovie, C., Loppes, R., Dept. of Botany, University of Liège, Belgium: Transmission of chloroplast genes and mtDNA in diploids resulting from artificial fusion or sexual crosses between *C. reinhardtii* and *C. smithii*.

Morawiec, A., Gerber, D., Perlman, P., Dept. of Molecular Genetics, Ohio State University, Columbus: Studies of petite formation in yeast mitochondria.

Kaplan, S.A., Blasko, K., Johnson, E.M., Lim, P.-O., Wolfson, R., Sears, B.B., Dept. of Botany and Plant Pathology, Michigan State University, East Lansing: The *Oenothera* plastome mutator line is heteroplasmic for chloroplast DNA restriction-fragment-length polymorphisms.

VanWinkle-Swift, K.P., Salinger, A.P., Dept. of Biology, Texas A & M University, College Station: Loss of mt$^+$-derived chloroplast DNA is associated with a lethal allele in *C. monoica*.

Wenzlau, J.,[1] Pohlman, J.,[2] Morawiec, A.,[1] Dietrich, R.,[1] Butow, R.,[2] Perlman, P.,[1] [1]Dept. of Molecular Genetics, Ohio State University, Columbus; [2]Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Conversion of optional sequences in mitochondrial crosses.

Uthayashanker, R., Zassenhaus, H.P., Dept. of Microbiology, St. Louis University, Missouri: Effect of nuclease deficiency on mitochondrial recombination and gene conversion.

SESSION 4 TRANSMISSION, SEGREGATION, AND RECOMBINATION OF ORGANELLE GENOMES

Chairman: R. Sager, Sidney Farber Cancer Institute

Coleman, A.W., Brown University, Providence, Rhode Island: Thoughts on how/why cells control plastid genome

number.

Boynton, J.E., Harris, E.H., Gillham, N.W., Depts. of Botany and Zoology, Duke University, Durham, North Carolina: Mating type and organelle genome transmission in *C. reinhardtii*.

Lemieux, C., Gauthier, A.,[1] Lee, R.W.,[2] Lemieux, B.,[1] Turmel, M.,[1] [1]Dépt. de biochimie, Faculté des sciences et génie, Université Laval, Québec, [2]Dept. of Biology, Dalhousie University, Halifax, Canada: Intron-mediated gene conversion in the chloroplast of *Chlamydomonas*.

Spreitzer, R.J., Zhang, D., Chastain, C.J., Dept. of Agriculture and Biochemistry, University of Nebraska, Lincoln: Heteroplasmicity mediates informational suppression of chloroplast nonsense mutations in *Chlamydomonas*.

Perrin, A., Monteilhet, C., Colleaux, L., Dujon, B., Center for Molecular Genetics, Université Pierre et Marie Curie, Gif-sur-Yvette, France: Biochemical activity of an intron-encoded transposase of *S. cerevisiae*.

Maliga, P., Fejes, E., Engler, D., Advanced Genetic Sciences, Inc., Oakland, California: Extensive homologous chloroplast DNA recombination in a *Nicotiana* somatic hybrid.

Laipis, P.J., Hauswirth, W.W., Depts. of Biochemistry and Molecular Biology and Immunology and Medical Microbiology, University of Florida, Gainesville: Transmission genetics of mtDNA in heteroplasmic animals.

Perlman, P.S.,[1] Butow, R.A.,[2] [1]Dept. of Molecular Genetics, Ohio State University, Columbus; [2]Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Recombination processes in yeast mitochondria.

SESSION 5 EXPRESSION OF ORGANELLE GENES. I. INTRON SPLICING

Chairman: P. Perlman, Ohio State University

Benne, R.,[1] Sloof, P.,[1] de Vries, B.F.,[1] Hakvoort, T.,[2] Muijsers, A.O.,[2] [1]Laboratory of Biochemistry, [2]Section for Molecular Biology and Enzymology, University of Amsterdam, The Netherlands: Unusual features of kinetoplast DNA from trypanosomes—Novel mechanism of gene expression (RNA-editing), the absence of tRNA genes, and the variable presence of a cytochrome oxidase subunit gene.

Wolf, K., Merlos-Lange, A.M., Ahne, A., Zimmer, M., Institut für Genetik und Mikrobiologie der Universität Munchen, Federal Republic of Germany: Introns as mobile genetic elements—DNA splicing and horizontal gene transfer.

Akins, R.A., Cherniack, A., Lambowitz, A.M., Depts. of Molecular Genetics and Biochemistry, Ohio State University, Columbus: A protein component required for splicing group I introns in *Neurospora* mitochondria is mitochondrial tyrosyl-tRNA synthetase or some derivative thereof.

Müller, M.W.,[1,2] Schmelzer, C.,[1] Augustin, S.,[1,2] Schweyen, R.J.,[2] [1]Institut für Genetik und Mikrobiologie, Universität München, Federal Republic of Germany: Institut für Mikrobiologie und Genetik, Universität Wien, Austria: Catalytic activities of group II introns in vitro.

Jacq, C., Banroques, J., Becam, A., Creusot, F., Goguel, V., Mendelzon, J., Perea, J., Center for Molecular Genetics, CNRS, Gif-sur-Yvette, France: Intron-encoded RNA maturases of yeast mitochondria. In vivo and in vitro properties of the bI4 RNA maturase.

Peebles, C.L.,[1] Benatan, E.J.,[1] Jarrell, K.A.,[2] Dietrich, R.C.,[2] Uhl, T.M.,[2] Perlman, P.S.,[2] [1]University of Pittsburgh, Pennsylvania; [2]Ohio State University, Columbus: *Cis* and *trans* reactions of mutant and wild-type forms of a group II intron.

Montandon, P.E., Stutz, E., Laboratoire de Biochimie, Université de Neuchâtel, Switzerland: *E. gracilis* chloroplast DNA—Analysis of the spliced *tufA*-ORF-206 mRNA.

Kück, U., Lehrstuhl für Allgemeine Botanik, Ruhr Universität Bochum, Federal Republic of Germany: The discontinuous chloroplast gene for the P700 chlorophyll a-apoprotein from *C. reinhardtii*—Evidence for *trans*-splicing of pre-mRNAs.

SESSION 6 POSTER SESSION

Expression I. Introns and Splicing

Bordonné, R., Amé, J.C., Schwob, E., Dirheimer, G., Martin, R.P., Laboratory of Biochemistry, CNRS, Strasbourg, France: Expression of yeast mitochondrial genes and characterization of RNA processing activities.

Hildebrand, M.,[1] Hallick, R.B.,[1] Passavant, C.W.,[2] Bourque, D.P.,[1] Depts. of Biochemistry, Molecular and Cellular Biology, and Nutrition and Food Sciences, [1]University of Arizona, Tucson; [2]Dept. of Microbiology, Oregon State University, Corvallis: *Trans*-splicing in chloroplasts—The *rps12* loci of *N. tabacum*.

Carignani, G.,[1] Bergantino, E.,[1] Netter, P.,[2] [1]Istituto di Chimica Biologia dell'Università, Padova, Italy: [2]Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Analysis of cis- and trans-acting mutations affecting the splicing of two class II introns.

Collins, R.A., Dept. of Botany, University of Toronto, Canada: Conserved secondary structures outside the "core" in a large subclass of group I introns.

Serizawa, N., Dobinson, K.F., Lambowitz, A.M., Dept. of Molecular Genetics and Biochemistry, Ohio State University, Columbus: Characterization of *cyt-4*, a *Neurospora* nuclear gene required for processing of mitochondrial rRNA.

Séraphin, B., Simon, M., Faye, G., Institut Curie-Biologie, Centre Universitaire, Orsay, France: *MSS18*, a yeast nuclear gene involved in the splicing of intron aI5β of the mitochondrial COX1 transcript.

Gampel, A., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: A nuclear gene product promotes splicing of the terminal intervening sequence of mitochondrial cytochrome *b* pre-mRNA.

Altamura, N., Ben-Asher, E., Dujardin, G., Groudinsky, O., Herbert, C.J., Kermorgant, M., Labouesse, M., Slonimski, P.P., Center for Molecular Genetics, CNRS, Gif-sur-Yvette,

France: *NAM1* and *NAM2*—Nuclear genes involved in mtRNA splicing and translation.

Kreike, J.,[1,2] Schmidt, N.,[1] Söllner, T.,[1] Schweyen, R.J.,[2] [1]Institute for Genetics and Microbiology, University of Munich, Federal Republic of Germany, [2]Institute for Microbiology and Genetics, University of Vienna, Austria: Involvement of nuclear genes in mtRNA splicing in yeast.

Morales, M.J., Hollingsworth, M.J., Chen, J.-Y., Martin, N.C., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Nuclear and mitochondrial gene products necessary for endonucleolytic cleavages that liberate mitochondrial tRNAs from their primary transcripts.

Kohchi, T.,[1] Ogura, Y.,[1] Ohyama, K.,[1] Umesono, K.,[2] Nakahigashi, K.,[2] Ozeki, H.,[2] [1]Research Center for Cell and Tissue Culture, Faculty of Agriculture, [2]Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Biogenesis of chloroplast ribosomes—In vivo expression of a *trans*-split gene, *rps12*, for chloroplast ribosomal protein S12.

Osiewacz, H.D., Kück, U., Esser, K., Lehrstuhl für Allgemeine Botanik, Ruhr Universität, Bochum, Federal Republic of Germany: Expression of an ORF encoded by the first intron (p1DNA) of the gene coding for cytochrome c oxidase subunit I of *P. anserina*.

Cushman, J.C., Price, C.A., Waksman Institut of Microbiology, Rutgers University, Piscataway, New Jersey: Development-specific splicing of plastid genes in *Euglena*.

Shaw, J.M., Simpson, L., Dept. of Biology and Molecular Biology Institute, University of California, Los Angeles: RNA editing of maxicircle ORF 3/4 and COII mRNAs in *L. tarentolae*—Evidence for translation of COII mRNA through the frameshift region.

Hofmann, T.J., Dake, E., Zassenhaus, H.P., Dept. of Microbiology, St. Louis University, Missouri: RNA processing catalyzed by mitochondrial extracts prepared from a *nuc1* yeast strain.

Expression II and III. Transcriptional and Translational Regulation

Akada, S., Xu, Y.Q., Machii, H., Kung, S.D., Agricultural Biotechnology Center and Dept. of Botany, University of Maryland, College Park: Studies of *rbcL* transcription termination in heterologous and homologous systems.

Bai, U., Brody, M., Dept. of Biological Sciences, Hunter College, City University of New York, New York, New York: II. *Ricinus* chloroplasts—Source of a lectin/β-galactosidase; in vitro translation studies.

Balaji, K.S.K., Rajamanickam, C., Dept. of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, India: Transcriptional stimulation of *CYC1* gene expression by hemin in cells of *S. cerevisiae*—Possible mediation by a hemoprotein.

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Berends, T., Gamble, P.E., Muilet, J.E., Dept. of Biochemistry and Biophysics, Texas A & M University, College Station: Characterization of the barley chloroplast transcription units containing *psaB-psaB* and *psbD-psbC*.

Bonen, L., Bird, S., Dept. of Biology, University of Ottawa, Canada: Transcript analysis of wheat mitochondrial protein-coding genes.

Brody, M., Bai, U., Dept. of Biological Sciences, Hunter College, City University of New York, New York, New York: I. *Ricinus* chloroplasts—Source of a lectin/β-galactosidase; in vitro translation studies.

Brown, T.A., Dyson, N.J., Waring, R.B., Davies, R.W., Dept. of Biochemistry and Applied Molecular Biology, UMIST, Manchester, England: The Mitochondrial ribosomal RNA molecules of *A. nidulans*.

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Erdös, G.,[1] Chen, H.-Q.,[1] Buetow, D.E.,[1,2] Depts. of [1]Physiology and Biophysics, [2]Plant Biology, University of Illinois, Urbana: Developmental-stage-specific controls regulate LHCII nuclear gene expression during light-induced chloroplast development in cultured soybean cells.

Castora, F.J., Brothers, P.N., Dept. of Chemistry, University of Maryland Baltimore County, Catonsville: A transcription promoter of rat mtDNA found using prokaryotic promoter identification vector pK01.

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Chanut, F., Grabau, E.A., Gesteland, R.F., Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City: Organization and transcription of the αATPase gene of soybean mitochondria.

Chiu, I., Fink, G.R., MIT/Whitehead Institute, Cambridge, Massachusetts: The cytoplasmic and mitochondrial histidine-tRNA synthetases can functionally replace each other in vivo.

Chomyn, A., Tarle, I., Strathman, M., Attardi, G., Division of Biology, California Institute of Technology, Pasadena: Translational regulation of mitochondrial gene expression in human cells.

Denslow, N.D.,[1] LiCata, V.J.,[2] Gualerzi, C.,[3] O'Brien, T.W.,[1] [1]University of Florida, Gainesville; [2]Johns Hopkins University, Baltimore, Maryland; [3]Max-Planck-Institute for Molecular Genetics, Berlin, Federal Republic of Germany: Interaction of *E. coli* IF3 with bovine mitochondrial ribosomes.

Feagin, J.E.,[1] Shaw, J.,[2] Simpson, L.,[2] Stuart, K.,[1] [1]Seattle Biomedical Research Institute, Washington; [2]Dept. of Biology, University of California, Los Angeles: Addition of nucleotides within cytochrome *b* transcripts of kinetoplastids.

Fisher, R.P., Topper, J.N., Clayton, D.A., Dept. of Pathology, Stanford University School of Medicine, California: Promoter selection of human mitochondria involves binding of a transcription factor to orientation-independent upstream regulatory elements.

Gamble, P.E., Berends, T., Mullet, J.E., Dept. of Biochemistry and Biophysics, Texas A & M University, College Station: Illumination of dark-grown barley causes the accumulation of two RNAs that hybridize to the *psbD-psbC* transcription unit.

Gounaris, I., Price, C.A., Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Plastid genes and gene transcripts in chromoplasts and chloroplasts of *C. annuum*.

Hadjeb, N., Price, C.A., Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Chromoplast-specific proteins in *C. annuum*.

Herrin, D.L., Battey, J.F., Greer, K.L., Schmidt, G.W., Botany Dept., University of Georgia, Athens: Pigment control of LHCII expression in *Chlamydomonas*.

Hosler, J.P., Boynton, J.E., Gillham, N.W., Depts. of Botany and Zoology, Duke University, Durham, North Carolina: Posttranscriptional regulation of protein synthesis in the chloroplast of the green alga *C. reinhardtii*.

Kobayashi, H.,[1] Ngernprasirtsiri, J.,[2] Macherel, D.,[2] Akazawa, T.,[2] [1]Radioisotope Center, [2]Research Institute for Biochemical Regulation, Nagoya University, Japan: Transcriptional regulation of genes for photosynthesis in nonphotosynthetic plastids.

Kobayashi, H.,[1] Viale, A.M.,[2] Valle, E.,[2] Akazawa, T.,[2] [1]Radioisotope Center, [2]Research Institute for Biochemical Regulation, Nagoya University, Japan: Gene expression and subunit assembly of RuBisCO.

Liu, X-Q., Hosler, J.P., Boynton, J.E., Gillham, N.W., Depts of Botany and Zoology, Duke University, Durham, North Carolina: Preferential translation of chloroplast ribosomal proteins in *C. reinhardtii*.

Neuhaus, H., Hughes, J.E., Link, G., University of Bochum, Plant Cell Physiology, Federal Republic of Germany: Structure, transcription, and expression in *E. coli* and in vivo of the split *trnK* gene from mustard (*S. alba*) chloroplasts.

Thomas, F., Briat, J.F., Massenet, O., Seyer, P., Mache, R., Laboratoire de Biologie Moleculaire Vegetale, CNRS, Universite de Grenoble, France: Expression of the chloroplastic S10-like operon in spinach.

Mannan, M.R., Krishnan, M., Gnanam, A., School of Biological Science, Madurai Kamaraj University, India: Synthesis of polypeptides associated with PSI by the isolated *S. vulgare* chloroplast.

Thompson, R.J., Mosig, G., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Repression of a *Chlamydomonas* chloroplast promoter in *E. coli* by torsional stress involves integration host factor.

Mulligan, R.M., Walbot, V., Dept. of Biological Sciences, Stanford University, California: Transcription initiation and RNA processing in maize mitochondria.

Narasimhan, N., Attardi, G., Division of Biology, California Institute of Technology, Pasadena: Specific requirement for ATP at an early step of in vitro transcription of human mtDNA.

Hu, M.-C.,[1] Orozco, E.M., Jr.,[1,2,3] [1]Depts. of Plant Biology, [2]Agronomy, University of Illinois, Urbana; [3]USDA Agricultural Research Service, Urbana, Illinois: In vitro transcription start sites of the spinach chloroplast *atpB* gene.

Ragnini, A.,[1] Francisci, S.,[1] Zennaro, E.,[1] Palleschi, C.,[1] Frontali, L.,[1] Bolotin Fukuhara, M.,[2] [1]Dept. of Cell and Developmental Biology, University of Rome, Italy; [2]Laboratoire de Génétique Moléculaire, Université Paris Sud, France: Expression of the mitochondrial tRNA genes in *S. cerevisiae*.

Schön, A.,[1] Kannangara, C.G.,[2] Gough, S.,[2] Söll, D.,[1] [1]Dept. of Molecular Biochemistry and Biophysics, Yale University, New Haven, Connecticut; [2]Dept. of Physiology, Carlsberg Research Laboratories, Copenhagen, Denmark: tRNA mischarging and a transamidase reaction are required for normal protein biosynthesis in organelles – A tRNA-dependent amidotransferase synthesizes Gln-tRNA^{Gln} from Glu-tRNA^{Gln}.

Silk, G.W., Wu, M.C., Dept. of Biological Sciences, University of Maryland, Baltimore County, Catonsville: Rapid light regulation of chloroplast transcript levels in *C. reinhardtii*.

Feagin, J.E., Stuart, K., Seattle Biomedical Research Institute, Washington: Developmental regulation of U addition within mitochondrial transcripts of *T. brucei*.

van Grinsven, M.Q.J.M., Kneppers, T.J.A., Hille, J., Nijkamp, H.J.J., Kool, A.J., Dept. of Genetics Vrije Universiteit de Boelelaan, Amsterdam, The Netherlands: Regulation of chloroplast gene expression during plastid biogenesis.

White, D., Michaels, A., Dept. of Biology, University of South Florida, Tampa: Constant and variable chloroplast mRNA abundance during the cell cycle of *C. reinhardtii*.

Zhu, H., Macreadie, I.G., Butow, R.A., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: RNA processing and expression of an intron-encoded protein in yeast mitochondria – Role of a conserved dodecamer sequence.

SESSION 7 EXPRESSION OF ORGANELLE GENES. II. TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION

Chairman: R. Herrmann, Botanisches Institut de Ludwig-Maximilians-Universität

Mullet, J.E., Klein, R.R., Gamble, P.E., Berends, T., Baumgartner, B.J., Dept. of Biochemistry and Biophysics, Texas A & M University, College Station: Transcriptional and posttranscriptional regulation of chloroplast gene expression during barley leaf biogenesis.

Rochaix, J.-D.,[1] Day, A.,[1] Mayfield, S.,[1] Goldschmidt-Clermont, M.,[1] Choquet, Y.,[1] Erickson, J.,[1] Kuchka, M.,[1] Kück, U.,[1] Girard-Bascou, J.,[2] Bennoun, P.[3] [1]Dept. of Molecular Biology, University of Geneva, Switzerland; [2]Ruhr Universität Bochum, Federal Republic of Germany; [3]Institut de Biologie Physico-Chimique, Paris, France: Structure and expression of genes involved in photosynthesis in *C. reinhardtii*.

Gruissem, W., Deng, X.W., Jones, H., Stern, D., Tonkyn, J., Dept. of Botany, University of California, Berkeley: Transcriptional and posttranscriptional control of chloroplast gene expression.

Whitfield, P.R., Hudson, G.S., Holton, T.A., Bottomley, W., CSIRO, Division of Plant Industry, Canberra, Australia: Are there two β'-type subunits in chloroplast RNA polymerases?

Westhoff, P.,[1] Herrmann, R.G.,[1] Eskins, K.,[2] Grune, H.,[1] Muller, M.,[3] Oswald, A.,[1] Schrubar, H.,[1] Streubel, M.,[1] Institutes of [1]Botany and [3]Genetics, University of Munich, Federal Republic of Germany, [2]USDA Northern Regional Research Laboratory, Peoria, Illinois: Gene expression during thylakoid membrane biogenesis.

Schmidt, G.W., Botany Dept., University of Georgia, Athens: Chloroplast transcription and translation – Dependence on nuclear gene products.

Grienenberger, J.M., Wintz, H., Runeberg-Roos, P., Maréchal, L., Jeannin, G., Weil, J.H., Institut de Biologie Moléculaire et Cellulaire, Université L. Pasteur, Strasbourg, France: Localization of tRNA genes present in the mitochondrial genomes of maize and wheat.

Hallick, R., Dept. of Biochemistry, University of Arizona, Tucson: Chloroplast *rpoA*, *rpoB*, and *rpo*C genes encode subunits of chloroplast DNA-dependent RNA polymerases.

SESSION 8A EXPRESSION OF ORGANELLE GENES. III. TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION

Chairman: N. Martin, University of Texas Health Science Center

Simpson, L., Simpson, A.M., Shaw, J., Dewes, H., Dept. of Biology and Molecular Biology Institute, University of California, Los Angeles: Transcription of mitochondrial maxicircle and minicircle DNAs in *L. tarentolae*.

Hauswirth, W.W., McCarty, D.M., Muise, R.C., Dickel, C.D., Dept. of Immunology and Medical Microbiology, University of Florida, Gainesville: Maize mitochondrial transcription initiation in vivo and in vitro.

Jaehning, J.A., Wilcoxen, S.E., Peterson, C.R., Schultz, P.W., Marczynski, G.T., Dept. of Biology, Indiana University, Bloomington: Transcription by the yeast mtRNA polymerase.

Bogenhagen, D.F., Romanelli, M., Insdorf, N.F., Dept. of Pharmacology, State University of New York, Stony Brook: Nucleotide sequence and protein requirements for in vitro transcription of *X. laevis* mtDNA.

Getz, G.S.,[1,2,3] Biswas, T.K.,[2] Ticho, B.,[3] Mueller, D.,[2] Wettstein-Edwards, J.,[2] Backer, J.,[2] Edwards, J.,[2] Sanyal, A.,[2] Rabinowitz, M.,[2] Depts. of [1]Pathology, [2]Medicine, [3]Biochemistry/Molecular Biology, University of Chicago, Illinois: Regulation of transcription in yeast mitochondria.

SESSION 8B IMPORT AND ASSEMBLY OF PROTEINS BY ORGANELLES. I.

Chairman: J.-D. Rochaix, Université de Geneve

Erickson, J.M.,[1] Rochaix, J.-D.,[2] [1]Dept. of Biology, University of California, Los Angeles; [2]Depts. of Molecular and Plant Biology, University of Geneva, Switzerland: PSII core proteins – Structure, function, assembly, and gene regulation.

Smeekens, S.,[1,3] Hageman, J.,[1] de Boer, D.,[1] Cremers, F.,[1] Bauerle, C.,[2] Keegstra, K.,[2] Weisbeek, P.,[1] [1]Dept. of Molecular Cell Biology, University of Utrecht, The Netherlands, [2]Dept. of Botany, University of Wisconsin, Madison, [3]Division of Molecular Plant Biology, University of California, Berkeley: Protein transport toward different chloroplast compartments – In vitro and in vivo analysis.

Kohorn, B.D.,[1] Tobin, E.M.,[2] [1]Dept. of Botany, Duke University, Durham, North Carolina; [2]Dept. of Biology, University of California, Los Angeles: Import and assembly of a nucleus-encoded LHCP into chloroplast membranes.

Herrmann, R.G., Westhoff, P., Bartling, D., Reilander, H., Botanical Institute, Ludwig-Maximilians-Universitat, Munich, Federal Republic of Germany: Structure and modification of genes for thylakoid membranes from spinach and *Oenothera*.

SESSION 9 POSTER SESSION

Import and Assembly

Archer, E.K., Hanson, M.R., Section of Genetics and Development, Cornell University, Ithaca, New York: GOT iso-

zymes in tomato as a system for studying protein targeting.

Bedwell, D.M., Emr, S.D., California Institute of Technology, Pasadena: Redundant mitochondrial import information is present at the amino terminus of the F_1-ATPase β-subunit in yeast.

Berthier, F., Sebald, W., Physiologisch-Chemisches Institut der Universität Koellikerstr., Würzburg, Federal Republic of Germany: Retargeting of human IL-2 into mitochondria depends on the type of targeting sequence.

Buvinger, W.E., Michel, H.P., Bennett, J., Dept. of Biology, Brookhaven National Laboratories, Upton, New York: A functional analysis of transit peptide sequences from an LHCP.

Glaser, S.M., Miller, B.R., Foreman, J.E., Cumsky, M.G., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: The amino-terminal five residues of the *COX4* precursor constitutes a fully functional mitochondrial import signal.

Dumont, M.E., Das, G., Sherman, F., Dept. of Biochemistry, University of Rochester Medical Center, New York: Heme attachment is required for import of cytochrome c into yeast mitochondria.

Hendrick, J., Sztul, E., Kraus, J., Kalousek, F., Rosenberg, L.E., Yale School of Medicine, New Haven, Connecticut: Two cleavage steps are required for removal of the ornithine transcarbamylase leader peptide.

Kraus, J.P.,[1] Novotny, J.,[2] Kalousek, F.,[1] Swaroop, M.,[1] Rosenberg, L.E.,[1] [1]Yale University School of Medicine, New Haven, Connecticut; [2]Massachusetts General Hospital, Harvard Medical School, Boston: The amino-terminal domain of ornithine transcarbamylase leader is involved in both mitochondrial import and carboxy-terminal cleavage.

Lamppa, G., Abad, M., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Cleavage of the LHCP precursor in vitro.

Leu, S., Michaels, A., Dept. of Biology, University of South Florida, Tampa: Reconstitution of in-vitro-synthesized ps1A2 into CPI.

Linnane, A.W., Willson, T., Marzuki, S., Lukins, H.B., Meltzer, S., Dept. of Biochemistry, Monash University, Clayton, Victoria, Australia: Subunit composition and assembly of yeast mitochondrial ATP synthase.

Liu, X.,[1] Freeman, K.,[2] Shore, G.,[1] [1]Dept. of Biochemistry, McGill University, Montreal, [2]Dept. of Biochemistry, McMaster University, Hamilton, Canada: Topogenesis of mitochondrial inner membrane uncoupling protein.

Toukifimapa, R.,[1] Romby, P.,[2] Rozier, C.,[1] Ehresmann, C.,[2] Westhof, E.,[2] Ebel, J.P.,[2] Ehresmann, B.,[2] Mache, R.,[1] [1]Laboratoire de Biologie Moléculaire Végétale, Université de Grenoble, [2]Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: High-order structure of chloroplastic 5S rRNA and binding to two homologous ribosomal proteins.

Skerjanc, I., Silvius, J., Shore, G., Dept. of Biochemistry, McGill University, Montreal, Canada: The interaction of a synthetic mitochondrial signal peptide with lipid membranes is independent of transbilayer potential.

Sutton, A., Michel, H.P., Buvinger, W.E., Bennett, J., Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Organization and assembly of PSII.

Vainstein, A.,[1] Ferreira, P.,[1] Morishige, D.,[1] Verbeke, J.,[2] Peterson, C.C.,[1] Thornber, J.P.,[1] [1]Dept. of Biology, University of California, Los Angeles; [2]Dept. of Biological Sciences, University of Illinois, Chicago: Expression and import of the PSII LHCIIβ into maize mesophyll and bundle sheath cells.

Viitanen, P.V.,[1] Bacot, K.O.,[1] Dunsmuir, P.,[2] [1]Central Research and Development Dept., Du Pont Experimental Station, Wilmington, Delaware; [2]Advanced Genetic Sciences, Oakland, California: What is the role of the transit peptide in thylakoid integration of the LHCP?

Nuclear-Organelle Interactions

Barkan, A.,[1] Rock, C.,[2] Martienssen, R.,[1] Taylor, W.,[1] [1]Dept. of Genetics, University of California, Berkeley; [2]DOE-Plant Research Laboratory, Michigan State University, East Lansing: Influence of nuclear mutations and cellular differentiation of plastid gene expression in maize.

Daignan-Fornier, B., Su, I., Contamine, V., Bolotin-Fukuhara, M., Laboratoire de Génétique Moléculaire, Université de Paris-Sud, France: Cloning and expression of a yeast mitochondrial ribosomal protein.

Breen, G.A.M., Garnett, K.E., Holmans, P.L., Biology Programs, University of Texas at Dallas, Richardson: Isolation of cDNAs for the α and β subunits of the bovine mitochondrial ATP synthase complex.

Chow, C.M.,[1] Benarous, R.,[2] RajBhandary, U.L.,[1] [1]Dept. of Biology, Massaschusetts Institute of Technology, Cambridge; [2]Institut de Pathologie Moleculaire, Faculte de Medecine Cochin Port Royal, Paris, France: Nuclear genes for the cytoplasmic and mitochondrial forms of leucyl-tRNA synthetase in *N. crassa* map to two different linking groups.

di Rago, J.-P., Meunier, B., Colson, A.-M., Laboratoire de Génétique Microbienne, University of Louvain, Belgium: Molecular basis for resistance to antimycin and diuron inhibitors of the mitochondrial ubiquinol-cytochrome c reductase in *S. cerevisiae*.

Crivellone, M.D., Wu., M., Txagoloff, A., Columbia University, New York, New York: Mechanism of assembly of coenzyme QH_2-cytochrome c reductase in yeast mitochondria.

Kuiper, M.T.R.,[1] Holtrop, M.,[1] Vennema, H.,[1] Lambowitz, A.R.,[2] de Vries, H.,[1] [1]Laboratory of Physiological Chemistry, State University Groningen, The Netherlands; [2]Dept. of Genetics, Ohio State University, Columbus: Characterization of the nuclear gene for mito-ribosomal protein S24 of *N. crassa* – Analysis of its function and its regulation.

Dieckmann, C.L., Cavenagh, M., Gandy, B.J., Dept. of Biochemistry, University of Arizona, Tucson: Deletion of a gene adjacent to *CBP1* results in overproduction of an 86-kD cytoplasmic protein in yeast.

Ellis, S.R.,[1] Hopper, A.K.,[2] Martin, N.C.,[3] [1]Dept. of Biochemistry, University of Louisville, Kentucky; [2]Dept. of Biological Chemistry, Hershey Medical Center, Pennsylvania; [3]Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Characterization of the mitochondrial targeting signals of the *trm1* gene product of *S. cerevisiae*.

Evans, C.T., Srere, P.A., Veterans Administration Medical Center and University of Texas Health Science Center, Dallas: Isolation and nucleotide sequence of a cDNA encoding pig citrate synthase.

Gantt, J.S., Botany Dept., University of Minnesota, St. Paul: Nucleus-encoded plastid ribosomal proteins – Transit sequences and homology with *E. coli*.

Heckmann, J., Langer, C., Weaver, W., Kreader, C., Dept. of Chemistry, Indiana University, Bloomington: Analysis of cDNA clones for two mitochondrial ribosomal proteins of *N. crassa*.

Kispal, G.,[1] Rosenkrantz, M.,[2] Guarente, L.,[2] Srere, P.A.,[1] [1]Veterans Administration Medical Center and University of Texas Health Science Center, Dallas; [2]Massachusetts Institute of Technology, Cambridge: Metabolic effects of citrate synthase deletions in *S. cerevisiae*.

Krishnasamy, S.,[1] Mannan, R.M.,[1] Krishnan, M.,[2] Gnanam, A.,[1] Dept. of Plant Sciences, Madurai Kamaraj University, India; [2]Dept. of Plant and Soil Sciences, College of Agricultural Sciences, Texas Tech University, Lubbock: Stability of chloroplast-associated heat-shock protein in *V. sinensis*.

Kubelik, A.R., Lambowitz, A.M., Depts. of Molecular Genetics and Biochemistry, Ohio State University, Columbus: Cloning and characterization of *Neurospora* nuclear genes that are required for mitochondrial ribosome assembly and protein synthesis.

Kuchka, M.R., Mayfield, S.P., Rochaix, J.-D., Dept. of Molecular Biology, University of Geneva, Switzerland: Nuclear PSII mutants of *C. reinhardtii* reduced in D2 polypeptide synthesis.

Lomax, M.I.,[1] Bachman, N.J.,[2] Grossman, L.I.,[3] [1]Dept. of Microbiology and Immunology, University of Michigan Medical School, [2]Dept. of Biology, Franklin and Marshall College, [3]Dept. of Molecular Biology and Genetics, Wayne State University School of Medicine: Molecular genetics of mammalian cytochrome *c* oxidase.

Lukins, H.B., Payne, M.J., Ooi, B.G., Nagley, P., Linnane, A.W., Dept. of Biochemistry, Monash University, Clayton, Australia: Yeast nuclear and mitochondrial mutations affecting expression of the *oli1* gene and synthesis of H^+-ATPase subunit 9.

Partaledis, J., Fearon, K., Cui, Z., Mason, T., Dept. of Biochemistry, University of Massachusetts, Amherst: Cloning and expression of nuclear genes for components of the yeast mitochondrial translation system.

Merchant, S., Bogorad, L., Harvard University, Cambridge, Massachusetts: Characterization of a cDNA clone for pre-apocytochrome *c*-552 from *C. reinhardtii*.

Kang, Y.-W., Miller, D.L., Biology Programs, University of Texas, Dallas: Nuclear and mitochondrial revertants of a mitochondrial tRNA mutant in *S. cerevisiae*.

Conrad, H.M.,[1] Zhu, H.,[2] Butow, R.A.,[2] Perlman, P.S.,[1] [1]Ohio State University, Columbus; [2]University of Texas Health Science Center, Dallas: Nuclear gene affecting mtRNA metabolism.

Poyton, R.O., Trueblood, C.E., Wright, R.M., Trawick, J.D., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Organization and differential expression of nuclear genes for cytochrome *c* oxidase in *S. cerevisiae*.

Repetto, B., Tzagoloff, A., Akai, A., Kurkulos, M., Dept. of Biological Science, Columbia University, New York, New York: Homology of yeast mitochondrial leucyl-tRNA synthetase and isoleucyl- and methionyl-tRNA synthetase of *E. coli*.

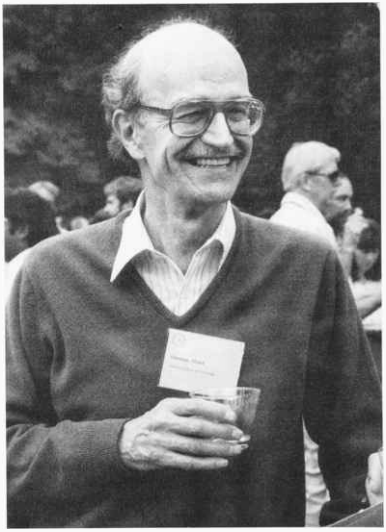
Schuster, G.,[1] Even, D.,[1] Kloppstech, K.,[2] Ohad, I.,[1] [1]Dept. of Biological Chemistry, Hebrew University, Jerusalem, Israel, [2]Institute of Botany, University of Hanover, Federal Republic of Germany: A nucleus-coded 22-kD heat-shock protein is transported into the chloroplasts, localized in the thylakoids, and might be involved in protecting photosynthesis against photoinhibition during heat shock.

Sieburth, L.E., Herrin, D.L., Schmidt, G.W., Botany Dept., University of Georgia, Athens: Nuclear mutants affected in chloroplast gene expression in *C. reinhardtii*.

Sumegi, B., Malloy, C., Inman, L., Srere, P.A., Veterans Administration Medical Center and University of Texas Health Science Center, Dallas: Pleiotropic effect of three different mutations in cytochrome oxidase.

Whitfield, C., Jefferson, L., Dept. of Biochemistry and Cancer Center, Howard University, Washington, D.C.: Characterization of mtRNA in a Chinese hamster mutant deficient in NADH dehydrogenase and cytochrome *c* oxidase.

Videira, A., Filser, M., Werner, S., Institut für Physiologische Chemie, University of Munich, Federal Republic of Germany: On the structure and function of the respiratory chain complex I of *N. crassa*.



G. Attardi

Wu, M., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: Mitochondrial and cytoplasmic fumarases in *S. cerevisiae* are encoded by a single nuclear gene *FUM1*.

Vincent, R.D., Hofmann, T.J., Zassenhaus, H.P., Dept. of Microbiology, St. Louis University, Missouri: Characterization of a yeast mutant deficient in the mitochondrial nuclease encoded by *NUC1*.

Evolution

Baldauf, S.L., Manhart, J.R., Palmer, J.D., Dept. of Biology, University of Michigan, Ann Arbor: Movement of the chloroplast *tufA* gene to the nucleus.

Garesse, R., Batuecas, B., Calleja, M., Balberde, J.R., Marco, R., Dept. de Bioquimica and Instituto de Investigaciones Biomedicas, Facultad de Medicina, UAM, Madrid, Spain: mtDNA from *D. melanogaster* and *Artemia*—Molecular characterization and its relation with the nuclear genome.

Gellissen, G., Haucke, R., Rippe, R.M., Uhlenbusch, I., Institute for Zoology, University of Düsseldorf, Federal Republic of Germany: Evolution of the mitochondrial genome of the orthopteran insect *L. migratoria*.

Pumo, D.E.,[1] Phillips, C.J.,[1] Genoways, H.H.,[2] [1]Dept. of Biology, Hofstra University, Hempstead, New York; [2]State Museum, University of Nebraska, Lincoln: Application of mtDNA analysis to the understanding of island biogeography, dispersal, and speciation of neotropical fruit bats.

Randolph-Anderson, B.L., Boynton, J.E., Gillham, N.W., Depts. of Botany and Zoology, Duke University, Durham, North Carolina: Immunological and electrophoretic comparisons of chloroplast and prokaryotic ribosomal proteins.

Yonekawa, H., Fischer-Lindahl, K., Howard Hughes Medical Institute, Depts. of Microbiology and Biochemistry, University of Texas Health Science Center, Dallas: Molecular evolution of mtDNA among closely related *Mus* species.

SESSION 10 IMPORT AND ASSEMBLY OF PROTEINS BY ORGANELLES. II.

Chairman: A. Linnane, Monash University

Chen, W., Hoyt, D., Gierasch, L., Douglas, M., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Protein structure during mitochondrial import.

Eilers, M., Verner, K., Ohba, M., Roise, D., Biocenter, University of Basel, Switzerland: Import of proteins into yeast mitochondria.

Horwich, A.L., Pollack, R.A., Cheng, M.Y., Yale School of Medicine, New Haven, Connecticut: Targeting the human ornithine transcarbamylase precursor to the mitochondrial matrix.

Neupert, W., Institute for Physiological Chemistry, Physical Biochemistry and Cell Biology, University of Munich, Federal Republic of Germany: Mechanisms of protein translocation into mitochondria.

Lewin, A.S., Burns, D.J., Modrak, D., Dept. of Chemistry, Indiana University, Bloomington: Import and assembly of the mitochondrial ATPase of baker's yeast.

Nagley, P., Farrell, L.B., Gearing, D.P., Nero, D., Braidotti, G., Meltzer, S., Devenish, R.J., Dept. of Biochemistry and Centre for Molecular Biology and Medicine, Monash University, Clayton, Victoria, Australia: Functional replacement of a mitochondrially encoded inner membrane protein by an imported version encoded by an artificial nuclear gene.

Shore, G., Nguyen, M., Liu, X., Skerjanc, I., Sheffield, W., Dept. of Biochemistry, McGill University, Montreal, Canada: Protein sorting between mitochondrial membranes.

Grivell, L.A., Oudshoorn, P., Meijer, M., Laboratory of Biochemistry/Biotechnology Centre, University of Amsterdam, The Netherlands: Mutations affecting protein import by yeast mitochondria.

SESSION 11 INTERACTIONS BETWEEN NUCLEAR AND ORGANELLE GENOMES. I.

Chairman: N. Gillham, Duke University

Pierce, J., Carlson, T.J., Williams, J.G.K., Central Research and Development Dept., E.I. Du Pont de Nemours & Co., Inc., Wilmington, Delaware: RuBP carboxylase/oxygenase—Problems and possibilities.

von Wettstein, D., Dept. of Physiology, Carlsberg Laboratory, Copenhagen, Denmark: Synthesis of chlorophyll and chlorophyll binding proteins—Involvement of a chloroplast $tRNA^{Glu}$.

Raines, C.A., Longstaff, M., Lloyd, J.C., Dyer, T.A., Plant Breeding Institute, Trumpington, Cambridge, England: Isolation and manipulation of nuclear genes for Calvin cycle enzymes of wheat chloroplasts.

Attardi, G., Alziari, S., Chomyn, A., Gaines, G., King, M., Tarle, I., Division of Biology, California Institute of Technology, Pasadena: New approaches to the study of mitochondrial gene expression and nuclear-mitochondrial interactions in human cells.

Butow, R.A., Docherty, R., Parikh, V.S., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: A path from mitochondria to the yeast nucleus.

Fisher-Lindahl, K., Loveland, B., Richards, S., Yonekawa, H., Howard Hughes Medical Institute, Depts. of Microbiology and Biochemistry, University of Texas Health Science Center, Dallas: In search of the molecular basis for a mitochondrial transplantation antigen.

Fox, T.D., Costanzo, M.C., Strick, C.A., Seaver, E.C., Marykwas, D.L., Section of Genetics and Development, Cornell University, Ithaca, New York: Yeast nuclear genes that control translation of specific mitochondrial mRNAs.

Martin, N.C.,[1] Ellis, S.R.,[1] Li, J.M.,[1] Gillman, E.C.,[2]

D. Clayton, J. Boynton, N. Gillman, R. Butow

Slusher, L.,[2] Hopper, A.K.,[2] [1]Dept. of Biochemistry, University of Texas Health Science Center, Dallas; [2]Dept. of Biological Chemistry, Milton H. Hershey Medical Center, Pennsylvania: Origin and compartmentalization of tRNA modification enzymes in *S. cerevisiae*.

Schinkel, A.H., Koerkamp, M.J.A.G., Stuiver, M.H., Tabak, H.F., Laboratory of Biochemistry, University of Amsterdam, The Netherlands: mtRNA polymerase of yeast – Structure and promoter recognition.

SESSION 12 INTERACTIONS BETWEEN NUCLEAR AND ORGANELLE GENOMES. II.

Chairman: P. Laipis, University of Florida

Nargang, F.,[1] Drygas, M.,[1] Lemire, E.,[1] Nicholson, D.,[2] Neupert, W.,[2] [1]Dept. of Genetics, University of Alberta, Edmonton, Canada; [2]Institut fur Physiologische Cheie, Universitat Munchen, Federal Republic of Germany: Characterization of cytochrome-deficient mutants of *N. crassa*.

Wallace, D.C., Zheng, X., Neckelmann, S.N., Ye, J., Li, K., Lott, M.T., Singh, G.P., Dept. of Biochemistry, Emory University Medical School, Atlanta, Georgia: ATP synthase β, ADP-ATP translocator, and mitochondrial myopathy – Coevolution and developmental regulation of nuclear and mitochondrial DNA oxidative phosphorylation genes.

Guarente, L., Massachusetts Institute of Technology, Cambridge, DNA-binding and transcriptional activation by the yeast activators *HAP1*, *HAP2*, and *HAP3*.

Tzagoloff, A., Repetto, B., Vambuti, A., Dept. of Biological Sciences, Columbia University, New York: Novel aspects of yeast mitochondrial amino acyl tRNA synthetases.

RajBhandary, U., Sachs, N., Suarez, M., Kim, G., David, M., Dept. of Biology, MIT, Cambridge, Massachusetts: Cytochrome oxidase genes in *N. crassa*.

Bertrand, H., Dept. of Biology, University of Regina, Canada: Senescence factors of *Neurospora* – Nuclear plasmids that insert into mtDNA.

Cummings, D., Domenico, J., Turker, M., Nelson, J., Dept. of Microbiology, University of Colorado School of Medicine, Denver: Excision-amplification of mtDNA in senescent/longevity phenotypes of *P. anserina*.

SESSION 13 EVOLUTION OF ORGANELLE GENOMES

Chairman: W. Birky, Ohio State University

Saccone, C.,[1] Preparata, G.,[2] Attimonelli, M.,[1] Lanave, C.,[1] Pesole, G.,[1] [1]CSMME, CNR, and Dept. of Biochemistry and Molecular Biology, University of Bari, [2]Dept. of Physics, University of Milano, Italy: mtDNA in animal cells – Gene evolution and rearrangement.

Gray, M.W., Dept. of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada: rRNA genes and evolution of mitochondria.

Wilson, A., Palumbi, S., Universities of California, Cambridge and Hawaii: Forces molding the mitochondrial genome of animals – A model.

Brown, W., Cox, R., Moritz, C., Gharrett, A., Szura, L., Dept. of Biology, University of Michigan, Ann Arbor: Sequence duplications and rearrangements in animal mtDNA – Are tRNA genes mobilizing elements?

Palmer, J.D., Baldauf, S.L., Calie, P.J., Hampton, J.N., Milligan, B.G., Dept. of Biology, University of Michigan, Ann Arbor: Transfer of genes from the chloroplast to the nucleus and invasion of chloroplast genomes by transposable elements.

Clegg, M., Dept. of Botany and Plant Sciences, University of California, Riverside: Evolution of chloroplast DNA sequences.

de Zamaroczy, M., Bernardi, G., Laboratoire de Genetique Moleculaire, Institut de Recherche en Biologie Moleculaire, Paris, France: The evolutionary origin and mechanism of formation of the noncoding sequences from the mitochondrial genome of yeast.

Summary: G. Attardi, California Institute of Technology

Eukaryotic DNA Replication

September 2—September 6

ARRANGED BY

Thomas Kelly, Johns Hopkins University School of Medicine
Bruce Stillman, Cold Spring Harbor Laboratory

271 participants

The 1987 Cancer Cells meeting was the sixth meeting in this series and focused on eukaryotic DNA replication. Nearly 300 scientists were brought together for the first international meeting on this subject. The choice of this topic reflected the fact that this expanding area of research is an integral part of efforts to understand the abnormal proliferation found in cancer cells. The timing was apparently just right as many avenues of investigation have recently yielded exciting new results. Many areas were covered at this meeting, including studies on eukaryotic DNA viruses, which continue to provide valuable insight on the mechanism of DNA replication and the function of both virus-encoded and cellular proteins. The program also included sessions on the replication of stable, extrachromosomal elements such as papillomavirus and Epstein-Barr virus episomes, mitochondrial chromosomes, and the yeast autonomously replicating plasmids. Other sessions focused on the replication and amplification of cellular DNA and on the structures and functions of centromeres, telomeres, and origins within eukaryotic chromosomes. The structure and functions of the four eukaryotic cell DNA polymerases were also discussed in detail. Control of the cell-cycle progression and S-phase-specific regulatory events were also presented. A special session on prokaryotic DNA replication opened the meeting and provided an excellent summary of some of the best-characterized replication systems. In addition, two poster sessions contained a wealth of interesting information from many laboratories.

This meeting was supported in part by grants from the National Institute on Aging, the National Institute of General Medical Sciences and the National Science Foundation.

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SESSION 1 PROKARYOTIC MODEL SYSTEMS

Chairman: R. McMacken, Johns Hopkins University

Cha, T.-A., Alberts, B.M., Dept. of Biochemistry and Biophysics, University of California, San Francisco: In vitro studies of the T4 bacteriophage DNA replication system.

Huber, H.E., Beauchamp, B.B., Bernstein, J., Nakai, H., Rabkin, S.D., Tabor, S., Richardson, C.C., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Bacteriophage T7 DNA replication.

McHenry, C., Hawker, J., Flower, A., Dept. of Biochemistry, Biophysics, and Genetics, University of Colorado Health Sciences Center, Denver: Mechanism of coordination of leading with lagging-strand synthesis by the DNA polymerase III holoenzyme.

Baker, T.A., Bertsch, L.L., Bramhill, D., Sekimizu, K., Yung, B., Kornberg, A., Dept. of Biochemistry, Stanford University, California: Enzymatic replication of plasmids from the origin of the *E. coli* chromosome.

McMacken, R.,[1] Alfano, C.,[1] Mensa-Wilmot, K.,[1] Dodson, M.,[2] Echols, H.,[2] [1]Dept. of Biochemistry, Johns Hopkins University, Baltimore, Maryland: [2]Dept. of Molecular Biology, University of California, Berkeley: Initiation of bacteriophage λ DNA replication with a set of nine purified λ and *E. coli* proteins.

M. Lusky

A. Spradling

P. Nurse

M. Challberg

SESSION 2 REPLICATION OF VIRUS DNA

Chairman: R. Knippers, University of Konstanz

Stahl, H., Wiekowski, M., Scheffner, M., Knippers, R., Division of Biology, University of Konstanz, Federal Republic of Germany: DNA helicase acivity of SV40 large T antigen.

Dean, F.,[1] Dodson, M., Echols, H., Hurwitz, J.,[1] [1]Graduate Program in Molecular Biology and Virology, Sloan-Kettering Cancer Center, New York; [2]Dept. of Molecular Biology and Virology, University of California, Berkeley: SV40 DNA replication-origin-dependent DNA unwinding by SV40 T antigen.

DePamphilis, M.L.,[1,3] Gutierrez, C.,[1,3] Cupo, D.Y.,[1] Fritze, C.E.,[1] Guo, Z.-S.,[1,3] Heine, U.,[1,3] Hendrickson, E.A.[1] Martinez-Salas, E.,[1,3] [1]Harvard Medical School, Boston, Massachusetts; [2]University of Texas, Austin; [3]Roche Institute of Molecular Biology, Nutley, New Jersey: Origins of polyomavirus and SV40 DNA replication and their regulation during early mammalian development.

McFadden, G., Stuart, D., Upton, C., Dickie, P., Morgan, A.R., Dept. of Biochemistry, University of Alberta, Canada: Replication and resolution of poxvirus telomeres.

Merchlinsky, M., Moss, B., NCI, National Institutes of Health, Bethesda, Maryland: Resolution of concatemer junctions of vaccinia virus DNA into mature terminal hairpins.

Lavi, S., Berko-Flint, Y., Kleinberger, T., Blank, M., Karby, S., Dept. of Microbiology, Tel Aviv University, Israel: Activation of SV40 DNA amplification and replication in CHO cells by carcinogen-induced factors.

Dermody, J.,[1] Lawlor, K.,[1] Du, H.,[1] Wojcik, B.,[1] Jha, K.,[1] Malkas, L.,[2] Hickey, R.,[2] Baril, E.,[2] Ozer, H.L.,[1] [1]Dept. of Biological Sciences, Hunter College, City University of New York, New York; [2]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Cell mutants temperature-sensitive in cellular and viral DNA synthesis.

Lusky, M., Heise, J., Pesold-Hurt, B., Heidelberg University, Federal Republic of Germany: Characterization of BPV replication functions by site directed mutagenesis.

Berns, K.I.,[1] Labow, M.A.,[2] [1]Dept. of Microbiology, Cornell University Medical College, New York, New York; [2]Dept. of Molecular Biology, Princeton University, New Jersey: Negative regulation of adenovirus-associated DNA replication.

SESSION 3 POSTER SESSION

Borowiec, J., Dean, F., Hurwitz, J., Dept. of Molecular Biology, Sloan-Kettering Cancer Center, New York, New York: Formation of an ATP-dependent complex of SV40 T antigen with the SV40 replication origin.

Murakami, Y.,[1] Hurwitz, J.,[2] [1]RIKEN Institute, Tsukuba Science City, Japan; [2]Molecular Biology Program, Sloan-Kettering Cancer Center, New York, New York: In vitro replication of SV40-origin-containing DNA in extracts from various cell lines—A further analysis of a permissive factor.

Prelich, G., Stillman, B., Cold Spring Harbor Laboratory, New York: Analysis of PCNA function during SV40 replication in vitro.

Sarkar, N.,[1,2] Taljanidisz, J.,[1,2] Decker, R.S.,[2] Guo, Z.-S.,[2,3] DePamphilis, M.L.,[2,3] [1]Dept. of Metabolic Regulation, Boston Biomedical Research Institute, [2]Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Initiation of SV40 DNA replication in vitro—Identification of RNA-primed nascent DNA chains.

Roberts, J.D., Kunkel, T.A., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Accuracy of DNA synthesis during in vitro replication from the SV40 origin.

Weiner, B., Bradley, M.K., Dana Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Analyses of T antigen activities involved in SV40 DNA replication in vitro.

Auborn, K., Markowitz, R., Guo, M., Wang, E., Yu, Y.-T., Prives, C., Dept. of Biological Sciences, Columbia University, New York, New York: Characterization of mutant and wild-type SV40 tumor antigens.

Gluzman, Y., McVey, D., Mohr, I., Strauss, M., Argani, P.,

W. Earnshaw

J. Broach

Frank, R., Admon, A., Cold Spring Harbor Laboratory, New York: An analysis of large T antigen functions required for SV40.

Graesser, F.,[1] Mann, K.,[2] Walter, G.,[1] [1]Dept. of Pathology, University of California, San Diego, La Jolla; [2]Dept. of Biology, University of Alaska, Anchorage: Removal of serine phosphates from SV40 large T antigen increases its ability to stimulate SV40 DNA replication in vitro but has no effect on ATPase and DNA binding.

Schneider, J., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: Enhanced SV40 DNA replication in vivo and novel DNA-binding properties of mutant T antigens with point mutations in the carboxy-terminal phosphorylated region.

Chandrasekharappa, S.C., Subramanian, K.N., Dept. of Microbiology and Immunology, University of Illinois College of Medicine, Chicago: Effects of position and orientation of the 72-bp repeat translational enhancer on replication from the SV40 core origin.

Cheng, L., Kelly, T.J., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Stimulation of SV40 DNA replication in simian and human cells by the transcriptional enhancer of BK virus.

Bennett, E.R., Hassell, J.A., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Activation of SV40 and polyomavirus origins for DNA replication by heterologous enhancer and promoter elements.

Powelson, M., Strayer, J., Snapka, R.M., Dept. of Radiology, Ohio State University, Columbus: SV40 DNA replication in the presence of topoisomerase inhibitors.

Rao, B.S.,[1] Manor, H.,[2] Martin, R.G.,[1] [1]NIDDKD, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biology, Technion-Israel Institute of Technology, Haifa: Pausing of DNA replication by a sequence containing (dG-dA)$_{27}$·(dT-dC)$_{27}$ in an SV40 model system.

Kalvonjian, S., Fluck, M., Dept. of Microbiology, Michigan State University, East Lansing: Interviral recombination associated with the transformation of rat fibroblasts by polyomavirus.

Hartl, M., Huber, B., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: A cellular DNA sequence inhibits SV40 DNA replication in *cis*.

Maulbecker, C., Yokota, H., Botchan, M., Bartholomew, J., Laboratory of Chemical Biodynamics, Dept. of Molecular Biology, University of California, Berkeley: Human cells transformed with wild-type SV40 harbor a mutated extrachromosomal SV40 in high copy number.

Lambert, P., Hermonat, P., Howley, P., Laboratory of Tumor Virology, NCI-National Institutes of Health, Bethesda, Maryland: Role of BPV-1 E1 ORF in the control of viral transcription.

Holmes, A., Wietstock, S., Ruyechan, W., Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Maryland: Identification of a DNA primase activity present in HSV-infected cells and associated with HSV DNA polymerase.

Lockshon, D., Galloway, D., Fred Hutchinson Cancer Research Center, Seattle, Washington: Sequence requirements for an HSV DNA replication origin.

Knipe, D.,[1] deBruynKops, A., Gao, M.,[1] Villarreal, E.,[1] Su, L.,[1] Yager, D.,[2] Coen, D.,[2] Depts. of [1]Microbiology and Molecular Genetics, [2]Pharmacology, Harvard Medical School, Boston, Massachusetts: Nuclear localization of HSV DNA replication proteins.

Coen, D., Chiou, H., Gibbs, J., Yager, D., Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts: Functional sites and control of expression of the HSV DNA polymerase gene.

Kunzi, M., Shaffer, R., Traktman, P., Depts. of Cell Biology and Anatomy and Microbiology, Cornell University Medical College, New York, New York: Purification and analysis of two vaccinia-encapsidated enzymes involved in DNA metabolism.

Evans, E., Greenberg, A., Traktman, P., Dept. of Cell Biology and Anatomy, Cornell University Medical College, New York, New York: Molecular genetic analysis of two genes with an essential function in vaccinia DNA replication.

Traktman, P., McDonald, B., Crozel, V., Depts. of Cell Biology and Anatomy and Microbiology, Cornell University Medical College, New York, New York: Molecular genetic analysis of the vaccina virus DNA polymerase.

Stuart, D., Upton, C., McFadden, G., Dept. of Biochemistry, University of Alberta, Canada: Mutagenesis of poxvirus telomeres in vitro.

O'Neill, E.A., Burrow, C.R., Kauffman, M.G., Wu, D.G., Kelly, T.J., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Purification of nuclear factor III, a cellular protein required for efficient initiation of adenovirus DNA replication in vitro.

Meisterernst, M., Gander, I., Donath, C., Müller, U., Rogge, L., Schneider, H., Winnacker, E.L., Dept. of Biochemistry, University of Munich, Federal Republic of Germany: Approaches to the cloning of nuclear factor I and to its functional identification.

Shu, L.,[1] Pettit, S.C.,[1] Meyers, M.,[2] Horwitz, M.S.,[2] Engler, J.A.,[1] [1]Dept. of Biochemistry, University of Alabama, Birmingham; [2]Dept. of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York: Expression of active adenovirus DNA polymerase and preterminal protein from cloned DNA requires DNA

sequences near adenovirus genome coordinate 39.

Zhao, L., Sripad, G., Padmanabhan, R., Dept. of Biochemistry, University of Kansas Medical Center, Kansas City: Expression of the replication proteins of adenovirus in *E. coli* and mammalian cells.

Pearson, G.D., Xu, F.-Y., Hu, C.-H., Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Adenovirus strand-displacement in vivo.

Faust, E.A.,[1] Brudzynska, K.,[1] Salvino, R.,[1] Astell, C.,[2] Tiberiis, B.,[2] St. Amand, J.,[2] [1]Cancer Research Laboratory and Dept. of Biochemistry, University of Western Ontario, [2]Dept. of Biochemistry, University of British Columbia, Canada: Site-specific cleavage, DNA strand transfer, and packaging of a short palindromic terminal sequence of MVM DNA.

Focher, F.,[1] Gassmann, M.,[1] Spadari, S.,[2] Ferrari, E.,[1] Brown, N.C.,[3] Hübscher, U.,[1] [1]Institut für Pharmakologie und Biochemie, University of Zürich, Switzerland; [2]Istituto di Genetica Biochimica e Evoluzionistica del CNR, Pavia, Italy; [3]University of Massachusetts Medical School, Worcester: In vitro replication of PCV DNA, a naturally occurring mammalian single-stranded circular genome.

Malkas, L.H., Baril, E.F., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: A DNA sequence-recognition protein associated with HeLa cell DNA polymerase α.

Davey, S.K., Faust, E.A., Cancer Research Laboratory, Dept. of Biochemistry, University of Western Ontario, Canada: Sequence requirements for ψ-dependent RNA-primed initiation by DNA polymerase α-primase.

Dresler, S.L., Frattini, M.G., Kimbro, K.S., Dept. of Pathology, Washington University School of Medicine, St. Louis, Missouri: Studies in permeable human cells implicate DNA polymerases α and δ in DNA replication.

Krauss, S.W., Linn, S., Dept. of Biochemistry, University of California, Berkeley: Changes in HeLa DNA polymerase α after exposure to protein kinase C.

Prussak, C.E., Almazan, M.T., Tseng, B.Y., University of California School of Medicine, San Diego, La Jolla: Mouse primase p49 subunit, molecular cloning and cell-cycle expression.

Widen, S., Kedar, P., Zmudzka, B., Wilson, S., NCI, National Institutes of Health, Bethesda, Maryland: Transcription control of human DNA polymerase β.

Yamaguchi, M.,[1] Hirose, F.,[2] Hayashi, Y.,[1] DePamphilis, M.L.,[3] Matsukage, A.,[1] [1]Aichi Cancer Center Research Institute, [2]Nagoya University School of Medicine, Japan; [3]Roche Institute of Molecular Biology, Nutley, New Jersey: Roles of positive and negative regulatory elements in the expression of DNA polymerase β gene.

Elie, C., Forterre, P., Salhi, S., Rossignol, J.M., de Recondo, A.M., Laboratoire de Biologie Moléculaire de la Réplication, IRSC-CNRS, France: DNA polymerase from the archaebacterium *S. acidocaldarius* – DNA synthesis at extremely high temperature.

Elder, R.H.,[1] Mezzina, M.,[2] Lisandri, A.,[2] Sarasin, A.,[2] Rossignol, J.M.,[1] Laboratoires de [1]Biologie Moléculaire de la Réplication, [2]Mutagenèse, Moléculaire, IRSC-CNRS, France: Identification and partial purification of two DNA ligase species from rat liver.

Wahl, A.F., Geis, A.M., Wong, S.W., Wang, T.S.-F., Laboratory of Experimental Oncology, Dept. of Pathology, Stanford University School of Medicine, California: Transcriptional regulation of human DNA polymerase α during cell proliferation and cell cycle.

Ciarrocchi, G., Montecucco, A., Pedrali-Noy, G., Spadari, S., Istituto di Genetica Biochimica e Evoluzionistica del CNR, Pavia, Italy: Do DNA ligases and topoisomerases act through a common mechanism of action?

Melendy, T., Ray, D.S., Molecular Biology Institute and Dept. of Biology, University of California, Los Angeles: A homogeneous type II topoisomerase from the trypanosomatid *C. fasciculata*.

Ackerman, P.,[1] Glover, C.V.C.,[2] Osheroff, N.,[1] [1]Dept. of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee; [2]Dept. of Biochemistry, University of Georgia, Athens: Phosphorylation of DNA topoisomerase II by total homogenates of *Drosophila* Kc cells.

Gutierriz, C.,[1] Guo, Z.-S.,[1] Farrell-Towt, J.,[2] DePamphilis,

SESSION 4 VIRUS CHROMOSOME REPLICATION

Chairman: **P. Tegtmeyer,** State University of New York, Stony Brook

Dean, F.B.,[1] Hurwitz, J.,[1] Deb, S.,[2] Tegtmeyer, P.,[2] [1]Graduate Program in Molecular Biology, Sloan-Kettering Cancer Center, New York, [2]Dept. of Microbiology, State University of New York, Stony Brook: The SV40 replicon.

Wold, M.S., Li, J.J., Weinberg, D.H., Kelly, T.J., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Analysis of SV40 DNA replication in vitro.

Stillman, B., Fairman, M., Prelich, G., Tsurimoto, T., Cold Spring Harbor Laboratory, New York: SV40 DNA replication – Identification of cellular replication components.

Gough, G., Gannon, J., Lane, D.P., Imperial Cancer Research Fund, London, England: Cellular proto-oncogene, p53, blocks the binding of SV40 T antigen to DNA polymerase α.

Sturzbecher, H.-W., Braithwaite, A.W., Palmer, C., Addison, C., Jenkins, J., Marie Curie Research Institute, Oxted,

J. Hamlin, M. Fairman

K. Berns, C. Prives, R. Martin, C. Schildkraut

M.L.,[1] [1]Roche Institute of Molecular Biology, [2]Roche Research Center, Nutley, New Jersey: c-myc protein and DNA replication—Separation of c-myc antibodies from an inhibitor of DNA synthesis.

England: p53 inhibits DNA synthesis from the SV40 origin of replication.

Challberg, M., Nelson, N., Olivo, P., Wu, C., NIAID, National Institutes of Health, Bethesda, Maryland: Viral genes required for HSV DNA replication.

Weller, S., Carmichael, E., Goldstein, D., Zhu, L., Seghatoleslami, R., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Genetic analysis of HSV-1 DNA replication.

van der Vliet, P.C., de Vries, E., Leegwater, P.A.J., Pruijn, G.J.M., van Driel, W., van Miltenburg, R.T., Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands: Protein-DNA interactions at the adenovirus DNA replication origin.

Hay, R., Clark, L., Cleat, P., Harris, M., Robinson, C., Watson, C., Dept. of Biochemistry and Microbiology, University of St. Andrews, Scotland: Requirements for the initiation of Ad2 and Ad4 DNA replication.

Fanning, E., Traut, W., Alliger, P., Dehde, S., Dornreiter, I., Institute for Biochemistry, Munich, Federal Republic of Germany: Sequence-specific interactions between a cellular DNA-binding protein and the SV40 origin of DNA replication.

SESSION 5 REPLICATION OF EXTRA-CHROMOSOMAL ELEMENTS

Chairman: D. Clayton, Stanford University

Botchan, M., University of California, Berkeley: Regulation of DNA replication in papillomaviruses.

Roberts, J., Weintraub, H., Fred Hutchinson Cancer Research Center, Seattle, Washington: Regulation of DNA replication.

Yates, J., Dept. of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York: Activation of origin-specific DNA replication and transcriptional enhancement by EBV nuclear antigen 1.

Chang, D.D., Topper, J.N., Clayton, D.A., Dept. of Pathology, Stanford University School of Medicine, California: Sequence analysis of a nuclear gene that encodes the RNA component of a mitochondrial RNA processing enzyme.

Volkert, F.C., Armstrong, K., Som, T., Broach, J.R., Dept. of Molecular Biology, Princeton University, New Jersey: Mechanism of stable propagation of the yeast plasmid 2-μm circle.

Huberman, J.A., Spotila, L.D., Nawotka, K.A., El-Assouli, S., Davis, L.R., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: In vitro replication origin and terminus of the yeast 2-μm plasmid.

Brewer, B.J., Fangman, W.L., Dept. of Genetics, University of Washington, Seattle: Physical mapping of replication origins on yeast ARS plasmids.

Kimmerly, W.,[1] Buchman, A.,[2] Kornberg, R.,[2] Rine, J.,[1] [1]University of California, Berkeley; [2]Stanford University, California: Coincidence of sites and factors for mitotic segregation and position-dependent repression in yeast.

Diffley, J.F.X., Stillman, B., Cold Spring Harbor Laboratory, New York: DNA-protein interactions at the chromosomal replicator, ARS1 and their effect on DNA bending.

Sheehan, M.A., Dilworth, S.M., Mills, A.D., Sleeman, A.M., Blow, J.J., Laskey, R.A., CRC Molecular Embryology Group, Dept. of Zoology, Cambridge, England: Mechanisms of assembly of nuclei and nucleosomes in cell-free extracts of *Xenopus* eggs.

Mignotte, B., Barat-Gueride, M., Mounolou, J.C., Laboratorie de Biologie Générale, Université de Paris-Sud, France: A single-stranded DNA-binding protein from *X. laevis* oocyte mitochondria.

Orr-Weaver, T.,[1] Spradling, A.,[2] [1]Whitehead Institute and Dept. of Biology, Massachusetts Institute of Technology, Boston; [2]Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Control elements regulating *Drosophila* chorion gene amplification.

Swimmer, C.,[1] Martinez, J.-C.,[1] Fahrner, K.,[1] Fenerjian, M.,[1] Kafatos, F.C.,[1,2] [1]Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts; [2]Institute of Molecular Biology and Biotechnology and Dept. of Biology, University of Crete, Greece: A functional analysis of an evolutionarily conserved amplification control region in *Drosophila*.

H. Huber, R. McMacken

Zannis-Hadjopoulos, M., Frappier, L., Khoury, M., Price, G.B., McGill Cancer Centre, Dept. of Medicine, McGill University, Montreal, Canada: Effect of anti-cruciform DNA monoclonal antibodies on DNA replication.

Frappier, L., Landry, S., Zannis-Hadjopoulos, M., McGill Cancer Centre, Dept. of Medicine, McGill University, Montreal, Canada: Autonomous replication of plasmids bearing monkey DNA origin-enriched sequences (*ors*).

Braithwaite, A.W., Jenkins, J.R., Marie Curie Research Institute, Oxted, England: Isolation of mammalian DNA sequences that can function as origins of DNA replication.

Sykes, R.C.,[1] Lin, D.,[1] Chinault, A.C.,[1,2] [1]Dept. of Biochemistry, [2]Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas: Properties of a potential replication origin from the human X chromosome.

Romanczuk, H., Tassiopoulos, K., Wormington, M., Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts: *Trans*-activation of BPV-1 DNA replication in *Xenopus* eggs.

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Ascenzioni, F.,[1] Schmid, M.,[2] Lipps, H.J.,[2] [1]Gruppo di Microbiologia, University of Rome, Italy; [2]Institut für Biologie, University of Tübingen, Federal Republic of Germany: A linear plasmid containing the BPV-1 genome.

Sheline, C.T., Ray, D.S., Dept. of Biology and Molecular Biology Institute, University of California, Los Angeles: Characterization of *C. fasciculata* minicircle replication intermediates.

Cooney, C.A., Eykholt, R.L., Bradbury, E.M., Dept. of Biological Chemistry, University of California School of Medicine, Davis: DNA methylation in control of *Physarum* rDNA replication.

Cunningham, D., Dove, W.F., McArdle Laboratory for Cancer Research and the Laboratory of Genetics, University of Wisconsin, Madison: Replication patterns of developmentally regulated α-tubulin genes in the *Physarum* plasmodium.

Shipley, G.L., Flanagan, R.R., Diller, J.D., Jones, K.B., Sauer, H.W., Dept. of Biology, Texas A & M University, College Station: The order of replication of cell-type-specific genes in *P. polycephalum*.

Biamonti, G., Tribioli, C., Giacca, M., Colonna, M., Riva, S., Falaschi, A., Istituto di Genetica Biochimica ed Evoluzionistica CNR, Pavia, Italy: Isolation and characterization of human DNA replicated at the onset of the S phase.

Dhar, V.,[1] Iqbal, M.A.,[1] Mager, D.,[2] Schildkraut, C.L.,[1] [1]Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York; [2]Terry Fox Laboratory, British Columbia Cancer Research Centre, Vancouver, Canada: Temporal order of DNA replication in the human β-like globin region in K562 cells.

Foreman, P.K., Hamlin, J.L., Dept. of Biochemistry, University of Virginia, Charlottesville: Transcriptional activity in and around the origin of replication isolated from the amplified DHFR domain in CHO cells.

Burhans, W.C., Caddle, M.S., Lussier, R.H., Jackman, J., Brinton, B., Heintz, N.H., Dept. of Pathology, University of Vermont, Burlington: Replication of the amplified DHFR domain of CHOC 400 cells—Synthesis commences within a specific restriction fragment and proceeds bidirectionally both in vivo and in vitro.

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Wani, M., Snapka, R.M., Dept. of Radiology, Ohio State University, Columbus: Methotrexate resistance and polyoma oncogene expression.

Kenter, A.L.,[1,2] Watson, J.V.,[3] [1]Dept. of Microbiology and Immunology, University of Illinois College of Medicine, Chicago; [2]MRC, Laboratory of Molecular Biology, [3]MRC, CORU, Cambridge, England: Mitogens induce immunoglobulin switch region rearrangements during S phase of the cell cycle in B lymphocytes.

Allshire, R.,[1] Cranston, G.,[1] Hastie, N.,[1] Fantes, P.,[2] [1]MRC Clinical and Population Cytogenetics Unit, Western General Hospital, [2]Dept. of Zoology, University of Edinburgh, Scotland: Alteration of the *S. pombe* telomere in mouse cells—A route to mouse telomere sequences?

Allshire, R.,[1] Cranston, G.,[1] Gosden, J.,[1] Maule, J.,[1] Hastie, N.,[1] Fantes, P.,[2] [1]MRC Clinical and Population Cytogenetics Unit, Western General Hospital, [2]Dept. of Zoology, University of Edinburgh, Scotland: Maintenance of a fission yeast chromosome in mouse cells.

Raveh, D., Strathern, J.N., NCI-Frederick Cancer Research Facility, Frederick, Maryland: DNA intermediates generated during the mating-type switch in yeast.

Clarke, L., Fishel, B., Amstutz, H., Baum, M., Carbon, J., Dept. of Biological Sciences, University of California, Santa Barbara: Structural studies on centromeric DNA in fission yeast (*S. pombe*.)

Newlon, C.S., Dershowitz, A., Dept. of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark: Contribution of ARSs to chromosome stability in yeast.

Palzkill, T.G.,[1,2] Newlow, C.S.,[1] [1]Dept. of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark; [2]Genetics Ph.D. Program, University of Iowa, Iowa City: A new sequence important for yeast ARS function.

Pluta, A.F.,[1,2] Zakian, V.A.,[2] [1]Dept. of Pathology, University of Washington, [2]Fred Hutchinson Cancer Center, Seattle: DNA sequence and structure requirements for telomere formation in yeast.

Baldacci, G., Madelin, E., de Recondo, A.M., Laboratoire de Biologie Moléculaire de la Réplication, IRSC-CNRS, France: An *S. cerevisiae* sequence selected with an *ets* gene probe shows local homology with two *CDC* genes.

Thrash-Bingham, C., Fangman, W.L., Dept. of Genetics, University of Washington, Seattle: Identification and mapping of a mutation that stabilizes an ARS1 plasmid in yeast.

McCarroll, R.M.,[1] Ferguson, B.M.,[1] Reynolds, A.E.,[1] Newlon, C.S.,[2] Fangman, W.L.,[1] [1]Dept. of Genetics, University of Washington, Seattle; [2]Dept. of Microbiology, UMDNJ-New Jersey Medical School, Newark: Analysis of early and late replication transitions in yeast chromosomes.

Kearsey, S.E., Edwards, J., Dept. of Zoology, Oxford, England: Characterization of mutations that affect ARS function in *S. cerevisiae*.

Gottschling, D.E., Zakian, V.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: DNA substrate specificity and possible yeast homologs of *O. nova* telomere-binding proteins.

Berezney, R., Tubo, R.A., Dept. of Biological Sciences, State University of New York, Buffalo: Replicative dependency of DNA polymerase—primase megacomplexes associated with the nuclear matrix—The clustersome model.

Nakayasu, H., Martelli, A.M., Buchholtz, L.A., Berezney, R., Dept. of Biological Sciences, State University of New York, Buffalo: Antibodies as probes for defining the structural topography of replicational sites in eukaryotic cells.

Jackson, D.A., Cook, P.R., Sire William Dunn School of Pathology, Oxford, England: Replication centers are organized by a nucleoskeleton.

Ng, L., Fisher, P.A., Dept. of Pharmacological Sciences, Health Sciences Center, State University of New York, Stony Brook: Heat shock promotes the association of DNA polymerase α with karyoskeletal protein (nuclear matrix) fractions prepared from *D. melanogaster*.

Thömmes, P., Schray, B., Fett, R., Knippers, R., Division of Biology, University of Konstanz, Federal Republic of Germany: Studies on the cell-cycle-dependent expression of human DNA polymerase α.

Strauss, P.R., Northeastern University, Boston, Massachusetts: Detergent-soluble DNA is enriched in sequences actively being transcribed.

Vos, J.-M., Wauthier, E., Hanawalt, P.C., Dept. of Biological Sciences, Stanford University, California: Bypass replication of psoralen adducts in active human and rodent genes.

Wood, R.D., Imperial Cancer Research Fund, Clare Hall Laboratories, London, England: UV-stimulated repair replication in mammalian cell extracts.

Ma, D., Campbell, J.L., Divisions of Chemistry and Biology, California Institute of Technology, Pasadena: Role of the *dnaA* protein in the replication of pBR322.

Hupp, T.R., Kaguni, J.M., Dept. of Biochemistry, Michigan State University, East Lansing: Genetic analysis of *dasC*, an extragenic suppressor of the *dnaA*46 gene of *E. coli*.

Hwang, D.S., Kaguni, J.M., Dept. of Biochemistry, Michigan State University, East Lansing: Purification and biochemical characterization of the *E. coli dnaA*46 gene product in initiation of DNA replication.

Wang, Q., Kaguni, J.M., Dept. of Biochemistry, Michigan State University, East Lansing: Transcriptional repression of *dnaA*, *htpR*, and other genes of *E. coli* by dnaA protein.

Masai, H., Arai, K., Dept. of Molecular Biology, DNAX Research Institute, Palo Alto, California: Initiation of R1 plasmid DNA replication in vitro—Interaction of *repA* and *dnaA* protein with DNA at *oriR*.

M. DePamphilis, D. Knipe, M. Horwitz

SESSION 7 CONTROL OF CELL CYCLE AND S PHASE

Chairman: P. Nurse, Imperial Cancer Research Fund

Lee, M., Norbury, C., Spurr, N., Nurse, P., Imperial Cancer Research Fund, London, England: Isolation and characterization of a human homolog of the fission yeast cell-cycle control gene *cdc2*.

Mendenhall, M., Wittenberg, C., Hadwiger, J., Reed, S., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Regulation of the *S. cerevisiae* cell cycle by the *CDC28* protein kinase.

Draetta, G., Brizuela, L., Beach, D., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Biochemical characterization of human p34, a homolog of the p34^{cdc2}/p36^{cdc28} cell-cycle regulator of yeast.

Ryder, K., Nathans, D., Howard Hughes Medical Institute and Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Genes acivated by growth factors.

Heintz, N., Dailey, L., Fletcher, C., Gallinari, P., Hanly, S., LaBella, F., Pierani, A., Roberts, S., Roeder, R.G., Sive, H., Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, New York: Factors regulating histone gene expression during the cell cycle.

Bonner, W.M., Wu, R.S., Panusz, H.T., Muneses, C., NCI, National Institutes of Health, Bethesda, Maryland: Histone pools, cell cycle, and the balance between protein and DNA synthesis.

Earnshaw, W.C., Heck, M.M.S., Dept. of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland: Cell biology of DNA topoisomerase II.

J. Kaguni, J. Rossignol, L. Kaguni

Sherley, J.L., Kelly, T.J., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Analysis of the mechanisms that regulate the expression of thymidine kinase during the mammalian cell cycle.

Celis, J.E., Madsen, P., Nielsen, S., Celis, A., Neilsen, H.V., Gesser, B., Dept. of Medical Biochemistry, Aarhus University, Denmark: Cyclin (PCNA, auxiliary protein of DNA polymerase *d*) and dividin are central components of the pathway(s) leading to DNA replication and cell division.

SESSION 8 REPLICATION AND AMPLIFICATION OF CHROMOSOMAL DNA

Chairman: A. Spradling, Carnegie Institution of Washington

Blow, J.J.,[1] Watson, J.V.,[2] Laskey, R.A.,[1] [1]CRC Molecular Embryology Research Group, Dept. of Zoology, [2]MRC Clinical Oncology Unit, MRC Centre, Cambridge, England: Initiation of DNA replication in a cell-free extract of *Xenopus* eggs.

Kelley, R., Cooley, L., Leys, G., Orr-Weaver, T., Spradling, A., Dept. of Embryology, Carnegie Institute of Washington, Baltimore, Maryland: Regulation of *Drosophila* chorion gene amplification.

Delidakis, C.,[1] Kafatos, F.C.,[1,2] [1]Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts; [2]Institute of Molecular Biology and Biotechnology and Dept. of Biology, University of Crete, Greece: Amplification of the chorion gene cluster in *D. melanogaster* is subject to multiple control elements and long-range position effects.

Schimke, R.T., Hill, A., Sherwood, S., Johnston, R., Feder, J., Farnham, P., Hoy, C., Rice, G., Schumacher, I., Assaraf, Y., Dept. Biol. Sci, Stanford University, California: Perturbation of DNA replication, enhancement of gene amplification, and generation of chromosomal aberrations.

Rolfe, M., Knights, C., Stark, G., Imperial Cancer Research Fund, London, England: Somatic-cell genetic studies of amplificator cell lines.

Plevani, P., Foiani, M., Francesconi, S., Mazza, C., Pizzagalli, A., Valsasnini, P., Lucchini, G., Dipt. di Genetica e Biologia dei Microrganismi, Università di Milano, Italy: Biochemical and genetic characterization of the yeast DNA polymerase–DNA primase complex.

Campbell, J.L., Sweder, K., Budd, M., Gordon, C., Jong, A., Clark, M., Divisions of Chemistry and Biology, California Institute of Technology, Pasadena: Use of reverse genetics to dissect the yeast replication apparatus.

Brooks, M., Pausch, M.H., Peterson, B.C., Dumas, L.B., Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois: The yeast DNA primase–DNA polymerase complex—Immunoaffinity purification and analysis of RNA primer synthesis.

Hatton, K.S., Dhar, V., Stuart, S., Brown, E.H., Iqbal, M.A., Gahn, T., Schildkraut, C.L., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Replication program of active and inactive multigene families in mammalian cells.

Hamlin, J.L., Leu, T.-H., Foreman, P.S., Anachkova, B.B., Vaughn, J.P., Dijkwel, P.A., Dept. of Biochemistry, University of Virginia, Charlottesville: Analysis of a mammalian origin of replication isolated from the amplified DHFR domain in CHO cells.

SESSION 9 REPLICATION PROTEINS

Chairman: R. Lehman, Stanford University

Cotterill, S.M.,[1] Reyland, M.E.,[2] Loeb, L.A.,[2] Lehman, I.R.,[1] [1]Dept. of Biochemistry, Stanford University, California; [2]Dept. of Pathology, University of Washington, Seattle: Enzymatic activities associated with the 182-kD polymerase subunit of the DNA polymerase–primase of *D. melanogaster*.

Baril, E.F.,[1] Malkas, L.H.,[1] Hickey, R.,[1] Li, C.,[1] Vishwanatha, J.K.,[2] Coughlin, S.,[3] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]Dept. of Biochemistry, University of Nebraska Medical Center, Omaha; [3]Dept. of Microbiology, Sterling-Winthrop Research Institute, Rensselaer, New York: Multiprotein form of DNA polymerase α from human cells – Interaction with other proteins in DNA replication.

Wong, S.W., Wahl, A.F., Wang, T.S.-F., Laboratory of Experimental Oncology, Dept. of Pathology, Stanford Medical School, California: Human DNA polymerase α – Primary structure, functional expression, and related functional domains with other DNA polymerases.

Goulian, M., Carton, C., DeGrandpre, L., Heard, C., Olinger, B., Richards, S., University of California School of Medicine, San Diego, La Jolla: Discontinuous DNA synthesis by purified proteins from mammalian cells.

Grosse, F., Nasheuer, H.-P., Dept. of Chemistry, Max-Planck-Institute for Experimental Medicine, Goettingen, Federal Republic of Germany: Structure and properties of the immunoaffinity-purified DNA polymerase α–primase complex.

Downey, K.M., Tan, C.-K., Li, X., Andrews, D., So, A.G., Depts. of Medicine and Biochemistry and Center for Blood Diseases, University of Miami School of Medicine, Florida: Comparative studies of DNA polymerases α and δ from fetal calf thymus.

Nishida, C,[1] Reinhard, P.,[2] Linn, S.,[1] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Dept. of Nutrition, Wander AG, Bern, Switzerland: DNA repair synthesis in human fibroblasts specifically requires DNA polymerase δ.

Huebner, K.,[2] Chang, L.M.S.,[1] Bollum, F.J.,[1] Xiu, X.X.,[1] Cheung, L.C.,[1] Croce, C.M.,[2] Hecht, B.K.,[3] Hecht, F.,[3] Cannizzaro, L.A.,[3] [1]Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Maryland: [2]Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania; [3]Southwest Biomedical Research Institute, Scottsdale, Arizona: Molecular cloning and chromosome sublocalization to 8p11→p12 of a cDNA for human DNA polymerase β.

Kaguni, L.S., Wernette, C.M., Conway, M.C., Yang-Cashman, P., Dept. of Biochemistry, Michigan State University, East Lansing: Structural and catalytic features of the mitochondrial DNA polymerase from *D. melanogaster* embryos.

SESSION 10 CHROMOSOME STRUCTURE AND FUNCTION

Chairman: E. Blackburn, University of California, Berkeley

Clarke, L., Fishel, B., Ng, R., Tschumper, G., Carbon, J., Dept. of Biological Sciences, University of California, Santa Barbara: Centromere structure and function in budding and fission yeasts.

Spencer, F.A.,[1] Connelly, C.J.,[1] Lee, S.,[1] Gerring, S.L.,[1] Snyder, M.,[2] Hieter, P.,[1] [1]Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Yale University, New Haven, Connecticut: Chromosome transmission fidelity mutants in *S. cerevisiae*.

Greider, C., Henderson, E., Larson, D., Shampay, J., Spangler, E., Blackburn, E., Dept. of Molecular Biology, University of California, Berkeley: Telomere synthesis and regulation.

Grummt, F.,[1] Holst, A.,[1] Müller, F.,[1] Zastrow, G.,[1] Luksza, H.,[1] Schwender, S.,[1] Wegner, M.,[1] Klavinius, A.,[1] Zentgraft, H.,[2] [1]Institute of Biochemistry, University of Würzburg, [2]German Cancer Research Center, Heidelberg, Federal Republic of Germany: Murine ARS elements and ARS-binding proteins.

Duncan, C., Leffak, M., Dept. of Biochemistry, Wright State University, Dayton, Ohio: A sequence 5′ to the human c-myc gene allows autonomous replication of a selectable plasmid in HeLa cells.

Umek, R.M., Eddy, M., Kowalski, D., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: DNA sequences required for unwinding prokaryotic and eukaryotic origins of replication.

Carroll, S.M., Gaudray, P., DeRose, M.L., Emery, J.F., Meinkoth, J.L., Nakki, E., Subler, M., Von Hoff, D.D., Wahl, G.M., Salk Institute for Biological Studies, San Diego, California: Characterization of an episome produced in hamster cells that amplify a transfected CAD gene at high frequency – Functional evidence for a mammalian replication origin.

Almouzni, G., Méchali, M., Institut Jacques Monod, Paris, France: Assembly of spaced chromatin coupled or uncoupled to DNA replication in a cell-free system from *Xenopus* eggs.

A. Wahl, I.R. Lehman, C.C. Richardson, D. Clayton

Modern Approaches to New Vaccines Including Prevention of AIDS

September 9—September 13

ARRANGED BY

Robert M. Chanock, NIAID, National Institutes of Health
Richard A. Lerner, Research Institute of Scripps Clinic
Fred Brown, Wellcome Biotechnology Ltd.
Harold Ginsberg, Columbia University

329 participants

The annual meeting on Modern Approaches to New Vaccines goes from strength to strength. More than 350 people attended the meeting held in September, 1987. About one half of those attending were from commercial organizations, an indication that the concept of vaccines based on the new technology is regarded as a realistic practical possibility.

More than 150 papers were submitted for oral presentation. Even with the packed sessions for which Cold Spring Harbor meetings are famous or notorious, it was impossible to accept more than 60 papers. Disappointment was ameliorated somewhat by allowing a few 5-minute talks, but this meant that the sessions became sprints as well as marathons. The program included a poster session (more than 90 posters were on display) and sessions on Immunology, Parasitology, Bacteria and Bacterial Diseases, three sessions on Virology, and three sessions on AIDS.

This meeting was supported in part by The Rockefeller Foundation.

SESSION 1 IMMUNOLOGY

Chairman: R. Lerner, Research Institute of Scripps Clinic

Satterthwait, A., Hogotian, R., Zavala, F., Nussenzweig, V., Lerner, R., Medical Research Institute of Scripps Clinic, La Jolla, California; [2]Dept. of Medical and Molecular Parasitology, New York University Medical Center, New York: Building synthetic peptides shaped in three-dimension by covalent replacements for hydrogen bonds.

Francis, M.J., Hastings, G.Z., Syred, A.D., McGinn, B., Brown, F., Rowlands, D.J., Wellcome Biotechnology Ltd., Pirbright, England: Peptides with added T-cell epitopes can overcome genetic restriction of the immune response.

Houghten, R.A., Research Institute of Scripps Clinic, La Jolla, California: Determination of the precise amino acids involved, and their relative importance, in peptide antigen/monoclonal antibody interactions.

Milich, D.R.,[1] McLachlan, A.,[1] Thornton, G.B.,[2] Hughes, J.,[1] [1]Dept. of Basic and Clinical Research, Research Institute of Scripps Clinic, La Jolla, [2]Johnson and Johnson Biotechnology Center, Sorrento Valley, California: Celfular mechanisms of the enhanced immunogenicity of the nucleocapsid of HBV.

Bernards, R., Destree, A., McKenzie, S., Gordon, E., Weinberg, R., Panicali, D., Whitehead Institute for Biomedical

D. Brown, R. Chanock, N. Letvin

Research, Applied bioTechnology, Inc., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Effective tumor immunotherapy directed against an oncogene-encoded product using a vaccinia virus vector.

Williams, W.V.,[1] Rubin, D.H.,[1] Robey, F.,[2] Greene, M.I.,[1] [1]University of Pennsylvania School of Medicine and Veterans Administration Medical Center, Philadelphia; [2]NCI, National Institutes of Health, Bethesda, Maryland: Use of shared primary structure to predict the oligopeptide-neutralizing epitope on the type-3 reovirus hemagglutinin.

Field, H.,[1] Yarranton, G.,[2] Rees, A.R.,[1] [1]Laboratory of Molecular Biophysics, University of Oxford, [2]Celltech Ltd., Slough, England: Production of mini-antibodies in *E. coli*.

Meloen, R.H., Puyk, W.C., Schaaper, W.M.M., Lankhof, H., Meijer, D.J.A., Posthumus, W.P.A., Central Veterinary Institute, Lelystad, The Netherlands: Use of peptides to locate and characterize epitopes.

SESSION 2 PARASITOLOGY

Chairman: M. Good, National Institutes of Health

Capron, A., Institute Pasteur, Lille, France: Newly recognized schistosomiasis antigen and its role in resistance.

Scott, P., Pearce, E., Natovitz, P., Sher, A., NIAID, National Institutes of Health, Bethesda, Maryland: Identification of T-cell-reactive antigens in a partially defined vaccine against *L. major*.

Zavala, F.,[1] Tam, J.P.,[2] Nussenzweig, V.,[1] Nussenzweig, R.S.,[1] [1]New York University Medical Center, [2]Rockefeller University, New York, New York: Synthetic vaccine induces protective immunity against *P. berghei* sporozoites.

Lal, A.A., de la Cruz, V.F., Good, M.F., Weiss, W.J., Maloy, W.L., Welsh, J.A., Lunde, M., McCutchan, T.F., NIAID, National Institutes of Health, Bethesda, Maryland: Antibodies against the repeat region of circumsporozoite protein are not protective against challenge of sporozoites—A rodent model study.

Schofield, L., Nussenzweig, V., Nussenzweig, R.S., Dept. of Medical and Molecular Parasitology, New York University Medical Center, New York, New York: Antimalarial activity of Lyt2+ (suppressor/cytotoxic) T cells required for immunity to sporozoite challenge.

Good, M.F.,[1] Pombo, D.,[1] Quakyi, I.A.,[1] Riley, E.M.,[4] Menon, A.,[4] Maloy, W.L.,[2] Houghten, R.,[5] de la Cruz, V.F.,[1] Berzofsky, J.A.,[3] Miller, L.H.,[1] [1]Laboratory of Parasitic Diseases, NIAID, [2]Laboratory of Immunogenetics, [3]Metabolism Branch, NCI, National Institutes of Health, Bethesda, Maryland; [4]Medical Research Council Laboratories, The Gambia; [5]Scripps Clinic and Research Foundation, La Jolla, California: Identification of the polymorphic regions of circumsporozoite protein as the immunodominant domains recognized by human and murine T cells.

Brake, D., Burns, J.M., Vidaya, A., Weidenz, W.P., Malaria Research Group, Dept. of Microbial and Immunology, Hahnemann University, Philadelphia, Pennsylvania: Adoptive protection in nude mice against a murine malaria parasite by a cloned T-cell line.

Langford, C.,[1] Edwards, S.,[2] Corcoran, L.,[1] Peterson, G.,[1] McIntrye, P.,[1] Kemp, D.,[1] Anders, R.,[1] [1]Walter and Eliza Hall Institute of Medical Research, [2]Commonwealth Serum Laboratories, Victoria, Australia: Recombinant protein, synthetic peptide, recombinant virus, and hybrid "cocktail" antigen vaccines against falciparum malaria.

SESSION 3 AIDS. I.

Chairman: E. Norrby, Karolinska Institutet

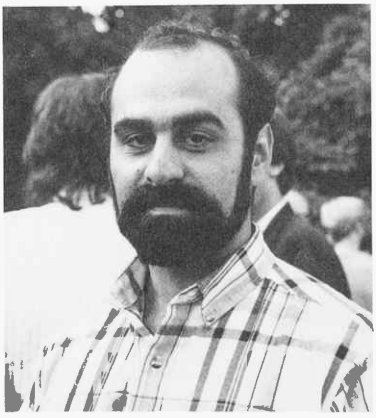
Schiller, P.,[1] Geffin, R.,[2] Parks, W.,[2] Voellmy, R.,[1] Depts. of [1]Biochemistry, [2]Pediatrics, University of Miami School of Medicine, Florida: Inducible expression of HIV envelope glycoproteins in a mammalian cell-culture system.

Rice, A.P., Mathews, M.B., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Use of a recombinant adenovirus vector to analyze the mechanism of tatII *trans*-activation of HIV LTR gene expression.

Kowalski, M.,[1] Potz, J.,[1] Basiripour, L.,[1] Dorfman, T.,[1] Goh, W.C.,[1] Dayton, A.,[1] Terwilliger, E.,[1] Rosen, C.,[1] Haseltine, W.,[2] Sodroski, J.,[1] [1]Dana-Farber Cancer Institute, Dept. of Pathology, Harvard Medical School, [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Structure-function relationships of the HIV envelope glycoproteins.

Clavel, F., Alizon, M., Guyader, M., Emerman, M., Sonigo, P., Montagnier, L., Unite d'oncologie virale, Institute Pasteur, Paris, France: Molecular and biological characterization of HIV-2.

Miyazawa, M., Nishio, J., Chesebro, B., NIAID, National In-



W. Williams

stitutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Antigen-presenting cells control T-cell responsiveness to Friend viral antigens.

Cheng-Mayer, C., Levy, J.A., Cancer Research Institute, University of California School of Medicine, San Francisco: Biologic and serologic properties of neurotropic HIV.

Letvin, N.L., Kannagi, M., Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts: Functional role of DC8+ lymphocytes in SIV infection of rhesus monkeys.

SESSION 4 VIROLOGY. I.

Chairman: A. Kapikian, NIAID, National Institutes of Health

Clarke, B.E., Newton, S.E., Carroll, A.R., Francis, M.J., Appleyard, G., Syred, A.D., Highfield, P.E., Rowlands, D.J., Brown, F., Wellcome Biotechnology Ltd., Pirbright, England: Chimeric proteins based on HBcAg form highly immunogenic particles.

Cohen, J., Rosenblum, B., Feinstone, S., Ticehurst, J., Purcell, R., NIAID, National Institutes of Health, Bethesda, Maryland: Use of infectious HAV cDNA to study viral attenuation.

Mason, B., Molnar-Kimber, K.L., Morin, J.E., Lubeck, M.D., Conley, A.J., Davis, A.R., Hung, P.P., Wyeth Laboratories, Inc., Division of Microbiology, Philadelphia, Pennsylvania: Adenovirus type 4 and type 7 as viral vectors.

Cox, N.J.,[1] Kitame, F.,[2] Naeve, C.,[3] Kendal, A.P.,[1] [1]Influenza Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia; [2]Dept. of Bacteriology, Yamagata University School of Medicine, Japan; [3]Division of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Sequence changes in genes of the attenuated influenza-A vaccine donor strain, A/Ann Arbor/6/60.

Snyder, M.H.,[1] Betts, R.F.,[2] Clements, M.L.,[3] Herrington, D.,[4] Sears, S.D.,[3] Maassab, H.F.,[5] Murphy, B.R.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]University of Rochester School of Medicine, New York; [3]Center for Immunization Research, Johns Hopkins University School of Public Health, [4]University of Maryland School of Medicine, Baltimore; [5]University of Michigan School of Public Health, Ann Arbor: identification of four genes that attenuate influenza A/Ann Arbor/6/60 (H2N2) cold-adapted reassortant viruses.

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Markoff, L.J.,[1] Bray, M.,[1] Gordon, S.W.,[1] Lai, C.J.,[1] Gentry, M.K.,[2] Echols, K.,[2] Summers, P.,[2] Houghton, R.,[3] Lerner, R.,[3] Chanock, R.M.,[1] [1]NIAID, National Institute of Health, Bethesda, Maryland; [2]Walter Reed Army Research Institute, Washington, D.C.; [3]Research Institute of the Scripps Clinic, La Jolla, California: Antigenic analysis of the dengue envelope glycoprotein using synthetic peptides.

SESSION 5 AIDS. II.

Chairman: H. Ginsberg, Columbia University

Fultz, P.N.,[1] Switzer, W.,[1] McClure, H.,[2] Reed, C.,[1] Montagnier, L.,[3] [1]AIDS Program, Centers for Disease Control, [2]Yerkes Primate Research Center, Emory University, Atlanta, Georgia; [3]Institut Pasteur, Paris, France: Simian models for AIDS–SIV/SMM and HIV-2 infection of rhesus macaques.

Arthur, L., Robey, W., Pyle, S., Bess, J., Jr., Nara, P., Kelliher, J., Gilden, R., Fischinger, P., NCI-Frederick Cancer Research Facilities, Frederick, Maryland: Preparation and evaluation of an HIV outer envelope glycoprotein prototype vaccine.

Estin, C.D., Hu, S.-L., Stevenson, U.S., Plowman, G.D., Hellström, I., Hellström, K.-E., ONCOGEN, Seattle, Washington: Recombinant vaccinia virus expressing the human melanoma-associated antigen p97 as a therapeutic tumor vaccine.

Luciw, P.,[1] Tong-Starksen, S.,[2] Peterlin, B.M.,[2] [1]University of California, Davis; [2]Howard Hughes Medical Institute, University of California, San Francisco: The HIV LTR responds to T-cell-activation signals.

Linnette, G.,[1] Pierce, P.,[1] Wagner, K.,[2] Wong, D.,[1] Folks, T.,[3] Hartzman, R.,[2] [1]Georgetown University, Washington, D.C.; [2]Naval Medical Command and Naval Medical Research Institute, [3]NIAID, National Institutes of Health, Bethesda, Maryland: Evidence for HIV-specific cellular immunity in seropositive asymptomatic individuals.

Payne, S.,[1] Ball, J.,[1] Issel, C.,[2,3] Montelaro, R.,[2,3] Depts. of [1]Biochemistry, [2]Veterinary Science, Louisiana State University and Louisiana Agricultural Experiment Station, [3]Dept. of Veterinary Microbiology and Parasitology, Louisiana State University School of Medicine, Baton Rouge: Envelope gene variation in EIAV–Implications for vaccine development.

Sadaie, M.R., Benter, T., Wong-Staal, F., NCI, National Insti-

S. Grant, S. Alpert, E. Wetzel

tutes of Health, Bethesda, Maryland: Direct mutagenesis analysis of conserved domains of *tat* in a cloned HIV-1 provirus.

Guy, B.,[1] Kieny, M.P.,[1] Rivière, Y.,[2] Girard, M.,[3] Montagnier, L.,[2] Lecocq, J.P.,[1] [1]Transgene S.A., Strasbourg, [2]Institut Pasteur, Paris, [3]Pasteur-Vaccins, France: Phosphorylation of the F protein (3′ *orf*) of HIV by protein kinase C and down-regulation of the lymphocyte T4 antigen.

Smith, T.F.,[1] Myers, G.,[2] [1]Harvard Medical School, Boston, Massachusetts; [2]Theoretical Division, Los Alamos National Laboratory, New Mexico: Phylogenetic analysis of HIV-1 and HIV-2.

Plata, F.,[1] Hoffenbach, A.,[1] Pedroza-Martins, L.,[1] Langlade-Demoyen, P.,[1] Autran, B.,[2] Garcia-Pons, F.,[1] Debré, P.,[2] Wain-Hobson, S.,[1] [1]Laboratoire de Biologie et d'Immunologie Moléculaires des Rétrovirus, Institut Pasteur, [2]Laboratoire d'Immunologie Cellulaire et Tissulaire, Hôpital Pitié-Salpé-trière, Paris, France: Quantitative analysis of AIDS virus-specific cytotoxic T lymphocytes in human and murine experimental systems.

SESSION 6 POSTER SESSION

Adams, S.E.,[1] Malim, M.H.,[2] Senior, J.M.,[1] Kingsman, S.M.,[1,2] Kingsman, A.J.,[1,2] [1]Depts. of Molecular Biology and Immunology, British Bio-technology Ltd., [2]Dept. of Biochemistry, University of Oxford, England: Induction of influenza HA antibodies by Ty–HA hybrid virus-like particles.

Bertonis, J.,[1] Costopoulos, D.,[1] Liu, T.,[1] Meier, W.,[1] Kowalski, M.,[2] Fisher, R.,[1] [1]Biogen Research Corp., Cambridge, [2]Dana Farber Cancer Institute, Boston, Massachusetts: Development of recombinant soluble T4 as a novel AIDS therapeutic.

Broekhuijsen, M.P.,[1] Blom, A.J.M.,[1] van Rijn, J.M.M.,[1] Pouwels, P.H.,[1] Francis, M.J.,[2] Brown, F.,[2] Enger-Valk, B.E.,[1] [1]TNO Medical Biological Laboratory, Rijswijk, The Netherlands; [2]Wellcome Biotechnology Ltd., Pirbright, England: Neutralization and protection induced by fusion proteins containing FMDV epitopes synthesized in *E. coli*.

Burnette, W.N.,[1] Mar, V.L.,[1] Morris, C.F.,[1] Cieplak, W.,[2] Kaljot, K.,[2] Sato, H.,[3] Keith, J.M.,[2] [1]Amgen, Thousand Oaks, California; [2]NIAID, Rocky Mountain Laboratory, Hamilton, Montana; [3]National Institute of Health, Tokyo, Japan: Recombinant strategy for development of pertussis vaccine components.

Campbell, J.I.A., Binns, M., Boursnell, M., Tomley, F.M., Dept. of Microbiology, Institute for Animal Disease Research, Huntington, England: Nucleotide sequence of the terminal region of the fowl poxvirus genome–Arrangement of repeated and unique sequences.

Cieplak, W., Locht, C., Lobet, Y., Kaljot, K.T., Keith, J.M., NIAID, Rocky Mountain Laboratories, Hamilton, Montana: Structure-function relationships of the S1 subunit of pertussis toxin.

Dale, J.B., Bronze, M.S., Beachey, E.H., Veterans Administration Medical Center and University of Tennessee, Memphis: Protective and heart-cross-reactive epitopes of type-19 streptococcal M protein.

de la Cruz, V., Lal, A.A., McCutchan, T.F., NIAID, National Institutes of Health, Bethesda, Maryland: Sequence variation in putative functional domains of the circumsporozoite protein of *P. falciparum*.

Delaporte, E.,[1] Dazza, M.C.,[1] Wain-Hobson, S.,[2] Brun-Vézinet, F.,[3] Larouzé, B.,[1] Saimot, A.G.,[1] Dupont, A.,[4] Roelants, G.E.,[4] [1]INSERM, Hôpital Claude Bernard, [2]Unité de Biologie Moléculaire et d'Immunologie des Rétrovirus, Institut Pasteur, [3]Laboratoire de Virologie, Hôpital Claude Bernard, Paris, [4]International Centre for Medical Research, Gabon, France: HIV-related viruses in Gabon.

Dietzschold, B., Wang, H., Rupprecht, C.E., Koprowski, H., Wistar Institute, Philadelphia, Pennsylvania: Immunization with rabies nucleocapsid protein and synthetic nucleocapsid-protein fragments—A novel approach to vaccination against rabies.

Duchêne, M.,[1] Schweizer, A.,[1] Mertz, R.,[1] von Specht, B.U.,[2] Lottspeich, F.,[3] Domdey, H.,[1] [1]Laboratorium für molekulare Biologie-Genzentrum-der Universität München, Martinsried, [2]Chirurgische Universitätsklinik, Freiburg, [3]Gentechnologische Arbeitsgruppe am Max Planck Institut, Martinsried, Federal Republic of Germany: Immunization against *P. aeruginosa* with peptides derived from outer-membrane porin protein F.

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Johnson, J.M.,[1] Harmon, S.A.,[2] Richards, O.C.,[2] Ehrenfeld, E.,[2] Summers, D.F.,[2] [1]Dept. of Medicine, University of Texas Health Science Center, San Antonio; [2]Dept. of Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City: Expression of the HAV VP1 gene in prokaryotic and eukaryotic vectors.

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Rupprecht, C.,[1] Lord, R.,[2] Papo, S.,[3] Dietzschold, B.,[1] Koprowski, H.,[1] [1]Wistar Institute, Philadelphia, [2]Indiana University of Pennsylvania; [3]Instituto de Investigaciones Veterinarias, Maracay, Venezuela: Bovine paralytic rabies control – Immunization of vampire bats with an inactivated oral rabies vaccine.

Sattentau, Q.J.,[1] Weber, J.N.,[2] Clapham, P.R.,[2] Weiss, R.A.,[2] Beverley, P.C.L.,[1] [1]University College Hospital

J. Ivy

and Middlesex Hospital Medical School, [2]Chester Beatty Laboratories, London, England: Anti-idiotype antisera and monoclonal antibodies raised against CD4 antibodies recognize a conserved neutralization site on the envelope glycoprotein of diverse isolates of HIV-1 and HIV-2.

Scherf, A.,[1] Sieg, K.,[1] Pohl, I.,[1] Kun, J.,[1] Schreiber, M.,[1] Mercereau-Puijalon, O.,[2] Pereira da Silva, L.,[2] Muller-Hill, B.,[1] [1]Institute für Genetik, Universität zu Köln, Federal Republic of Germany; [2]Unite de Parasitologie Experimentale, Institut Pasteur, Paris, France: Vectors for genomic expression cloning and purification of polymerized antigenic determinants.

Scholtissek, S., Weber, J., Grosse, F., Max-Planck-Institute for Experimental Medicine, Dept. of Chemistry, Göttingen, Federal Republic of Germany: A novel expression vector designed for the production of antigens in *E. coli*.

Shaw, M.W., Centers for Disease Control, Atlanta, Georgia: Expression and antigenic characterization of the influenza virus small membrane proteins M2 and NB.

Shibley, G.P., Espeseth, D.A., Joseph, P.L., Gay, C.G., Animal and Plant Health Inspection Service, Veterinary Biologics, Veterinary Services, Hyattsville, Maryland: Guidelines for field testing and licensing of live genetically engineered veterinary biologics.

Sinigaglia, F., Guttinger, M., Gillessen, D., Trzeciak, A., Doran, D., Pink, J.R.L., Central Research Units, Hoffman-La Roche & Co., Ltd., Basel, Switzerland: Anti-*P. falciparum* sporozoite vaccine—Identification of a new epitope recognized by human T cells.

Sinigaglia, F.,[1] Matile, H.,[1] Pink, J.R.L.,[1] Jacot, H.,[1] Takacs, B.,[1] Crisanti, A.,[2] [1]Central Research Units, Hoffmann-La Roche & Co., Ltd., Basel, Switzerland; [2]AMBH, Heidelberg, Federal Republic of Germany: Nonpolymorphic sequences of p190, a protein of the *P. falciparum* erythrocytic stage, contain both T- and B-cell epitopes.

Smith, D.P.,[1] Bennett, J.W.,[1] Giri, C.,[2] Kaufman, J.,[2] Wright, S.E.,[1,3] [1]Viral Oncology Laboratory, Veterans Administration Medical Center, Salt Lake City, Utah; [2]Division of Virology, Office of Biologics, DHHS, Bethesda, Maryland; [3]Depts. of Internal Medicine and Cellular and Molecular Biology, University of Utah School of Medicine, Salt Lake City: Expressing Pr ASV-C envelope glycoprotein by vaccinia.

Stanberry, L.R.,[1] Harrison, C.J.,[1] Bernstein, D.I.,[1,2] Burke, R.L.,[3] Van Nest, G.,[3] Myers, M.G.,[1] [1]Children's Hospital Research Foundation, [2]Gamble Institute, Cincinnati, Ohio; [3]Chiron Corporation, Emeryville, California: Glycoprotein immunotherapy of recurrent genital HSV infection.

Steinhauer, E.H.,[1] Calvelli, T.A.,[1,2] Wiznia, A.,[1] Rubenstein, A.,[1] Depts. of [1]Pediatrics, [2]Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York: Differences in antibody reactivity to reduced native and recombinant HIV gp120 in HIV-positive mothers and their children.

Sylvan, S.P.E., Hellström, U.B., Elias Bengtsson Research Unit, Dept. of Infectious Diseases, Karolinska Institute, Roslagstull Hospital, Stockholm, Sweden: In vitro systems for evaluation of new HB-vaccine candidates.

Taylor, J.,[1] Weinberg, R.,[1] Desmettre, P.,[3] Paoletti, E.,[1,2] [1]Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany; [2]Virogenetics Inc., Albany, New York; [3]Rhone Merieux, Lyon, France: Development of genetically engineered *Avipoxvirus* vectors.

Thiel, H.-J.,[1] Pfaff, E.,[2] Schaller, H.,[2] [1]Federal Research Centre for Virus Diseases of Animals, Tübingen, [2]ZMBH, Heidelberg, Federal Republic of Germany: Molecular aspects of FMDV neutralization.

van Binnendijk, R.S.,[1] Langeveld, S.A.,[2] Osterhaus, A.D.M.E.,[1] UytdeHaag, F.C.G.M.,[1] Versteeg, J.P.M.,[2] Voorma, H.O.,[2] de Vries, P.,[1] Weisbeek, P.J.,[2] [1]National Institute of Public Health and Environmental Hygiene, [2]Bilthoven and Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, The Netherlands: Development and production of an improved vaccine against measles.

Voegtline, M.S., Minden, P., Houghten, R.A., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Alteration of binding between antibodies and a synthetic mycobacterial peptide by amino acid deletion.

Wasserman, G.F., Inacker, R., Silverman, C.C., Sitrin, R., Dept. of Protein Biochemistry, Smith Kline and French Laboratories, Swedeland, Pennsylvania: Recombinant human malaria vaccine candidates purified from *E. coli*.

Webster, D.M., Roberts, S., Cheetham, J.C., Rees, A.R., Laboratory of Molecular Biophysics, Oxford, England: Fine structure of antibody combining sites.

Weiss, W.R.,[1,2] Sedegah, M.,[1] Good, M.F.,[2] Fowlkes,

B.J.,[2] Kruisbeek, A.,[2] Beaudoin, R.,[1] Berzofsky, J.A.,[2] Miller, L.H.,[2] [1]Naval Medical Research Institute, [2]NCI, National Institutes of Health, Bethesda, Maryland: CD8+ T lymphocytes are critical effector cells in sporozoite immunized mice.

Werner, G., McCray, J.W., Sandoz Forschungsinstitut, Vienna, Austria: Antipeptide antibodies to a hypothetical receptor-binding site neutralize rhinoviruses.

Williamson, C.M.,[1] Pullinger, G.D.,[1] Lax, A.J.,[1] Manning, E.J.,[2] Baird, G.D.,[3] [1]AFRC Institute of Animal Disease Research, Berkshire, [2]John Radcliffe Hospital, Oxford, England; [3]Moredun Research Institute, Edinburgh, Scotland: Plasmid-encoded virulence determinants as potential components of a subunit vaccine against cattle salmonellosis.

SESSION 7 BACTERIA AND BACTERIAL DISEASES

Chairman: J. Keith, NIAID, National Institutes of Health

Beachey, E.H., Dale, J.B., Veterans Administration Medical Center and University of Tennessee, Memphis: Protective immunogenicity of a trivalent hybrid vaccine containing amino-terminal sequences of types 5, 6, and 24 streptococcal M protein synthesized in tandem.

De Magistris, M.T., Romano, M., Rappuoli, R., Tagliabue, A., Sclavo Research Center, Siena, Italy: Analysis at the T-cell clonal level of immune responses to *B. pertussis* in man.

Keith, J.M.,[1] Cieplak, W.,[1] Mar, V.L.,[2] Kaljot, K.,[1] Marchitto, K.S.,[1] Sato, H.,[3] Burnette, W.N.,[2] [1]NIAID, Rocky Mountain Laboratory, Hamilton, Montana; [2]Amgen, Thousand Oaks, California; [3]National Institutes of Health, Tokyo, Japan: Biochemical and immunological analyses of *B. pertussis* toxin subunits produced in recombinant *E. coli*.

Haron, J.,[1] Bohart, C.,[1] Staffileno, L.,[1] VanHook, P.,[1] Lehner, T.,[2] [1]Johnson and Johnson Biotechnology Center, La Jolla, California; [2]Dept. of Immunology, Guy's Hospital Medical and Dental Schools, London, England: Humoral responses to synthetic peptides representing the amino terminus of the 3800-molecular-weight antigen from *S. mutans*.

Lehner, T.,[1] Childerstone, A.,[1] Haron, J.,[2] [1]Dept. of Immunology, United Medical and Dental Schools of Guy's and St. Thomas Hospitals, London, England; [2]Biotechnology Center Inc., Johnson & Johnson, La Jolla, California: Human CD4 cell responses and serum antibodies to synthetic peptides derived from a streptococcal cell surface antigen.

Vodkin, M.H.,[1] Williams, J.C.,[1,2] [1]United States Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, [2]NIAID, National Institutes of Health, Bethesda, Maryland: Cloning and expression of a major antigen gene of *C. burnetii* homologous to a protein in *Mycobacteria* and *E. coli*.

Hedegaard, L., Pedersen, P.A., Klemm, P., Dept. of Microbiology, Technical University of Denmark, Copenhagen: Export of antigens to the *E. coli* surface by means of fimbriae protein systems.

Charbit, A.,[1] Sobczak, E.,[2] Michel, M.L.,[2] Molla, A.,[1] Tiollais, P.,[2] Hofnung, M.,[1] [1]UPMTG, [2]UREG, CNRS, INSERM, Institute Pasteur, Paris, France: Presentation of a foreign epitope at the surface of live recombinant bacteria—Immunization studies.

Finberg, R.,[1] Haregewoin, A.,[1] Crabbe, J.,[2] Onderdonk, A.,[3] Kasper, D.,[2] [1]Laboratory of Infectious Diseases, Dana-Farber Cancer Institute, [2]Channing Laboratories, [3]Tufts Veterinary School, Boston, Massachusetts: Use of T-cell hybridomas to prevent infections—Hybridoma supernatants prevent the development of abscesses.

SESSION 8 AIDS. III.

Chairman: F. Brown, Wellcome Biotechnology Ltd.

Lyerly, H.K., Matthews, T.J., Langlois, A.J., Bolognesi, D.P., Weinhold, K.J., Dept. of Surgery, Duke University Medical Center, Durham, North Carolina: Resolution of virus neutralization and anti-HIV ADCC activities in patient sera.

Gnann, J.W.,[1] McCormick, J.B.,[2] Nelson, J.A.,[1] Oldstone, M.B.A.,[1] [1]Research Institute for Scripps Clinic, La Jolla, California; [2]Centers for Disease Control, Atlanta, Georgia: HIV-1 and HIV-2 share a conserved immunogenic domain in their transmembrane glycoproteins.

Norrby, E.,[1] Biberfeld, G.,[2] Chiodi, F.,[1] Gegerfeldt, A.V.,[1] A. Nauclér,[3] Parks, E.,[4] Lerner, R.,[5] [1]Dept. of Virology, Karolinska Institutet, Stockholm, [2]Dept. of Immunology, National Bacteriological Laboratory, Stockholm, Sweden; [3]National Laboratory of Public Health, Bissau, Guinea Bissau, West Africa; [4]Johnson & Johnson, Biotechnology Center, La Jolla, [5]Dept. of Molecular Virology, Research Institute of Scripps Clinic, La Jolla, California: Site-directed serology discriminating between antibodies against HIV and related West African human retroviruses.

Gendelman, H.E.,[1] Meltzer, M.S.,[2] Leonard, J.,[1] Ferrua, C.,[1] Martin, M.A.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Immunology, Walter Reed Army Institute of Research, Washington, D.C.: Simultaneous isolation of HIVs tropic for CD4+ lymphocytes or macrophages from blood of AIDS patients.

Steimer, K.S., Van Nest, G.A., Barr, P.J., George-Nascimento, C., Haigwood, N., Dina, D., Tillson, E.M., Chiron Corporation, Emeryville, California: Recombinant nonglycosylated envelope polypeptides of HIV as targets of virus-neturalizing antibodies.

Krohn, K.,[1] Cease, K.,[2] Arthur, L.,[3] Fischer, R.,[4] Ranki, A.,[1] Putney, S.,[5] Lusso, P.,[1] Fischinger, P.,[3] Gallo, R.C.,[1] Berzofsky, J.,[2] [1]Laboratory of Tumor Cell Biology, [2]Metabolic Branch, [3]Office of the Director, NCI, National Institutes of Health, Bethesda, Maryland; [4]Biogen Corp.,

[5]Repligen Corp., Cambridge, Massachusetts: T-cell-specific epitopes on HIV envelope glycoprotein recognized by immunized chimpanzees.

Lusso, P.,[1] Markham, P.D.,[2] Ranki, A.,[1] Kueberuwa, S.S.,[2] Gallo, R.C.,[1] Krohn, K.J.E.,[1] [1]Laboratory of Tumor Cell Biology, NCI, National Institutes of Health, Bethesda, [2]Bionetics Research, Rockville, Maryland: Cell-mediated immune response toward viral envelope and core proteins in gibbon apes (*Hylobates lar*) chronically infected with HIV.

Homsy, J., Cheng-Mayer, C., Levy, J.A., Cancer Research Institute, University of California School of Medicine, San Francisco: Characterization of HIV serotypes with relevance to AIDS vaccines.

Lee, T.H.,[1] Redfield, R.,[3] Chou, M.J.,[1] Huang, J.H.,[1] Saah, A.,[4] Yu, X.F.,[1] Hsieh, C.C.,[2] McLane, M.F.,[1] Burke, D.S.,[3] Essex, M.,[1] Depts. of [1]Cancer Biology, [2]Epidemiology, Harvard School of Public Health, Boston, Massachusetts; [3]Dept. of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C.; [4]NIAID, National Institutes of Health, Bethesda, Maryland: Antibody reactivity to the carboxyl end of gp120 and the outcome of HIV infection.

Huet, T.,[1] Delaporte, E.,[2] Dazza, M.-C.,[2] Roelants, G.,[3] Brun-Vezinet, F.,[2] Wain-Hobson, S.,[1] [1]Institut Pasteur, Paris, [2]Hôpital Claude Bernard, Paris, France; [3]International Centre for Medical Research, Gabon, Africa: Genetic structure of an atypical HIV-1 from Gabon.

SESSION 9 VIROLOGY. II.

Chairman: K. Coelingh, NIAID, National Institutes of Health

Coelingh, K.,[1] Battey, J.,[2] LeBacq, A.,[2] Collins, P.,[1] Murphy, B.,[1] [1]Laboratory of Infectious Diseases, NIAID, [2]NMOB, NCI, Bethesda, Maryland: Development of live virus and subunit vaccines for PIV3.

Flexner, C., Hugin, A., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: IL-2 expression markedly attenuates the virulence of recombinant vaccinia virus and can increase antibody titers to coexpressed influenza antigens.

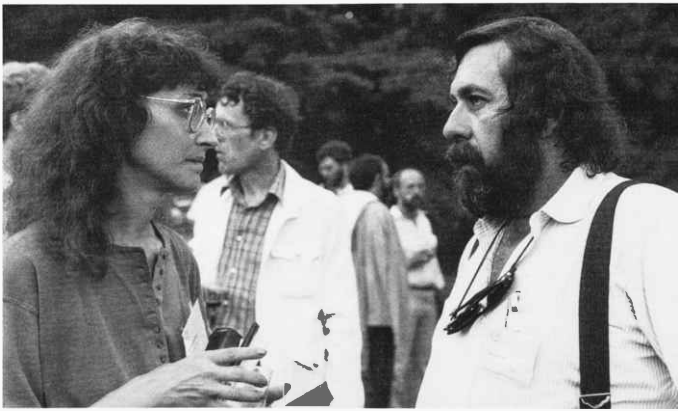
Lowe, R.S., Keller, P.M., Provost, P.J., Banker, F.S., Han, J., Eminı, E.A., Ellis, R.W., Dept. of Virus and Cell Biology, Merck, Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Immunogenicity of a recombinant varicella-zoster virus in the common marmoset.

Johnson, M.P., Meitin, C.A., Small, P.A., College of Medicine, Dept. of Immunology and Medical Microbiology, University of Florida, Gainesville: Passive antibody inhibits immune response to recombinant vaccinia.

Koszinowski, U.H.,[1] Volkmer, H.,[1] Jonjic, S.,[1] Wittek, R.,[2] Keil, G.M.,[1] [1]Federal Research Centre for Virus Diseases of Animals, Tubingen, Federal Republic of Germany; [2]Institut de Biologie Animale, Lausanne, Switzerland: Cytolytic T lumphocytes induced by vaccinia recombinant expressing a herpesvirus nonstructural immediate-early protein protect mice against lethal cytomegalovirus infection.

Meignier, B.,[1] Longnecker, R.,[2] Roizman, B.,[2] [1]Institut Mérieux, Lyon, France; [2]University of Chicago, Illinois: The R7017 and R7020 HSV recombinant prototype vaccine strains—Animal studies.

Murray, M.G., Kuhn, R.J., Wimmer, E., Dept. of Microbiology, State University of New York, Stony Brook: Poliovirus type 1/type 3 antigenic hybrid virus—Characterization and properties.



K. Coelingh, J. Keith

SESSION 10 VIROLOGY. III.

Chairman: R. Chanock, NIAID, National Institutes of Health

Olmsted, R.A.,[1] Murphy, B.R.,[1] Buller, R.M.,[2] London, W.T.,[3] Prince, W.T.,[3] Beeler, J.A.,[1] Collins, P.L.,[1] Laboratories of [1]Infectious Diseases, [2]Viral Diseases, NIAID, [3]NINCDS, National Institutes of Health, Bethesda, Maryland: Evaluation in nonhuman primates of the safety, immunogenicity, and efficacy of recombinant vaccinia viruses expressing the F and G glycoproteins of respiratory syncytial virus.

Vijaya, N.,[1] Elango, N.,[1] Moss, B.,[1] Zavala, F.,[2] Nussenzweig, V.,[2] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Depts. of Molecular Parasitology and Pathology, New York University Medical Center, New York, New York: Engineering vaccinia virus vectors that express hybrid proteins containing immunogenic sequences on the cell surface.

Whitton, J.L., Lewicki, H., Southern, P.J., Oldstone, M.B.A., Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Precise localization of cytotoxic T lymphocyte epitopes in the LCMV glycoprotein.

Weeks-Levy, C., Mento, S.J., Detjen, B.M., Cano, F.R., Lederle Biologicals, Pearl River, New York: Molecular characterization of Sabin type-3 poliovirus strains—Sequence correlates to neurovirulence testing.

Neurath, A.R.,[1] Kent, S.B.H.,[2] Strick, N.,[1] Parker, K.,[2] [1]New York Blood Center, New York, New York; [2]California Institute of Technology, Pasadena: Design of synthetic peptides mimicking the immunologic and biologic functions of the pre-S sequence of the HBV envelope protein.

Stapleton, J., Lange, D., Dept. of Internal Medicine, University of Iowa, Iowa City: Trypsin sensitivity of the HAV immunodominant neutralization site.

Summary: R. Lerner, Research Institute of Scripps Clinic

Translational Control

September 16—September 20

ARRANGED BY

Michael Mathews, Cold Spring Harbor Laboratory
John Hershey, University of California, Davis
Brian Safer, National Institutes of Health

277 participants

It has been more than ten years since a meeting on the topic of protein synthesis was last held at Cold Spring Harbor. That this year's meeting could concentrate on the regulatory aspects of the process, rather than on its mechanism, is a token of how far the field has advanced over the years. Of course, it is impossible to consider regulation without a knowledge of mechanism, and so an afternoon Workshop was included in the program to discuss recent ideas on the action of the protein synthesis factors. These enzymes mediate the initiation, elongation, and termination phases of messenger RNA decoding on the ribosome: Their activities are subject to controls of several different kinds, in organisms from the unicellular to the most complex, and under a large variety of circumstances.

The meeting was distinguished by the beginnings of the molecular genetic approach to problems that were previously amenable only to biochemical techniques, often of the most demanding sort. The genes or cDNAs for some of the protein synthesis factors themselves have been cloned and sequenced, and more are on the way. The first results to stem from a mutational analysis were presented, and we can now confidently look forward to answers to hitherto inaccessible questions from the application of reverse genetics strategies. Directed mutagenesis of messenger RNA genes, carried out over the last several years, has already disclosed numerous features of RNA sequence and structure that govern its efficiency as a template for protein synthesis in both prokaryotic and eukaryotic cells. A broad-ranging survey of this topic did as much to show how much we

need to know as how much we already know. As in so many other fields, viral systems were very much in evidence, lending themselves to both genetic and biochemical analyses. These systems offer insights into the range of regulatory processes open to the cell, serving as models for the more complex systems that participate in the developmental and growth regulatory programs which were also the subject of lively debate.

At its conclusion, both the participants and the Laboratory agreed that we should not wait another decade for the next meeting on this subject at Cold Spring Harbor. A second Translational Control meeting has been scheduled for 1989, and we will then see whether the rate of advance continues to accelerate. This year's meeting was the fortunate beneficiary of a generous grant from ICN Biomedicals, Inc., which covered all the costs of the conference.

SESSION 1 mRNA STRUCTURE AND REGULATION

Chairman: N. Sonenberg, McGill University

van Duijn, L.P., Voorma, H.O., Dept. of Molecular Cell Biology, Utrecht, The Netherlands: Secondary structure and expression in vivo and in vitro of mRNAs with newly inserted upstream AUG codons.

Tahara, S.M., Dietlin, T.A., Worrilow, L.M., Sijuwade, T., Harney, P., Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: Parameters governing start-site preference in bicistronic eukaryotic mRNAs.

Sonenberg, N., Darveau, A., Parkin, N., Nicholson, R., Pelletier, J., Dept. of Biochemistry, McGill University, Montreal, Canada: *cis*-Acting elements in the 5′ noncoding region of eukaryotic mRNAs modulate translation.

Clements, J., Laz, T., Sherman, F., Depts. of Biochemistry and Biophysics, University of Rochester Medical Center, New York: Role of mRNA sequences in yeast translation.

Cigan, A.M., Donahue, T.F., Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: The scanning process in yeast and the role of the initiator tRNA.

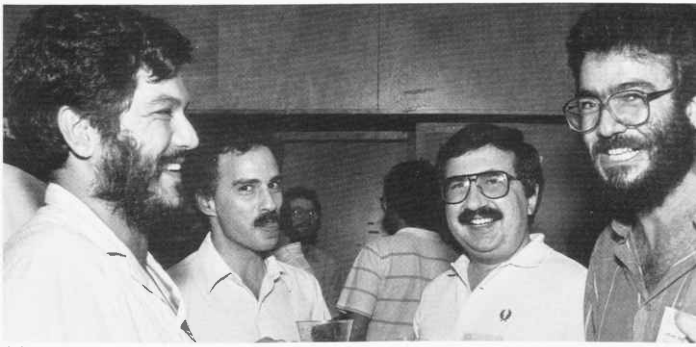
Costanzo, M.C., Fox, T.D., Section of Genetics and Development, Cornell University, Ithaca, New York: Three yeast nuclear gene products act on the 5′-untranslated leader of a specific mitochondrial mRNA to promote translation.

Lawson, T.G.,[1] Cladaras, M.H.,[1] Ray, B.K.,[1] Lee, K.A.,[1] Abramson, R.D.,[2] Merrick, W.C.,[2] Thach, R.E.,[1] [1]Dept. of Biology, Washington University, St. Louis, Missouri; [2]Dept. of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio: Unwinding of mRNA structure of eIF-4F, eIF-4A, and eIF-4B and the role of structure in determining translation rates.

Seal, S.N., Marcus, A., Institute for Cancer Research, Philadelphia, Pennsylvania: Structural features of an mRNA that determine ribosome binding.

Harvey, R.J., Short, S.A., Wellcome Research Laboratories, Research Triangle Park, North Carolina: IF1 and IF3 act in the recognition of translational initiation sequences of mRNA by *E. coli* ribosomes.

Chitpatima, T., Brawerman, G., Dept. of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts: Shifts in conformation of the 5′ non-coding region of a mouse mRNA under translational control.



J. Martin-Perez, A. Dancis, C. de Haro, J. Barriocanal

SESSION 2 REGULATION OF PROKARYOTIC mRNAs

Chairman: L. Gold, University of Colorado, Boulder

Denoya, C.,[1] Bechhofer, D.,[2] Breidt, F.,[1] Narayanan, C.S.,[2] Dubnau, D.,[1] [1]Dept. of Microbiology, Public Health Institute, [2]Dept. of Biochemistry, Mt. Sinai School of Medicine, New York, New York: Multiple mechanisms of posttranscriptional regulation of erythromycin resistance.

Matthews, L.C., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Translational retroregulation of ribosomal protein genes in *E. coli*.

Wu, H.N., Uhlenbeck, O.C., Dept. of Chemistry/Biochemistry, University of Colorado, Boulder: Specific coat protein–RNA interactions in the translational repression of two RNA bacteriophages.

Zengel, J.M., Lindahl, L., Dept. of Biology, University of Rochester, New York: Autogenous control of the *E. coli* S10 ribosomal protein operon at the translational and transcriptional levels.

Karam, J.,[1] Andrake, M.,[1] Wei, R.,[1] Guild, N.,[2] Gold, L.,[2] [1]Dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston; [2]Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Translational regulation of the DNA replication complex of bacteriophage T4.

Webster, K.R., Chen, W., Konigsberg, W.H., Spicer, E.K., Yale University School of Medicine, New Haven, Connecticut: In vitro studies of T4 RegA protein translational repression and protein-RNA interactions.

Draper, D.E., Tang, C.K., Dept. of Chemistry, Johns Hopkins University, Baltimore, Maryland: Structure of the α operon leader mRNA and mechanism of its repression by ribosomal protein S4.

Donly, B.C., Parsons, G.D., Mackie, G.A., Dept. of Biochemistry, University of Western Ontario, London, Canada: Mutations affecting the autogenous translational control of ribosomal protein S20.

Gold, L., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Translational regulation–Strategy and tactics.

SESSION 3 POSTER SESSION

Ahmad, M.F., Pierce, M.W., Diabetes Unit, Massachusetts General Hospital, Boston: Protein synthesis initiation in yeast–Overlapping sequences between α and γ subunits of eIF-2 and isolation of the yeast gene encoding the γ-subunit.

Akusjärvi, G.,[1] Svensson, C.,[1] Larsson, S.,[1] Nygård, O.,[2] [1]Dept. of Microbial Genetics, Medical Nobel Institute, Karolinska Institutet, Stockholm; [2]Dept. of Zoological Cell Biology, University of Stockholm, Sweden: Controls of protein synthesis and mRNA abundance by adenovirus VA RNA_I.

Andrews, D., Lingappa, V.R., Dept. of Physiology, University of California, San Francisco: Signal peptide function is effected by sequences beyond the cleavage site.

Arya, S.K., NCI, National Institutes of Health, Bethesda, Maryland: 3′ *orf* and *sor* genes of HIV-1–In vitro transcription-translation and immune reactive domains.

Bablanian, R.,[1] Goswami, S.K.,[1] Esteban, M.,[1] Banerjee, A.K.,[2] [1]Dept. of Microbiology, State University of New York Health Science Center, Brooklyn; [2]Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey: Mechanism of inhibition of protein synthesis by poly(A).

Baer, M.L.,[1] Jobling, S.A.,[1] Liem, K., Jr.,[1] Gehrke, L.,[1,2] [1]Harvard-Massachusetts Institute of Technology, Division of Health Sciences and Technology, Cambridge, [2]Dept. of Anatomy and Cellular Biology, Harvard Medical School, Boston: Characterization of a specific mRNA-protein interaction using the mobility band-shift assay.

Bag, J., Pramanik, S., Dept. of Molecular Biology and Genetics, University of Guelph, Ontario, Canada: Translation of mRNA coding for a 40,000-dalton polypeptide is regulated during differentiation of rat L6 myoblasts.

Benavente, J.,[1] Shatkin, A.J.,[2] [1]Dept. de Bioquimica, Facultad de Biologia, Universidad de Santiago, Spain; [2]New Jersey Center for Advanced Biotechnology and Medicine, Piscataway: Avian reovirus mRNAs are nonfunctional in infected mouse L cells–A novel translational basis for virus hostrange restriction.

Bennett, V.D., Adams, S.L., Dept. of Human Genetics, University of Pennsylvania, Philadelphia: Translational control of type I collagen synthesis in vertebral chondrocytes.

Berkner, K.L.,[1] Boel, E.,[2] Yarnold, S.,[1] Prunkard, D.E.,[1] [1]ZymoGenetics, Inc., Seattle, Washington; [2]Novo Industri A/S, Bagsvaerd, Denmark; Efficiency of expression of proteins encoded by polycistronic messages in mammalian cells.

Berry, J.O., Carr, J.P., Klessig, D.F., Waksman Institute of Microbiology, Rutgers State University of New Jersey, Piscataway: Regulation by light of translational initiation and elongation of RuBPCase synthesis in *A. cotyledons*.

Boal, T.R., Safer, B., LMH, NHLBI, NIH, Bethesda, Maryland: Inhibition of translation by anti-eIF-2B monoclonal antibodies–Mechanistic studies.

Boissonneault, G., Rogers, P.A., Tremblay, R.R., Laboratory of Hormonal Regulation, Laval University Hospital Centre, Quebec, Canada: Depressed translational capacity in an androgen-sensitive rat skeletal muscle.

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Jeffrey, I., Kelly, F., Pain, J., Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Brighton, England: Effects of starvation, diabetes, and acute insulin treatment on initiation of protein synthesis in rat skeletal muscle.

SESSION 4 REGULATION OF EUKARYOTIC mRNAs

Chairman: S. Lindquist, University of Chicago

Yen, T.J., Pachter, J.S., Gay, D.A., Cleveland, D.W., Johns Hopkins University School of Medicine, Baltimore, Maryland: A novel autoregulatory mechanism for controlling expression of tubulin—Sequences sufficient to confer modulated stability of polyribosome-bound β-tubulin mRNAs are contained within the first four codons.

Werner, M.,[1] Fuller, A.,[1] Piérard, A.,[1,2] [1]Laboratoire de Microbiologie, Université Libre de Bruxells, [2]Institut de Recherches du CERIA, Belgium: Study of the role of the leader peptide in the arginine-specific repression of yeast gene *CPA1*.

Theordorakis, N.G., Banerji, S.S., Morimoto, R.I., Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois: Posttranscriptional regulation of hsp70 expression in human cells—Effects of heat shock, inhibition of protein synthesis, and adenovirus infection on translation and mRNA stability.

Sanders, M.M., Bruederle, L.P., Dept. of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey: Translational regulation in heat-shock *Drosophila* cells.

Hinnebusch, A.G., Mueller, P.P., Williams, N.P., Laboratory of Molecular Genetics, National Institutes of Health, Bethesda, Maryland: Sequence requirements for translational control of *GCN4* mRNA.

Hentze, M.W., Caughman, S.W., Rouault, T.A., Dancis, A., Barriocanal, J., Harford, J.B., Klausner, R.D., NICHD, National Institutes of Health, Bethesda, Maryland: Translational regulation of human ferritin expresion by iron—Identification and characterization of the iron-responsive element and in vivo modeling of possible mechanisms.

Leibold, E.A.,[1] Munro, H.N.,[1,2] [1]Dept. of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, [2]USDA Human Nutrition Research Center on Aging, Boston: Identification of cytoplasmic factors that regulate the 5′-untranslated region in the rat liver ferritin L-subunit mRNA.

Strick, C.A.,[1] Fox, T.D.,[2] Sections of [1]Biochemistry, Molecular and Cell Biology, [2]Genetics and Development, Cornell University, Ithaca, New York: The yeast regulatory gene *PET111* encodes a mitochondrial protein that is translated from an mRNA with a long 5′-leader sequence.

Steel, L.F., Jacobson, A., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Translational regulation of ribosomal protein gene expression during *Dictyostelium* development.

SESSION 5 CONTROL AT ELONGATION AND TERMINATION

Chairman: R. Gesteland, University of Utah Medical Center

Bosch, L.,[1] Kraal, B.,[1] Vijgenboom, E.,[1] Swart, G.,[2] van Noort, J.M.,[1] Abrahams, J.P.,[1] Parmeggiani, A.,[2] [1]Dept. of Biochemistry, University of Leiden, The Netherlands; [2]Ecole Polytechnique, Laboratoire de Biochimie, Palaiseau, France: Novel functions of EF-TuA and EF-TuB.

Spanjaard, R.A., van Duin, J., Dept. of Biochemistry, Leiden, The Netherlands: The 50% frameshift during translation of the tandem minor codons AGG.AGG is not related to base complementarity of this sequence with the 3′ end of 16S rRNA.

Weiss, R.,[1] Dunn, D.,[1] Atkins, J.,[2] Gesteland, R.,[1] [1]Dept. of Human Genetics, University of Utah, and Howard Hughes Medical Institute, Salt Lake City; [2]Dept. of Biochemistry, University College, Cork, Ireland: Ribosomes can sense mRNA sequence during translation.

Jacks, T., Madhani, H., Varmus, H.E., Depts. of Biochemistry and Biophysics and Microbiology, University of California, San Francisco: Ribosomal frameshifting in RSV requires sequences at the frameshift site and downstream RNA secondary structure.

Smith, D., Yarus, M., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: tRNA-tRNA interactions alter the rate of translation.

Prats, A.C.,[1] Capdevielle, J.,[2] Ferrara, P.,[2] Darlix, J.L.,[1] [1]Labo-Retro, CRBGC-CNRS, Toulouse, [2]Sanofi-ELFBR, Toulouse, France: Translational initiation at AUG and GUG codons and ribosomal frameshifts take place in the 630-nucleotide leader of MLV RNA.

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Ivey, M.R., Steege, D.A., Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: An inherently inactive initiation site made functional by translational coupling.

Diener, D.R., Baker, E.J., Rosenbaum, J.L., Dept. of Biology, Yale University, New Haven, Connecticut: Translational selection between α1 and α2 tubulin mRNAs in *Chlamydomonas*.

Morch, M.D.,[1] Valle, R.P.C.,[1] Drugeon, G.,[1] Boyer, J.C.,[1] Legocki, A.,[1,2] Haenni, A.L.,[1] [1]Institut Jacques Monod, Paris, France; [2]University of Agriculture, Poznan, Poland: Termination of protein synthesis as a regulation step for the expression of viral RNAs.

SESSION 6 WORKSHOP: PROTEIN SYNTHESIS FACTORS

Chairman: W.C. Merrick, Case Western Reserve University

Jobling, S.A.,[1] Bendena, W.,[2] Pardue, M.L.,[2] Gehrke, L.,[1,3] [1]Harvard, Massachusetts Institute of Technology, Division of Health Sciences and Technology, [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge, [3]Dept. of Anatomy and Cellular Biology, Harvard Medical School, Boston: Analysis of mRNA translational efficiency in vitro using chimeric mRNAs containing heterologous untranslated leader sequences.

Kamath, A., Chakraburtty, K., Dept. of Biochemistry, Medical College of Wisconsin, Milwaukee: Effect of EF-3 on aminoacyl-tRNA binding to ribosomes.

Keierleber, C., Tran, H., McLaughlin, C.S., Moldave, K., Dept. of Biological Chemistry, University of California, Irvine: Role of protein synthesis in the control of cell division in yeast.

Klein, R.R., Gamble, P.E., Mullet, J.E., Dept. of Biochemistry and Biophysics, Texas A&M University, College Station: Regulation of chloroplast-encoded chlorophyll *a*-binding protein translation.

Klotsky, R.A., Wertheimer, S., Schwartz, I., Dept. of Biochemistry, New York Medical College, Valhalla: Transcriptional regulation of the *thrS-infC-rplT* operon of *E. coli*.

Kramer, G., Chen, S.-C., Hardesty, B., Clayton Foundation Biochemical Institute, Dept. of Chemistry, University of Texas, Austin: Phosphoprotein phosphatase activity as a potential mechanism for regulating the extent of eIF-2α phosphorylation.

Lane, R.M., Jackson, R.J., Dept. of Biochemistry, University of Cambridge, England: Control of initiation in heat-shocked and hypertonic-shocked mammalian cells.

Langstrom, N.,[1,2] Winblad, B.,[2] Wallace, W.,[1] [1]Dept. of Psychiatry, Mount Sinai Medical School, New York; [2]Dept. of Pathology, University of Umea, Sweden: Inhibition of elongation by polysomes from Alzheimer's disease brain tissues.

Lau, Y.T., Horowitz, S.B., Dept. of Physiology and Biophysics, Michigan Cancer Foundation, Detroit: Potassium modulation of cellular protein synthesis.

Liebhaber, S.A., Shakin, S.H., Howard Hughes Medical Institute and Depts. of Human Genetics and Medicine, University of Pennsylvania School of Medicine, Philadelphia: Influence of duplexes 3′ to the initiation codon on globin mRNA monosome formation.

Looman, A.C.,[1] Bodlaender, J.,[2] Comstock, L.J.,[3] Eaton, D.,[3] Jhurani, P.,[4] de Boer, H.A.,[3] van Knippenberg, P.H.[2] [1]Institut für Gärungsgewerbe und Biotechnologie, Berlin, Federal Republic of Germany; [2]Dept. of Biochemistry, University of Leiden, The Netherlands; Depts. of [3]Cell Genetics, [4]Organic Chemistry, Genentech, Inc., South San Francisco; Influence of the codon following the AUG initiation codon on the expression of a modified *lacZ* gene in *E. coli*.

Louis, B.G., Ganoza, M.C., Banting and Best Dept. of Medical Research, University of Toronto, Canada: Determinants of translational start-site recognition.

Lütcke, H.A.,[1] Chow, K.C.,[2] Mickel, F.S.,[2] Kern, H.F.,[1] Scheele, G.A.,[1] [1]Dept. of Cell Biology, Philips University, Marburg, Federal Republic of Germany: [2]Laboratory of Cell and Molecular Biology, Rockefeller University, New York, New York: Nucleotide – 3 modulates initiation of mRNA translation in vitro in reticulocyte lysate but not in wheat-germ extract.

Manchester, K.L., Dept. of Biochemistry, University of Witwatersrand, Johannesburg, South Africa: Kinetic constants required for the adequate functioning of eIF-2 and eIF-2B.

Merrick, W.C., Abramson, R.D., Dever, T.E., Dept. of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio: Initiation factors that bind mRNA – Possible new beginnings.

Milburn, S.,[1] Pelletier, J.,[2] Sonenberg, N.,[2] Hershey, J.,[1] [1]Dept. of Biological Chemistry, University of California School of Medicine, Davis; [2]Dept. of Biochemistry and McGill Cancer Center, McGill University, Montreal, Canada: eIF-4B is the 80-kD protein that interacts with the cap structure of eukaryotic mRNAs.

Mize, N.K., Lingappa, V.R., Dept. of Physiology, University of California, San Francisco: Reversal of transmembrane protein asymmetry.

Möller, W., Janssen, G., Zeelen, J., Maessen, G.D.F., Maasen, J.A., Dept. of Medical Biochemistry, Sylvius Laboratories, State University of Leiden, The Netherlands: Elongation of ribosomes during differentiation.

Morse, D., Milos, P., Roux, E., Hastings, J.W., The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Circadian rhythm of luciferin binding protein in *Gonyaulax* depends on translational control.

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Oppenheim, A.B., Altuvia, S., Locker-Giladi, H., Koby, S., Ben-Nun, O., Kornitzer, D., Teff, D., Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Translational control of bacteriophage λ cIII gene expression.

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Panniers, R., University of Rochester Cancer Center, New York: Dependence of protein synthesis on intracellular pH.

Pardigon, N., Vialat, P., Gerbaud, S., Girard, M., Bouloy, M., Unité de Virologie Moléculaire, CNRS, Institut Pasteur, Paris, France: In vitro translation of M and S mRNAs in *G. bunyavirus*.

Patrick, T.D., Pain, V.M., Biochemistry Laboratory, University of Sussex, School of Biological Sciences, Brighton, England: Preparation and characterization of cell-free protein synthesis systems from oocytes and eggs of *X. laevis*.

Perozzi, G.,[1] Mengheri, E.,[1] Colantuoni, V.,[2] Gaetani, S.,[1] [1]Instituto Nazionale della Nutrizione, Roma, [2]Instituto di Scienze Biochimiche, Università di Napoli, Italy: Expression of the retinol-binding protein gene in the liver of vitamin-A-deficient, zinc-deficient, and protein-depleted rats.

Persson, L.,[1] Holm, I.,[2] Heby, O.,[2] Depts. of [1]Physiology, [2]Zoophysiology, University of Lund, Sweden: Transla-

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tional control of ornithine decarboxylase by polyamines.

Poliquin, L.,[1] Francoeur, A.M.,[2] Stanners, C.P.,[1] [1]Dept. of Biochemistry, McGill University, Montreal, Canada; [2]Salk Institute, San Diego, California: Analysis of spontaneous VSV P function mutants isolated from wild-type strain.

Pollard, J.W.,[1] Galpine, A.,[2] Clemens, M.J.,[2] [1]MRC Group in Human Genetic Diseases, King's College, [2]CRC Mammalian Protein Synthesis and Interferon Research Group, St. George's Hospital Medical School, London, England: Control of protein synthesis initiation in CHO cells with temperature-sensitive aminoacyl-tRNA synthetase mutations.

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Qin, S.,[1] McLaughlin, C.S.,[2] Moldave, K.,[2] [1]Beiking University, People's Republic of China; [2]University of California, Irvine: EF-3 in yeast.

Rairkar, A., Lockard, R.E., Dept. of Biochemistry, Cornell University Medical College, New York, New York: Characterization of duck and mouse reticulocyte globin free mRNP particles.

Ramaiah, K.V.A., Levin, D.H., Chen, J.-J., London, I.M., Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, and Dept. of Biology, Cambridge: The eIF-2α kinase/RF·eIF-2(αP) phosphatase equilibrium in the regulation of RF (guanine nucleotide exchange) activity—Isolation of the physiological RF·eIF-2(αP) phosphatase.

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Rich, B.E., Steitz, J.A., Howard Hughes Medical Institute, Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Human ribosomal phosphoproteins P0, P1, and P2—Analysis of cDNA clones, in vitro synthesis, and assembly.

Rosenthal, E., Kewalo Marine Laboratory, University of Hawaii, Honolulu: Control of mRNA translation during oogenesis and fertilization in *U. caupo*.

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Samuel, C.E., Munemitsu, S.M., George, C.X., Atwater, J., Dept. of Biological Sciences, University of California, Santa Barbara: Regulation of the expression of reovirus S-class genes at the level of translation in untreated and interferon-treated cells.

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Schatz, J., Hurst, R., Matts, R., Dept. of Biochemistry, Oklahoma State University, Stillwater: Characterization of the effects of heavy metal ions on protein synthesis in the rabbit reticulocyte lysate.

Schwartz, D., Blobel, G., Laboratory of Cell Biology, Rockefeller University, New York, New York: Nucleotide requirements for detachment of membrane-bound polysomes.

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Simons, R.W., Case, C.C., Gonzalez, J.E., Dept. of Microbiology, University of California, Los Angeles: Translational control of IS*10* transposition.

Smith, R.C.,, Dworkin, M.B., Dworkin-Rastl, E., Ernst-Boehringer-Institut, Vienna, Austria: Translational control of the utilization of maternal mRNAs in early *Xenopus* development.

Steinhilber, W.,[1] Poensgen, J.,[1] Rausch, U.,[1] Kern, H.F.,[1] Scheele, G.A.,[2] [1]Dept. of Cell Biology, Philipps-University, Marburg, Federal Republic of Germany; [2]Laboratory of Cell and Molecular Biology, Rockefeller University, New York, New York: Translational control of anionic trypsinogen and amylase synthesis after caerulein stimulation in the rat pancreas.

Theodorakis, N.G., Banerji, S.S., Morimoto, R.I., Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illnois: hsp70 synthesis in chicken reticulocytes—Translational regulation at the level of elongation.

Traugh, J.A., Dept. of Biochemistry, University of California, Riverside: Regulation of protein synthesis by phosphorylation in response to growth-promoting compounds.

Trempe, J., Lally, C., Carter, B., NIDDKD, National Institutes of Health, Bethesda, Maryland: Posttranscriptional regulation of adeno-associated virus gene expression in 293 cells.

van Knippenberg, P.H., van Gemen, B., Koets, H.J., Plooy, C.A., Bodlaender, J., Dept. of Biochemistry, University of Leiden, The Netherlands: Characterization of the *ksgA* gene in *E. coli* encoding the 16S rRNA adenosine dimethyltransferase.

Verner, K., Eilers, M., Dept. of Biochemistry, Biocenter, University of Basel, Switzerland: Import of proteins into mitochondria.

Walden, W.E.,[1] McQueen, S.D.,[2] Russell, D.A.,[2] Smith, L.L.,[2] Thach, R.E.,[2] [1]University of Illinois College of Medicine, Chicago; [2]Washington University, St. Louis, Missouri: Regulation of mammalian ferritin synthesis at the translational level.

Waltz, K., Lopo, A.C., Division of Biomedical Sciences, University of California, Riverside: Change in covalent modification of eIF-4E with the activation of protein synthesis at fertilization in sea urchin eggs.

White, M., Kaspar, R., Morris, D., Dept. of Biochemistry, University of Washington, Seattle: Translational control in T lymphocytes.

Wu, Q.L., Raychowdhury, M., Eller, M.S., Sarkar, S., Dept. of Anatomy and Cellular Biology, Tufts University Medical School, Boston, Massachusetts: Resolution of cytoplasmic translation inhibitory RNA of chick embryonic muscle into biologically active subspecies.

SESSION 8 VIRAL mRNAs

Chairman: M. Kozak, University of Pittsburgh

Kozak, M., Biology Dept., University of Pittsburgh, Pennsylvania: Regulation of translation by structural features in mRNA.

Dabrowski, C., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania, Philadelphia: SV40 19S late mRNA translational start codon utilization—The efficiency of utilization of the agnoprotein AUG is affected by the 5′ end of the mRNA.

Geballe, A.P.,[1] Mocarski, E.S.,[2] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Dept. of Medical Microbiology, Stanford University, California: Translational control of a cytomegalovirus β gene is mediated by upstream AUG codons.

Hackett, P.B., Petersen, R.B., Moustakes, A., Hensel, C.H., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Translation of an ORF of the RSV leader RNA sequence affects transformation of chicken embryo fibroblasts.

Pelletier, J.,[1] Racaniello, V.R.,[2] Sonenberg, N.,[1] [1]Dept.of Biochemistry, McGill University, Montreal, Canada; [2]Dept. of Microbiology, Columbia University, New York, New York: Mutational analysis of the 5′-noncoding region of poliovirus RNA.

Jang, S.K.,[1] Kräusslich, H.G.,[1] Nicklin, M.J.H.,[1] Duke, G.M.,[2] Palmenberg, A.C.,[2] Wimmer, E.,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Dept. of Biochemistry, College of Agriculture and Life Sciences, University of Wisconsin, Madison: Studies on

the translational efficiency in vitro of poliovirus and EMCV 5′-nontranslated regions.

Roner, M.R., Gaillard, R.K., Joklik, W.K., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Regulation of translation of 5′ noncoding sequences in reovirus mRNAs.

Curran, J., Kolakofsky, D., Dept. of Microbiology, University of Geneva Medical School, Switzerland: Ribosomal initiation at alternate AUGs of the Sendai virus P/C mRNA.

Alonso-Caplen, F.V.,[1] Katze, M.G.,[2] Krug, R.M.,[1] [1]Sloan-Kettering Institute, New York, New York; [2]University of Washington, Seattle: Efficient mRNA translation in late adenovirus-infected cells that is independent of the tripartite leader.

Gallie, D.R.,[1] Sleat, D.E.,[1] Watts, J.W.,[1] Turner, P.C.,[2] Wilson, T.M.A.,[1] [1]Johns Hopkins Institute, AFRC Institute of Plant Science Research, Norwich, [2]Dept. of Biochemistry, University of Liverpool, England: Plant viral leader sequences that act as translational enhancers in vivo and in vitro.

Schultze, M., Hohn, T., Jiricny, J., Friederich Miescher Institute, Basel, Switzerland: Expression of the CaMV reverse transcriptase gene.

E. Darzynkiewicz

SESSION 9 GROWTH AND DEVELOPMENT

Chairman: M. Clemens, St. George's Hospital Medical School

Colin, A.M.,[1] Brown, B.D.,[1] Dholakia, J.N.,[2] Woodley, C.L.,[2] Wahba, A.J.,[2] Hille,, M.B.,[1] [1]Dept. of Zoology, University of Washington, Seattle; [2]Dept. of Biochemistry, University of Mississippi Medical Center, Jackson: mRNAs and guanine nucleotide exchange factors are repressed in sea urchin eggs.

Mandley, E., Lopo, A.C., Division of Biomedical Sciences, University of California, Riverside: Nuclease-sensitive inhibitor of protein synthesis in unfertilized eggs of the sea urchin *S. purpuratus*.

Jagus, R., Hansen, L.J., Huang, W.-I., Dept. of Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh School of Medicine, Pennsylvania: Regulation of maternal mRNA utilization in sea urchin eggs and embryos.

Galili, G.I.,[1] Kawata, E.E.,[1] Larkins, B.A.,[1] Smith, L.D.,[2] Depts. of [1]Botany and Plant Pathology, [2]Biological Sciences, Purdue University, West Lafayette, Indiana: 3′ poly(A) sequences enhance protein synthesis in *X. laevis* oocytes.

Richter, J.D., Swiderski, R.E., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: mRNP proteins from *Xenopus* oocytes bind specific message sequences.

Henshaw, E.C., Kumar, R., Panniers, R., Cancer Center and Dept. of Biochemistry, University of Rochester, New York: A step in protein synthesis dependent on calcium-calmodulin.

Brostrom, C.O., Chin, K.V., Cade, C., Boyer, I.J., Brostrom, M.A., Dept. of Pharmacology, Roher Wood Johnson Medical School, Piscataway, New Jersey: Calcium-dependent regulation of protein synthesis at initiation of translation of GH_3 pituitary cells.

Duncan, R.F., Hershey, J.W.B., Dept. of Biological Chemistry, University of California School of Medicine, Davis: Protein phosphorylation changes and the regulation of protein synthesis rate.

Petryshyn, R.,[1] Chen, J.-J.,[2] Judware, R.,[1] Tapp, P.,[1] [1]Dept. of Biochemistry and Molecular Biology, State University of New York Health Science Center, Syracuse; [2]Division of Health Science and Technology, Massachusetts Institute of Technology, Cambridge: Role of interferon during adipose differentiation.

SESSION 10 OTHER REGULATORY PROCESSES

Chairman: A.G. Hinnebusch, National Institutes of Health

Hovanessian, A.G.,[1] Galabru, J.,[1] Meurs, E.,[1] Williams, B.R.G.,[2] [1]Unité d'Oncologie Virale, Institute Pasteur, Paris, France; [2]Research Institute, Hospital for Sick Children and Dept. of Medical Genetics, University of Toronto, Canada: Characterization and cloning of the double-stranded RNA-dependent protein kinase induced by interferon.

Katze, M.G.,[1] Decorato, D.,[2] Krug, R.M.,[2] Boal, T.,[3] Safer, B.,[3] Akusjarvi, G.,[4] Galabru, J.,[5] Hovanessian, A.,[5] [1]University of Washington, Seattle; [2]Sloan Kettering Institute, New York, New York; [3]NHLBI, National Institutes of Health, Bethesda, Maryland; [4]University of Uppsala, Sweden; [5]Pasteur Institute, Paris, France: Translational control by adenovirus and influenza virus – Regulation of

M. Kosura

R. Reeves

P68 protein kinase autophosphorylation and activity.

Kostura, M., Mathews, M., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Partial purification and characterization of double-stranded RNA-activated kinase and its interaction with VA RNA.

Kaufman, R.J.,[1] Pittmann, D.D.,[1] Pathak, V.,[2] Hershey, J.,[2] [1]Genetics Institute, Cambridge, Massachusetts; [2]Dept. of Biological Chemistry, University of California School of Medicine, Davis: Role of phosphorylation of the α-subunit of eIF-2 in the translation of mRNAs in transfected cells.

Furtado, M.,[1] Subramanian, S.,[1] Bhat, R.,[1] Safer, B.,[2] Thimmappaya, B.,[1] [1]Dept. of Microbiology and Immunology, Northwestern University Medical School, Chicago, Illinois; [2]NHLB, National Institutes of Health, Bethesda, Maryland: Structural requirements of adenovirus VA RNA$_I$ for its translation modulation function – An in vivo mutational analysis of VA RNA$_I$ sequences.

Mellits, K.H., Mathews, M.B., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Structure of adenovirus VA RNA$_I$ and its function in translational control.

Rice, A.P., Mathews, M.B., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Use of a recombinant adenovirus vector to analyze the mechanism of tatIII *trans*-actvation of HIV LTR gene expression.

Marzluff, W., Chodchoy, N., Pandey, N., Dept. of Chemistry, Florida State University, Tallahassee: Structural requirements for regulation of histone mRNA degradation.

Reeves, R.,[1,2] Elton, T.S.,[1] Nissen, M.,[2] Lehn, D.,[2] Johnson, K.,[2] [1]Dept. of Biochemistry, [2]Program in Genetics and Cell Biology, Washington State University, Pullman: In vivo gene regulation and determination of posttranscriptional mRNA stability by the 3'-UTR of bIL-2.

Gonda, D.K., Bachmair, A., Varshavsky, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: In vitro analysis of amino-terminal targeting in selective protein degradation – Universality of the N-end rule in eukaryotes.

SESSION 11 TRANSLATIONAL COMPONENTS: GENES AND REGULATION

Chairman: H. Trachsel, Universität Bern

Cole, J.R.,[1] Olsson, C.L.,[2] Hershey, J.,[2] Grunberg-Manago, M.,[3] Nomura, M.,[1] University of California, [1]Irvine; [2]Davis; [3]Institut de Biologie Physico-Chimique, Paris, France: Feedback regulation of rRNA synthesis in *E. coli* requires IF-2.

Springer, M., Graffe, M., Dondon, J., Grunberg-Manago, M., Institut de Biologie Physico-Chimique, Paris, France: Genetic studies on the mechanism of *E. coli* threonyl-tRNA synthetase autoregulation.

Pathak, V., Ernst, H., Hershey, J.W.B., Dept. of Biological Chemistry, University of California School of Medicine, Davis: Cloning of HeLa cell cDNAs encoding eIF-2α and eIF-2β – Protein structures and the generation of eIF-2α lacking the HCR phosphorylation site.

Nielsen, P.,[1] Trachsel, H.,[2] [1]Max-Planck-Institut für Immunbiology, Freiburg, Federal Republic of Germany; [2]Institut für Biochemie and Molekularbiology, Bern, Switzerland: Genomic organization and tissue-specific expression of eukaryotic protein synthesis initiation factor 4A.

Altmann, M.,[1] Trachsel, H.,[2] Sonenberg, N.,[1] [1]Dept. of Biochemistry, McGill University, Montreal, Canada; [2]Institut für Biochemie und Molekularbiologie, Universität Bern, Switzerland: Functional studies using mutagenesis of the yeast *S. cerevisiae* 24-kD cap-binding protein.

Hiremath, L.S., Hiremath, S.T., Rychlik, W., Domier, L.L., Rhoads, R.E., Dept. of Biochemistry, University of Kentucky, College of Medicine, Lexington: Cell-free synthesis of human eIF-4E and effect of phosphorylation.

Richter, S., Hovemann, B., Center of Molecular Biology, ZMBH, University of Heidelberg, Federal Republic of Germany: Differential expression of two genes for EF-1α.

Slobin, L.I., Rao, T.R., Sunitha, I., Dept. of Biochemistry, University of Mississippi Medical Center, Jackson: Regulation of utilization and stability of mRNA for eEF-Tu in Friend erythroleukemia cells.

Dholakia, J.N., Woodley, C.L., Wahba, A.J., Dept. of Biochemistry, University of Mississippi Medical Center, Jackson: Regulation of polypeptide chain initiation in eukaryotes by the phosphorylation of the guanine nucleotide exchange factor and the redox state of the cell.

Hepatitis B Viruses

September 28—October 1

ARRANGED BY

William Mason, Fox Chase Cancer Center
Hans Will, Max-Planck Institut für Biochemie, Munich

203 participants

The Molecular Biology of Hepatitis-B Virus meeting covered a broad range of topics, from regulation of RNA and protein biosynthesis to vaccine development, to the molecular basis of liver damage, to hepatocellular carcinoma. Results were presented on the characterization of all four members of the hepatitis-B virus (hepadnavirus) family, as well as the related delta agent, and on cauliflower mosaic virus. Since last year's meeting, considerable progress has been made in defining viral gene products and functions. The role of signal sequences and posttranslational processing in virus maturation is now better understood, as are the host-range determinants of the viral envelope gene and the *trans*-acting regulatory potential of the viral *x* gene. The purification and characterization of the hepadnavirus reverse transcriptase also appear to be imminent and, along with genetic approaches made possible by the successful cultivation of hepadnaviruses in mammalian cells, should lead to a complete description of viral DNA synthesis from pregenomic RNA.

Progress has also been made in understanding the role of viral gene products and the immune response in progression to a chronic infection and associated liver disease. Along with the characterization of factors associated with the immune response to viral infection in man, a number of reports described mouse models as paradigms of the role of immunity and antigen expression in infection and disease. These studies led to the conclusion that both the immune response of the host and aberrant viral gene expression within individual cells may serve as major sources of liver damage during chronic infection.

The final session of the meeting focused on the role of chronic infection in hepatocellular carcinoma. Work presented at previous meetings had indicated that viral DNA integration was common in tumors; however, analysis of the structure and site of integration of viral DNA in tumors did not support the existence of a viral oncogene or of insertional activation of a cellular proto-oncogene. Several groups have now obtained evidence that insertional activation of oncogenes may play a role in some tumors and that *trans*-activation of cellular genes by a viral product may also have a role in tumor outgrowth.

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SESSION 1 HDV, CaMV, AND HEPADNAVIRUS REPLICATION

Chairman: J. Taylor, Fox Chase Cancer Center

Taylor, J.,[1] Kuo, M.,[1] Mason, W.,[1] Summers, J.,[1] Gowans, E.,[2] Cote, P.,[3] Gerin, J.,[3] [1]Fox Chase Cancer Center, Philadelphia, Pennsylvania; [2]Institute of Medical and Veterinary Science, Adelaide, Australia; [3]Georgetown University Medical Center, Rockville, Maryland: Structure and replication of HDV.

Robertson, H.D.,[1] Benenfeld, B.J.,[1] Branch, A.D.,[1] Wells, F.V.,[2] Gerin, J.L.,[2] Baroudy, B.M.,[2] [1]Rockefeller University, New York, New York; [2]Division of Molecular Virology and Immunology, Georgetown University, Rockville, Maryland: Structural analysis of HDV RNA.

Ponzetto, A., Forzani, B., Negro, F., Zyzik, E., Rasshofer, R.,

Roggendorf, M., Gerlich, W., Lavarini, C., Gerin, J.L., Callea, F., Verme, G., Division of Gastroenterology, Molinette Hospital, Turin, Italy: Biological characterization of acute and chronic HDV infection in the woodchuck.

Gordon, K.,[1] Bonneville, J.M.,[1] Fütterer, J.,[1] Pfeiffer, P.,[2] Pisan, B.,[1] Sansfacon, H.,[1] Hohn, R.,[1] [1]Friedrich Miescher-Institut, Basel, Switzerland; [2]Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France: Expression of the CaMV genome.

Miller, R.H., Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Maryland: Close evolutionary relatedness of the HBV and retroviral genomes.

Sprengle, R.,[1] Kaleta, E.F.,[2] Will, H.,[1] [1]Max-Planck-Institut für Biochemie, Martinsried/München, [2]Institut für Geflugelkrankheiten, Giesen, Federal Republic of Germany: Isolation and characterization of a DHBV-related virus endemic in herons.

Pugh, J.C., Summers, J., Fox Chase Cancer Center, Institute for Cancer Research, Philadelphia, Pennsylvania: Experimental infection of primary duck hepatocytes with DHBV in cultured medium containing DMSO.

Galle, P.R., Schlicht, H.J., Schaller, H., Zentrum für Molekulare Biologie, Heidelberg, Federal Republic of Germany: Production of DHBV in a human hepatoma cell line.

Sells, M.A., Zelent, A., Shvartsman, M., Acs, G., Dept. of Biochemistry, Mount Sinai School of Medicine, New York, New York: Characterization of HBV DNA and RNA transcripts in HepG2 cells that produce infectious virions.

Kay, A., Mandart, E., Shamoon, B., Galibert, F., Laboratoire d'Hématologie Expérimentale, Centre Hayem, Hôpital St-Louis, Paris, France: Transcription of WHV in the human cell line HepG2.

Seifer, M., Gerlich, W.H., Dept. of Medical Microbiology, University of Göttingen, Federal Republic of Germany: Replication of the HBV genome in transfected mouse fibroblasts.

SESSION 2 POSTER SESSION

Rapicetta, M.,[1] Forzani, B.,[2] Smedile, A.,[3] Hele, C.,[2] Morace, G.,[1] Di Rienzo, A.M.,[1] Buonavoglia, C.,[1] Avanzini, L.,[2] Gerin, J.L.,[3] Verme, G.,[2] Ponzetto, A.,[2] [1]Dept. of Virology, Instituto di Sanità, Roma, [2]Dept. of Glastroenterology, Molinette Hospital, Torino, Italy; [3]Dept. of Molecular Virology, Georgetown University, Rockville, Maryland: Pekin ducks are susceptible to HDV infection.

Forzani, B.,[1] Rapicetta, M.,[2] Rasshofer, R.,[3] Di Nardo, P.,[2] Hele, C.,[1] Callea, F.,[4] Roggendorf, M.,[3] Verano, F.,[2] Ciccaglione, A.R.,[2] Verme, G.,[1] Ponzetto, A.,[1] [1]Dept. of Gastroenterology, Molinette Hospital, Torino, [2]Dept. of Virology, Insituto di Sanità, Roma, Italy; [3]Max von Pettenkofer Institut, Munchen, Federal Republic of Germany; [4]Dept. of Pathology, Spedali, Civili, Brescia, Italy: Titration of woodchuck HDV in the woodchuck animal model.

Negro, F.,[1] Bergmann, K.F.,[1] Baroudy, B.,[1] Satterfield, W.C.,[2] Purcell, R.H.,[3] Gerin, J.L.,[1] [1]DMVI, Georgetown University, Rockville, Maryland; [2]University of Texas, Bastrop; [3]NIAID, National Institutes of Health, Bethesda, Maryland: Chronic HDV infection in HDV-superinfected HBV carrier chimpanzees.

Melegari, M., Pasquinelli, C., Manenti, F., Villa, E., Chair of Gastroenterology, University of Modena, Italy: HBV- and HDV-specific transcripts in chronic active hepatitis.

Di Bisceglie, A.M., Waggoner, J.G., Smedile, A., Bergman, K., Hoofnagle, J.H., NIDDK, National Institutes of Health, Bethesda, and Division of Molecular Virology and Immunology, Georgetown University, Rockville, Maryland: HBV status in liver and serum of patients with chronic δ hepatitis.

Nakajima, E.,[1] Thiers, V.,[1] Schellekens, H.,[2] Wands, J.,[3] Sninsky, J.,[4] Tiollais, P.,[1] Bréchot, C.,[1] [1]Recombinaison et Expression Génétique, Institute Pasteur, Paris, France; [2]TNO Primate Center, Rotterdam, The Netherlands; [3]Cetus Corp., San Francisco, California; [4]Massachusetts General Hospital, Boston, Massachusetts: HBV and HBV-related viruses in HBsAg-negative patients – Further characterization.

Spandau, D.F., Lee, C.H., Dept. of Pathology, Indiana University School of Medicine, Indianapolis: *trans*-Activation of gene expression by the HBV *X* gene product.

Lee, T.-H., Butel, J.S., Dept. of Virology, Baylor College of Medicine, Houston, Texas: *trans*-Activation of the HBV enhancer by the *X* gene of HBV.

Bishop, P., Bulla, G.A., Jameel, S., Siddiqui, A., Dept. of Microbiology and Immunology, University of Colorado School of Medicine, Denver: Repression of HBV enhancer-mediated transcription by adenovirus E1A and SV40 TAg proteins.

Twu, J.-S., Schloemer, R.H., Dept. of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis: Regulation of gene expression by HBV.

Louise, A., Tiollais, P., Buendia, M.A., Unité de Recombinaison et Expression Génétique, INSERM, CNRS, Institut Pasteur, Paris, France: HBsAg expression in cultured mammalian cells is strongly affected by *cis*-acting prokaryotic vector sequences.

F. Chisari

Jean-Jean, O.,[1] Levrero, M.,[2] Will, H.,[3] Perricaudet, M.,[2] Rossignol, J.-M.,[1] [1]Biologie Moleculaire de la Replication, [2]Virus Oncogenes, ER 272-IRSC, Villejuif, France; [3]Max Planck Institut für Biochemie, Martinsried, Federal Republic of Germany: Processing of the protein encoded by the pre-C sequence and C gene of HBV in human cells.

Shimoda, A.,[1] Kuroki, K.,[2] Unoura, M.,[1] Kobayashi, K.,[1] Hattori, N.,[1] Murakami, S.,[2] [1]First Internal Medicine, Kanazawa University Medical School, [2]Biophysics Division, Cancer Research Institute, Kanazawa, Japan: Expression of pre-C-C and C of human HBV and WHV in HeLa cells.

Pugh, J.C., Summers, J., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Amino acid composition of the major DHBV core particle polypeptide.

Feitelson, M.A., Millman, I., Duncan, G., Blumberg, B.S., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Presence of antibodies to the polymerase gene product(s) of HBV and WHV in natural and experimental infections.

Mohamad, A.A., Price, P.M., Dept. of Biochemistry, Mount Sinai School of Medicine, New York, New York: A baculovirus expression system to study the assembly of HBV in insect cells—Coexpression of the three HBV envelope proteins results in their coassembly and secretion as HBsAg particles.

Hsu, H.C.,[1] Gerlich, W.H.,[2] [1]Dept. of Pathology, National Taiwan University Hospital, Taipei, Republic of China; [2]Dept. of Medical Microbiology, University of Gottingen, Federal Republic of Germany: Correlation of pre-S proteins and glycosylation of hepatocyte HBsAg with viral replicative states.

Wang, Y.,[1] Wang, S.Q.,[1] Li, Z.P.,[1] Lu, X.Y.,[2] Yao, G.B.,[2] [1]Shanghai Institute of Biochemistry, Academic Sinica, [2]Jing-an Central District Hospital, Peoples Republic of China: Secretion of pre-S1 is cell-type-dependent.

Simon, K., Ganem, D., Dept. of Microbiology, University of California, San Francisco: Identification of intermediates in assembly of HBsAg particles.

Krone, B., Lenz, A., Heermann, K.H., Gerlich, W.H., Dept. of Medical Microbiology, University of Göttingen, Federal Republic of Germany: Binding of native serum albumin to HBsAg.

Lu, X.Y., Yao, G.B., Zhen, L.G., Wu, H.H., Clinical Immunology Research Unit, Jingan Central District Hospital, Shanghai, Peoples Republic of China: Binding characteristics of the HBV albumin receptor.

Wang, D.-F., Fasy, T., Hood, A., Thung, S.N., Gerber, M.A., Dept. of Pathology, Mount Sinai School of Medicine and City Hospital Center, Elmhurst, New York: HBsAg binding activity for transglutaminase cross-linked HSA.

Kniskern, P.J., Hagopian, A., Burke, P., Dunn, N., Markus, H., Montgomery, D., Bailey, J., Hurni, W., Miller, W., Emini, E., Wolanski, B., Wampler, E., Ellis, R.W., Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: A new hepatitis-B vaccine expressed in *S. cerevisiae*—Synthesis, assembly, and isolation of particles that display the immunogenic determinants of both the HBsAg and pre-S2 regions.

Goetz, A., Mimms, L., Whitters, E., Tyner, J., Wray, L., Sarin, V., Diagnostic Products Research and Development, Abbott Laboratories, Abbott Park, Illinois: Antigen and antibody assays for the pre-S2 epitope of HBV.

C. Pourcel

Chen, Y., Ma., X.K., Laboratory of Molecular Genetics, Institute of Basic Medical Sciences, Beijing, Peoples Republic of China: Synthesis of X-sequence protein and *X-Pol* fusion protein.

Stemler, M.,[1.3] Liang, X.-H.,[1] Hess, J.,[2] Braun, R.,[2] Will, H.,[3] Schröder, C.H.,[1] [1]Institut für Virusforschung, Deutsches Krebsforschungszentrum, [2]Institut für Medizinische Virologie der Universitat Heidelberg, [3]Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Antibodies to the HBV X protein.

Levrero, M.,[1.2] Jean-Jean, O.,[1] Franco, A.,[2] Blasano, F.,[2] Will, H.,[3] Perricaudet, M.,[1] [1]ER, Villejuif, France; [2]Instituto I Clinica Medica, Rome, Italy; [3]Max-Planck Institut für Biochemie, Martinsried, Federal Republic of Germany: Anti-X antibodies in chronic HBV infection.

Feitelson, M.A.,[1] Miller, R.H.,[2] [1]Fox Chase Cancer Center, Philadelphia, Pennsylvania; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Presence of sequences from the mammalian hepadnavirus *X* gene product in the major core product of DHBV.

Hadchouel, M.,[1] Farza, H.,[1] Simon, D.,[2] Tiollais, P.,[1] Pourcel, C.,[1] [1]Unité de Recombinaison et Expression Génétique, INSERM, CNRS, [2]Unité de Génétique des Mammifères, Institut Pasteur, Paris, France: Maternal inhibition of HBsAg gene expression in transgenic mice correlates with de novo methylation.

Deloia, J.A.,[1] Burk, R.D.,[2] Gearhart, J.D.,[1] [1]Johns Hopkins University, Baltimore, Maryland; [2]Albert Einstein College of Medicine, Bronx, New York: Relationship of developmental stage and hormonal status to HBsAg expression in HBV transgenic mice.

SESSION 3 STRATEGY AND CONTROL OF HEPADNAVIRUS GENE EXPRESSION

Chairman: H. Schaller, University of Heidelberg

Jameel, S., Patel, N.U., Siddiqui, A., Dept. of Microbiology/ Immunology, University of Colorado Medical School, Denver: Interactions between the HBV enhancer and cellular proteins.

Ostapchuk, P.,[1] Levine, A.J.,[2] Tackney, C.,[3] Hearing, P.,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Dept. of Molecular Biology, Princeton University, New Jersey; [3]ImClone Systems, New York, New York: Identification of a nuclear factor binding to a functional element of the HBV enhancer.

Karpen, S.,[1] Zelent, A.,[1] Banerjee, R.,[2] Acs, G.,[1,2] Depts. of [1]Biochemistry, [2]Neoplastic Diseases, Mount Sinai School of Medicine, New York, New York: Single nucleotide resolution of protein-binding sites in HBV enhancer and core promoter domains.

Shaul, Y., Ben-Levy, R., Faktor, O., Dickstein, R., Berger, I., Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Composition of the HBV enhancer element.

Murakami, S., Kaneko, S., Kodama, K., Harada, F., Kuroki, K., Biophysics Division, Cancer Research Institute, Kanazawa University, Japan: Detection of nuclear factor binding to the enhancer region of WHV DNA.

Bulla, G.A., Patel, N.U., Siddiqui, A., Dept. of Microbiology and Immunology, University of Colorado School of Medicine, Denver: Molecular analysis of HBsAg gene promoter regions.

Shaul, Y., De-Medina, T., Faktor, O., Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: The S promoter is regulated by positive and negative elements.

Molnar-Kimber, K., Jarocki-Witek, V., Dheer, S.K., Conley, A.J., Davis, A.R., Hung, P.P., Microbiology Division, Wyeth Laboratories, Inc., Philadelphia, Pennsylvania: Evidence of regulation of expression of hepatitis-B envelope proteins by X open reading frame in recombinant adenoviruses.

Zahm, P., Hofschneider, P.H., Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Transcriptional *trans*-activation by an HBV *X* gene product.

Wollersheim, M., Hofschneider, P.H., Max-Planck-Institut für Biochemie, München, Federal Republic of Germany: *trans*-Activation as a potential mechanism of HBV-associated oncogenesis.

Antonucci, T.K., Standring, D.N., Rutter, W.J., Hormone Research Institute, University of California, San Francisco: Tissue-specific expression in transient assays from four promoters and the enhancer of HBV.

SESSION 4 IN VIVO AND IN VITRO STUDIES ON VIRAL PROTEINS

Chairman: C. Seeger, Cornell University

Garcia, P.D., Ou, J.H., Rutter, W.J., Walter, P., Dept. of Biochemisty, University of California, San Francisco: Targeting of the HBV precore protein to the endoplasmic reticulum membrane – After signal peptide cleavage, translocation can be aborted and the product released into the cytoplasm.

Srandring, D.N., Ou, J.H., Walter, M., Rutter, W.J., Hormone Research Institute, University of California, San Francisco: Expression of HBV antigens in *X. laevis* oocytes.

Zyzik, E., Seifer, M., Taliani, G., Heermann, K.H., Gerlich, W.H., Dept. of Medical Microbiology, University of Göttingen, Federal Republic of Germany: Molecular structure of HBeAg.

Stemler, M.,[1,2] Weimer, T.,[1] Hess, J.,[3] Braun, R.,[3] Will, H.,[1] Schröder, C.H.,[2] [1]Max-Planck-Institut für Biochemie, Martinsried/München, [2]Institut für Virusforschung, Deutsches Krebsforschungszentrum, Heidelberg, [3]Institut für Medizinische Virologie, Universität Heidelberg, Federal Republic of Germany: Detection of antibodies directed against three domains of the hepatitis-B polymerase protein.

Chang, L.-J.,[1] Ganem, D.,[1,2] Varmus, H.E.,[1] Depts. of [1]Microbiology and Immunology. [2]Medicine, University of California, San Francisco: Study of the expression strategy of hepadnavirus *pol* genes.

Laub, O., Bavand, M., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: HBV-like particles contain proteins with reverse transcriptase activity.

Eble, B.,[1] Lingappa, V.,[2] Ganem, D.,[1] Depts. of [1]Microbiology, [2]Physiology, University of California, San Francisco: Membrane insertion of HBV pre-S proteins.

Pontisso, P.,[1] Petit, M.-A.,[2] Bankowski, M.,[1] Peeples, M.,[1] [1]Dept. of Immunology and Microbiology, Rush Medical Center, Chicago, Illinois; [2]INSERM, Clamart, France: Normal human liver plasma membranes bear two distinct receptors for HBV – One for pre-S1 and another for pre-S2.

M. Stemler, H. Will

Yokosuka, O., Omata, M., Ito, Y., Uchiumi, K., First Dept. of Medicine Chiba University School of Medicine, Japan: Expression of pre-S1/S2 and C peptides in the early phase of DHBV infection.

Katayama, K., Hayashi, N., Kasahara, A., Sasaki, Y., Ueda, Furusawa, S., Fusamoto, H., Sato, N., Kamada, T.,[1] Chisaka, O., Matsubara, K.,[2] [1]First Dept. of Medicine, Osaka University Medical School, [2]Institute for Molecular and Cellular Biology, Osaka University, Japan: Expression of the HBV *X* gene in type-B chronic liver disease.

Petit, M.A., Capel, F., Elfassi, E., Pillor, J., d'Immunologie Microbienne, Institut Pasteur, Paris, France: Isolation of the HBV X protein from an infected human liver and its use for anti-X antibody detection in patients.

SESSION 5 POSTER SESSION

Pontisso, P., Ogston, C.W., Peeples, M.E., Dept. of Immunology and Microbiology, Rush University, Chicago, Illinois: In vitro binding of HBV surface proteins to human leukocytes.

Colloca, S.,[1] Manzin, A.,[2] Vignetti, M.,[3] Amadori, S.,[3] Mandelli, F.,[3] Delfini, C.,[1] Clementi, M.,[2] Carloni, G.,[4] [1]Laboratory of Cell Biology, Istituto Superiore di Sanità, Rome, [2]Institute of Microbiology, University of Ancona, [3]Institute of Haematology, University of Rome, [4]Institute of Experimental Medicine, CNR, Rome, Italy: HBV DNA in mononuclear cells from HBV-infected subjects and leukemia patients.

Korba, B.E.,[1] Gowans, E.,[2] Tennant, B.C.,[3] Wells, F.V.,[1] Clarke, R.,[1] Gerin, J.L.,[1] [1]Georgetown University, DMVI Rockville, Maryland; [2]Institute of Medical Veterinary Science, Division of Virology, Adelaide, Australia; [3]Cornell University, College of Veterinary Medicine, Ithaca, New York: Tissue distribution of WHV in experimentally infected woodchucks.

Korec, E.,[1] Hložánek, I.,[1] Stará, J.,[1] Němeček, V.,[2] [1]Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Fremingovo, [2]Institute of Hygiene and Epidemiology, Prague, Czechoslovakia: Anti-idiotype antibody as a prospective vaccine against hepatitis B.

Schick, M.R., Kennedy, R.C., Dept. of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas: Genetic restriction of antibody production to an HBsAg epitope.

Schick, M.R., Kennedy, R.C., Dept. of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas: Induction of an anti-HBs response by an Ab-2α anti-idiotypic antibody.

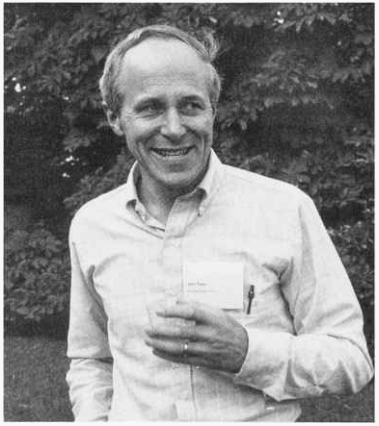
Nishioka, M., Kagawa, H., Third Dept. of Internal Medicine, Kagawa Medical School, Japan: IL-2 activity in patients with chronic hepatitis B.

Kagawa, H., Nishioka, M., Third Dept. of Internal Medicine, Kagawa Medical School, Japan: Effect of human recombinant IL-2 on patients with chronic active hepatitis B.

Actis, G.C., Maran, E., Rosina, F., Saracco, G., Brunetto, M., Bonino, F., Rizzetto, M., Verme, G., Division of Gastroenterology, Ospedale Molinette, Turin, Italy: Markers of response in HBV treated with interferon – Natural killer cells and aminotransferases (ALT).

Iino, S.,[1] Hino, K.,[2] Kurai, K.,[1] Miyakawa, H.,[2] Niwa, H.,[2] Oka, H.,[1] [1]First Internal Medicine, University of Tokyo, [2]Second Internal Medicine, National Defense Medical College, Japan: Effect of interferon therapy on vital responses in patients with chronic active hepatitis.

Kuhns, M.,[1] McNamara, A.,[1] Cabal, C.,[1] Perrillo, R.,[2] [1]Abbott Laboratories, North Chicago, Illinois; [2]Veterans Administration Medical Center and Washington University School of Medicine, St. Louis, Missouri: Quantitative monitoring of HBV DNA levels in chronic hepatitis-B patients undergoing prednisone withdrawal followed by IFN-α.



J. Taylor

Zhang, D., Liu, B., Institute for Viral Hepatitis, Chongqing Medical University, Peoples Republic of China: Serum inhibitory factor against IFN-α production in HBV infections.

Zhang, D., Liu, B., Liu, X.-y., Institute of Viral Hepatitis, Chongqing University of Medical Science, Peoples Republic of China: In vitro immunoregulation of anti-HBs synthesis by peripheral lymphocytes in HBV infections.

Chang, C.,[1,2] Sung, C.H.,[1] Hu, C.,[1,2] [1]Graduate Institute of Microbiology and Immunology, National Yang-Ming Medical College, [2]Dept. of Medical Research, Veterans General Hospital, Taipei, Taiwan, Republic of China: Induction of MHC gene expression on human hepatoma cell lines.

Hosoda, K., Imazeki, K., Omata, M., First Dept. of Medicine, Chiba University School of Medicine, Japan: Age-dependent infection by in vivo DHBV DNA transfection.

Brunetto, M.R., Saracco, G., Lattore, V., Torrani, M.C., Chiaberge, E., Maran, E., Bonino, F., Dept. of Gastroenterology, Molinette Hospital, Turin, Italy: Relationship between HBV replication and liver cell necrosis in HBsAg carriers.

L. Johnson, J. Newbold, W. Mason

Yong, H., Liu, Y.-f., Yang, S.-y., Dept. of Pathology, Fourth Military Medical College, Beijing, Peoples Republic of China: Detection of tissue HBsAg subtypes on paraffin sections of hepatocellular carcinoma and cirrhosis by monoclonal antibodies.

Chen, J.Y.,[1,5] Harrison, T.J.,[5] Hsu, H.C.,[2] Lai, M.Y.,[3] Lee, C.S.,[4] Chen, D.S.,[3] Yang, C.S.,[1] Zuckerman, A.J.,[5] Depts. of Bacteriology, [2]Pathology, [3]Medicine, [4]Surgery, College of Medicine, National Taiwan University, Republic of China; [5]Dept. of Medical Microbiology, London School of Hygiene and Tropical Medicine, England: Methylation of HBV DNA in hepatocellular carcinoma.

Koch, I., Koshy, R., Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Isolation of an HBV-sequence-containing cDNA clone from the human hepatoma cell line PLC/PRF/5, with flanking cellular sequences on both sides.

Fuchs, K., Roggendorf, M., Max von Pettenkofer Institute, University of Munich, Federal Republic of Germany: Transcription of WHV DNA in the hepatocellular carcinomas of woodchucks.

Chen, P.J., Lai, M.Y., Hsu, H.C., Lee, S.C., Chen, D.S., Graduate Institute of Clinical Medicine and Dept. of Pathology, National Taiwan University College of Medicine, Taipei, Republic of China: Active replication and accurate expression of the HBV genome in the cancerous tissue of hepatocellular carcinoma.

Shimoda, A.,[1] Kuroki, K.,[2] Uchijima, M.,[2] Kaneko, S.,[1] Unoura, M.,[1] Kobayashi, K.,[1] Hattori, N.,[1] Murakami, S.,[2] [1]First Internal Medicine, Kanazawa University Medical School, [2]Biophysics Division, Cancer Research Institute, Kanazawa, Japan: WHV DNA integration in multicentric primary hepatocellular carcinomas of woodchucks.

Gerin, J.L.,[1] Wells, F.V.,[1] Tennant, B.C.,[2] Baldwin, B.,[2] Rochee, M.,[1] Korba, B.E.,[1] [1]Georgetown University, DMVI, Rockville, Maryland; [2]Cornell University, College of Veterinary Medicine, Ithaca, New York: Integrated WHV DNA is present in hepatocellular carcinoma tissue from woodchucks that have serologically recovered from active WHV infections.

Nakai, S., Yamazoe, M., Ogasawara, N., Yoshikawa, H., Dept. of Genetics, Osaka University Medical School, Japan: Integration of WHV DNA into immunoglobulin V_K region of the chromosome of a hepatocarcinoma in woodchuck.

Mőrőy, T., Etiemble, J., Tiollais, P., Buendia, M.A., Unité de Recombinaison et Expression Génétique, INSERM, CNRS, Institut Pasteur, Paris, France: DNA rearrangement in a WHV-positive woodchuck hepatoma placed the proto-oncogene *c-myc* near the promoter region of a novel liver-specific gene.

Wang, H., Rogler, C.E., Albert Einstein College of Medicine, Bronx, New York: Loss of heterozygosity in chromosomes 11p and 13q in hepatocellular carcinomas.

Caselmann, W.H., Hofschneider, P.H., Koshy, R., Max-Planck-Institut für Biochemie, Martinsried/München, Federal Republic of Germany: Characterization of a single HBV DNA integrate with *trans*-activating potential from human hepatoma tissue.

Duelli, J., Koshy, R., Zahm, P., Hofschneider, P.H., Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: *cis* and *trans* studies of an integrated HBV DNA sequence.

Kaltschmidt, C., Hofschneider, P.H., Zahm, P., Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Chromosomally integrated HBV DNA with a truncated *X* gene retains *trans*-activating function.

SESSION 6 GENETIC APPROACHES TO VIRAL GENE FUNCTIONS

Chairman: C. Pourcel, Pasteur Institute

Pourcel, C.,[1] Hadchouel, M.,[1] Scotto, J.,[3] Tiollais, P.,[1] Babinet, C.,[2] Farza, H.,[1] [1]Unité de Recombinaison et Expression Génétique, INSERM, CNRS, [2]Unité de Génétique des Mammifères, Institut Pasteur, Paris, [3]Unité de Recherche d'Hépatologie Infantile, INSERM, Universite Paris-Sud, Hôpital d'Enfants, Bicêtre, France: Replication of HBV in a transgenic mouse.

Hino, O.,[1] Nomura, K.,[1] Ohtake, K.,[1] Sugano, H.,[1] Kitagawa, T.,[1] Kimura, S.,[2] Yokoyama, M.,[2] Katsuki, M.,[2,3] [1]Dept. of Pathology, Cancer Institute, Tokyo, [2]Central Institute for Experimental Animals, [3]Tokai University School of Medicine, Kanagawa, Japan: Instability of integrated HBV DNA in transgenic mice.

El-Ghor, A.A., Burk, R.D., Albert Einstein College of Medi-

cine, Bronx, New York: The DNase-I-hypersensitive site maps to the HBV enhancer in two independent lines of HBV transgenic mice.

Feitelson, M.A., Clayton, M.M., Zhou, X.-d., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Patterns of HBV gene expression and chronic hepatitis in viral-DNA-injected mice with different immune systems.

Chisari, F.V., Filippi, P., Buras, J., McLachlan, A., Pinkert, C.A., Palmitter, R.D., Brinster, R.L., [1]Research Institute of Scripps Clinic, La Jolla California; [2]University of Washington School of Medicine, Seattle; [3]University of Pennsylvania School of Veterinary Medicine, Philadelphia: Structural and pathological consequences of overexpression of the HBV large envelope polypeptide in transgenic mice.

Schlicht, H.-J., Schaller, H., ZMBH, University of Heidelberg, Federal Republic of Germany: Mutational analysis on the DHBV genome.

Chang, C., Enders, G., Sprengel, R., Varmus, H., Ganem, D., Dept. of Microbiology, University of California, San Francisco: Expression of the precore region of DHBV is not required for viral replication.

Yaginuma, K., Koike, K., Dept. of Gene Research, Cancer Institute, Tokyo, Japan: Putative promoter of the 3.6-kb RNA transcription for the HBV production found by in vitro mutagenesis.

Raney, A.K., Milich, D.R., Hughes, J.L., Sorge, J., Chisari, F.V., McLachlan, A., Dept. of Basic and Clinical Research, Research Institute of Scripps Clinic, La Jolla, California: High-efficiency retrovirus-mediated transfer of HBV antigen expression.

SESSION 7 TISSUE-SPECIFIC EXPRESSION, PATHOGENESIS, AND THE IMMUNE RESPONSE

Chairman: M. Omata, Chiba University

Onji, M.,[1] Lever, A.M.L.,[1] Saito, I.,[2] Thomas, H.C.,[1] [1]Dept. of Medicine, Royal Free Hospital, [2]Imperial Cancer Research Fund, London, England: Reduced sensitivity to interferon in cells containing the hepatitis-B genome.

Gerber, M.A., Thung, S.N., Hood, A., Price, P.M., Acs, G., Depts. of Pathology, Biochemistry, and Neoplastic Diseases, Mount Sinai School of Medicine, New York, New York: Induction of HLA-DR expression by IFN-γ in transfected hepatic cells.

Caselmann, W.H.,[1] Eisenburg, J.,[2] Hofschneider, P.H.,[1] Koshy, R.,[1] [1]Max-Planck-Institut für Biocheime, Martinsried/München, [2]Dept. of Internal Medicine, Krankenhaus der Barmherzigen Brüder, München, Federal Republic of Germany: Rapid elimination of HBV replication by IFN-β and IFN-γ.

Berninger, M.,[1] Di Bisceglie, A.M.,[2] Hoofnagle, J.H.,[2] Reyes, A.,[2] Schuster, D.,[1] Hartley, J.,[1] [1]Life Technologies, Inc., Gaithersburg, [2]NIDDK, National Institutes of Health, Bethesda, Maryland: Detection of HBV DNA in serum during therapy for chronic type-B hepatitis using a novel highly sensitive assay.

Fiume, L.,[1] Torrani, M.C.,[2] Baldi, M.,[2] Bonino, F.,[2] Verme, G.,[2] [1]Dept. of Experimental Pathology, University of Bologna, [2]Dept. of Gastroenterology, Molinette Hospital, Torino, Italy: Inhibition of HBV replication by ara-AMP conjugated with lactosaminated serum albumin.

Snyder, R.L.,[1] Newbold, J.E.,[2] Gowen, S.A.,[1] [1]Penrose Research Laboratory, Philadelphia, Pennsylvania; [2]Dept. of Microbiology, University of North Carolina, Chapel Hill: Acyclovir depresses DNA polymerase activity in woodchucks chronically infected with WHV.

Mason, W.S., Aldrich, C., Coates, L., Petcu, D., Lien, J.-M., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Effects of drugs on infection of cultured hepatocytes with DHBV.

Hosoda, K., Imazeki, F., Uchiumi, K., Omata, M., First Dept. of Medicine, Chiba University School of Medicine, Japan: More than expected extrahepatic DHBV replication.

O'Connell, A.P., London, W.T., Fox Chase Cancer Center, Philadelphia, Pennsylvania: DHBV in yolk sac endoderm and in cultured yolk sac cells.

Korba, B.E., Cote, P.J., Wells, F.V., Gerin, J.L., Georgetown University, DMVI, Rockville, Maryland: Mitogen-induced replication of WHV in cultured peripheral blood lymphocytes from chronically infected woodchucks.

SESSION 8 PATHOGENESIS AND THE IMMUNE RESPONSE

Chairman: F. Chisari, Scripps Clinic and Research Foundation

Anderson, S.A., Kennedy, R.C., Dept. of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas: A monoclonal anti-idiotypic antibody that recognizes a common human idiotype induces as in vitro antibody response specific for HBsAg.

Hoffmann, R.,[1,2] Jung, C.,[2] Rieber, E.P.,[2] Eisenburg, J.,[1] Paumgartner, G.,[1] Riethmüller, G.,[2] Pape, G.R.,[1,2] [1]Dept. of Internal Medicine, [2]Institute of Immunology, University of Munich, Federal Republic of Germany: Clonal analysis of T lymphocytes from liver tissue of patients with chronic hepatitis B.

Ferrari, C., Penna, A., Chisari, F.V., Mondelli, M.U., Dept. of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California: Amplification of the HBcAg T-cell clonal response by an autoreactive T-cell clone in man.

Yamauchi, K., Kamogawa, Y., Komatsu, T., Katoh, T., Obata, H., Institute of Gastroenterology, Tokyo Women's Medical College, Japan: HBsAg-specific suppressor T cells and their soluble factor in immunological nonresponsiveness to HBsAg.

Milich, D.R.,[1] McLachlan, A.,[1] Thornton, G.B.,[2] Moriarty,

A. O'Connell, P. Marionst

A.,[2] Hughes, J.,[1] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, [2]Johnson and Johnson Biotechnology Center, Sorrento Valley, California: Localization of multiple T-cell recognition sites confirms the cross-reactivity of HBcAg and HBeAg at the T-cell level.

Cote, P.J.,[1] Pohl, C.,[1] Gerin, J.L.,[1] Tennant, B.C.,[2] [1]DMVI, Georgetown University, Rockville, Maryland; [2]Cornell University School of Veterinary Medicine, Ithaca, New York: Serum AFP and γ-glutamyl transferase as markers for hepatocellular carcinoma in woodchucks.

Motte, P.,[1] Takahashi, H.,[1] Ozturk, M.,[1] Wilson, B.E.,[1] Frohlich, M.W.,[1] Kew, M.C.,[2] Wands, J.R.,[1] [1]GI Unit, Massachusetts General Hospital, Boston; [1]University of Witwatersrand, Johanesberg, South Africa: Characterization of human hepatoma-associated antigens using monoclonal antibodies.

Colucci, G.,[1] Tackney, C.,[2] Waksal, S.D.,[2] [1]Dept. of Biology and Genetics, University of Milan, Italy; [2]ImClone Systems Inc., New York, New York: Identification of a major HBcAg epitope using synthetic peptides.

Liu, Y.-F., Hou, Y., Yang, x.-Y., Dept. of Pathology, Fourth Military Medical College, Beijing, Peoples Republic of China: Presence of HBsAg in proliferating bile ductules.

SESSION 9 MECHANISMS OF ONCOGENESIS

Chairman: P. Marion, Stanford University

Marion, P.L.,[1] Salazar, F.H.,[2] Robinson, W.S.,[1] Will, H.,[3] Steevens, C.,[1] [1]Division of Infectious Diseases, Dept. of Medicine, Stanford University, [2]Syntex Inc., Palo Alto, California; [3]Max-Planck-Institut für Biochemie, Martinsried/Munchen, Federal Republic of Germany: Determination of the inversion-deletion sites in a GSHV novel DNA form cloned from chronically infected ground squirrel liver.

Wang, H., Rogler, C.E., Albert Einstein College of Medicine, Bronx, New York: Evidence for topoisomerase I cleavage of WHV virion DNA in the cohesive overlap region.

Imazeki, F.,[1] Omata, M.,[1] Koike, K.,[2] [1]First Dept. of Medicine, Chiba University School of Medicine, [2]Dept. of Gene Research, Cancer Institute, Tokyo, Japan: Cloning and structural analysis of integrated DHBV DNA in chronic hepatitis.

Zhou, Y.-z.,[1] Donehower, L.A.,[1] Slagle, B.L.,[1] vanTuinen, P.,[2] Ledbetter, D.H.,[2] Butel, J.S.,[1] [1]Dept. of Virology, [2]Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas: HBV DNA integration in chromosome 17p near the human p53 gene in a hepatocellular carcinoma.

Hsu, T.Y.,[1] Mōrōy, T.,[1] Etiemble, J.,[1] Trépo, C.,[2] Tiollais, P.,[1] Buendia, M.A.,[1] [1]Unité de Recombinaison et Expression Génétique, INSERM, CNRS, Institut Pasteur, Paris, [2]Unité de Recherches sur les Hépatites, UER Alex Carrel, Lyon, France: Insertional activation of c-*myc* by WHV by woodchuck hepatocellular carcinoma.

Lee, H.S., Rajagopalan, M.S., Vyas, G.N., Dept. of Laboratory Medicine, University of California, San Francisco: Lack of direct role of HBV in the activation of c-*myc*/*ras* oncogenes but a possible role of aflatoxin-DNA adducts in human hepatocellular carcinoma.

Dejean, A., de The, H., Marchio, A., Tiollais, P., INSERM, CNRS, Institut Pasteur, Paris, France: A novel steroid/thyroid hormone-receptor-related gene inappropriately expressed in human hepatocellular carcinoma.

Lasserre, C., Cariani, E., Hamelin, B., Brechot, C., INSERM, Paris, France: Reexpression of insulin-like growth factor II fetal transcripts in human primary liver tumors.

Su, T.,[1,2] Tsai, T.,[2] [1]Dept. of Medical Research, Veterans General Hospital, [2]Graduate Institute of Microbiology and Immunology, National Yang-Ming Medical College, Taiwan, Republic of China: Structure and expression of oncogenes (proto-oncogene) in human hepatoma cell lines.

Shirakata-Takasaki, Y.,[1] Yaginuma, K.,[1] Sano, H.,[2] Oda, M.,[2] Kobayashi, M.,[1] Koike, K.,[1] [1]Dept. of Gene Research, Cancer Institute, Tokyo, [2]Meiji Institute of Health Science, Odawara, Japan: Introduction of the *X* gene and enhancer sequence of HBV-stimulated growth and c-*myc* expression of mouse NIH-3T3 cells.

Molecular Neurobiology of *Drosophila*

October 4—October 8

ARRANGED BY

Barry Ganetzky, University of Wisconsin
Jeffrey Hall, Brandeis University

144 participants

The subject of this conference was genetic and molecular analysis of the development and function of the nervous system in *Drosophila*. The six sessions on the program proceeded from embryonic development, through aspects of later neural development, and then on to neural/functional topics, including sensory phenomena, membrane physiology, neurochemistry (neurotransmitters plus putative neuropeptides), and finally "higher" behaviors.

There were two 2-day poster sessions, involving about 90 presentations: These paralleled the oral parts of the program in that the posters covered development and then function of the fruit fly's nervous system.

Some of the substantive highlights of the conference involved efforts of participants not only to update well-known genetic and/or molecular "systems" that have come to the fore in the recent literature, but also to bring out new approaches and efforts that have begun to be developed in the last year or so. The first, more salient category included presentations on "neurogenic" genes; factors controlling the development of the visual system; manipulation of genes encoding opsin molecules; the definitive identification and characterization of a locus that encodes components of potassium channels; manipulation and expression studies revolving around genes involved in the biosynthesis or reception of nonpeptide neurotransmitters; and genes involved in learning and memory or in biological rhythms.

The second, newer category included presentations on one of the many genes in *Drosophila* known to disrupt muscle development (when mutated), which—unlike other "muscle genes"—has turned out to be molecularly expressed solely in the central nervous system; on the patterns of "births" plus later proliferations of definitively identified postembryonic neurons; on the first case of "reverse genetics" having been achieved in this organism (whereby the investigators moved from monoclonal antibody, to antigen, to gene isolation, and finally to the induction of interesting mutations at the locus, which influences photoreceptor cells); on the cloning of a gene encoding an apparent sodium/potassium ATPase; on the isolation of what seems to be a "G-protein" gene in this invertebrate; on the first cases of gene isolations for factors that produce what seem to be neuropeptides in *Drosophila* (starting with the relevant biochemical information from other organisms); and finally on some fascinating aspects of acoustical communication in species of Hawaiian *Drosophila* (which have long been studied evolutionarily, but now for the first time with regard to auditory signals that accompany reproductive behavior).

The conference seemed to be successful both in terms of revealing the frighteningly rapid progress that is occurring in studies of now-famous cloned "neural genes" in this organism and because the several new systems were announced not only by investigators who have been studying *Drosophila*

neurogenetics for some time (although they might have only recently begun to do so at the faculty level), but also by some neurobiologists who have moved portions of their research programs into genetic plus molecular analysis of excitable cells and tissues in the fruit fly.

SESSION 1 EMBRYONIC DEVELOPMENT

Chairman: R.J. Greenspan, Roche Institute of Molecular Biology

Hoppe, P.E.,[1] Greenspan, R.J.,[2] [1]Dept. of Biology, Princeton University, [2]Dept. of Neurosciences, Roche Institute of Molecular Biology, Nutley, New Jersey: Analysis of *Notch* gene action in neural-epidermal determination.

Yedvobnick, B., Smoller, D., Young, P., Mills, D., Dept. of Biology, Emory University, Atlanta, Georgia: Molecular analysis of *mastermind*, a locus essential for proper neurogenesis.

Modolell, J., Centro de Biologia Molecular, CSIC and Universidad Autonoma de Madrid, Spain: Involvement of the *achaete-scute* complex in neurogenesis.

Holmgren, R.,[1] Schafer, B.,[1] Patel, N.,[2] Goodman, C.,[2] [1]Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois; [2]Dept. of Biology, Stanford University, California:.Function of the segment polarity genes in the formation of the nervous system.

Fleming, R.J., DeSimone, S., White, K., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Molecular analysis of the *erect wing* locus.

SESSION 2 POSTEMBRYONIC DEVELOPMENT

Chairman: K.-F. Fischbach, Universität Freiburg

Fischbach, K.-F.,[1] Boschert, U.,[1] Tix, S.,[2] Technau, G.M.,[2] [1]Institute für Biologie, Universität Freiburg, [2]Institut für Entwicklungsphysiologie, Köln, Federal Republic of Germany: Mutant analysis of optic lobe development and larval visual neurons.

Posakony, J., Ellis, H., Hartenstein, V., Leviten, M., Spann, D., Dept. of Biology, University of San Diego, La Jolla: Genetic and developmental studies of the patterning and differentiation of epidermal sensilla.

Truman, J.W.,[1] Bate, M.,[2] Irish, V.,[2] [1]Dept. of Zoology, University of Washington, Seattle; [2]Dept. of Zoology, University of Cambridge, England: Neurogenesis and its relationship to *Ubx* expression in the larval CNS.

Zipursky, L., Reinke, R., Van Vactor, D., Krantz, D., Dept. of Biological Chemistry, University of California School of Medicine, Los Angeles: A molecular and genetic analysis of chaoptin—A photoreceptor-cell-specific glycoprotein required for cellular morphogenesis.

Katz, F.,[1] Moats, W.,[1] Jan, Y.M.,[2] [1]University of Texas Health Science Center, Dallas; [2]University of California, San Francisco: Biochemical and genetic studies of a neuronal cell-surface antigen.

C.-W. Wu, B. Ganetzky, S. Benzer, A. Ferris

D. Gailey, D. Siegel, J. Hall, B. Harris

SESSION 3 SENSORY SYSTEMS

Chairman: G.M. Rubin, University of California, Berkeley

Zuker, C.,[1] Cowman, A.,[2] Montell, C.,[2] Rubin, G.[2] [1]Dept. of Biology, University of California, San Diego, La Jolla; [2]Dept. of Biochemistry, University of California, Berkeley: Opsins and the phototransduction cascade in the visual system.

Feiler, R.,[1] Harris, W.,[2] Kirschfeld, K.,[1] Mismer, D.,[3] Wehrhahn, C.,[1] Rubin, G.,[3] Zuker, C.,[2] [1]Max Planck Institut für Biologische Kybernetik, Tübingen, Federal Republic of Germany; [2]Dept. of Biology, University of California, San Diego; [3]Dept. of Biochemistry, University of California, Berkeley: Genetically engineered cell-specific misexpression of a rhodopsin gene.

Stark, W.S., Sapp, R., Division of Biological Sciences, University of Missouri, Columbia: Turnover of photoreceptor membrane.

Tanimura, T., Dept. of Biology, Faculty of Science, Fukuoka University, Japan: Genetic dissection of the trehalose taste receptor.

Jallon, J.M., Boukella, H., Ferveur, J.F., Biologie et Génétique Evolutives du CNRS, Paris, France: Biosynthetic and genetic studies of pheromone production.

SESSION 4 MEMBRANE PHYSIOLOGY

Chairman: C.-F. Wu, University of Iowa

O'Dowd, D.K., Germeraad, S., Aldrich, R.W., Dept. of Neurobiology, Stanford University, California: Electrophysiological analysis of genes involved in sodium channel function.

Lebovitz, R.M., Fambrough, D.M., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Molecular cloning of gene and cDNA encoding the α-subunit of the Na^+/K^+ ATPase.

Wadsworth, S.C.,[1] Rosenthal, L.S.,[2] Kammermeyer, K.L.,[1] Potter, M.B.[1] Nelson, D.J.,[2] [1]Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, [2]Dept. of Chemistry, Clark University, Worcester, Massachusetts: CNS expression of an acetylcholine-receptor-related gene.

Pongs, O., Baumann, A., Krah, I., Müller, R., Kecskemethy, Müller-Holtkamp, F., Institut für Biochemie, Fakultät für Chemie, Ruhr-Universität Bochum, Federal Republic of Germany: Molecular analysis of the *Shaker* gene complex.

Schwarz, T.L., Papazian, D.M., Tempel, B.L., Jan, Y.N., Jan, L.Y., Howard Hughes Medical Institute and Dept. of Physiology, University of California, San Francisco: Cloning and sequences of cDNA from three probable potassium-channel components from the *Shaker* locus.

Iverson, L., Kamb, A., Ramaswami, M., Tseng-Crank, J., Tanouye, M., Division of Biology, Caltech, Pasadena: Ion channel genes.

SESSION 5 NEUROCHEMISTRY

Chairman: J. Hirsh, Harvard University Medical School

Morgan, B.A.,[1] Bray, S.J.,[1] Johnson, W.A.,[1] Beall, C.J.,[1] McCormick, C.A.,[1] Tully, T.,[2] Hirsh, J.,[1] [1]Dept. of Biological Chemistry, Harvard Medical School, Boston, [2]Dept. of Biology, Brandeis University, Waltham, Massachusetts: Neural expression of the *D. melanogaster DOPA decarboxylase* gene – Mechanisms and manipulation of gene expression.

Wolfgang, W.J., Gnagey, A., Forte, M., Volium Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland. Monoclonal antibodies reveal the presence of acetylcholinesterase very early in the developing optic system of *Drosophila*.

Thambi, N.,[1] Northup, J.,[2] Forte, M.,[1] [1]Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland; [2]Dept. of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: Characterization and purification of GTP-binding proteins from *Drosophila* neuronal membranes.

Yarfitz, S., Provost, N., Hurley, J.B., Dept. of Biochemistry and Howard Hughes Medical Institute, University of Washington, Seattle: Isolation and characterization of a *Drosophila* G protein β-subunit gene.

Nichols, R., Dept. of Biochemistry, Purdue University, West Lafayette, Indiana: Isolation of a vertebrate neuropeptide homolog present in *D. melanogaster*.

Nambu, J.R.,[1] Murphy-Erdosh, C.,[1] Andrews, P.C.,[2] Feistner, G.J.,[3] Scheller, R.H.,[1] [1]Dept. of Biological Sciences, Stanford University, California; [2]Dept. of Biochemistry, Purdue University, West Lafayette, Indiana; [3]Dept. of Psychiatry and Behavioral Sciences, Stanford Medical Center, California: Isolation and characterization of an FMRF amide-related neuropeptide gene from *Drosophila*.

SESSION 6 BEHAVIOR

Chairman: C.P. Kyriacou, University of Leicester

Yun, Y., Davis, R., Dept. of Biochemistry, Michigan State University, East Lansing: Abnormal expression of RNAs from copia transposable elements and serine protease genes in *dunce* mutants.

Ackerman, S.L., Siegel, R.W., Dept. of Biology, University of California, Los Angeles: Memory deficit in constitutive and conditional protein synthesis mutants.

Dowse, H., Kass, L., Ringo, J., Dept. of Zoology, University of Maine, Orono: Close parallels between puparial heart rate and circadian rhythms in clock mutants.

Thackeray, J., Greenacre, M., Hill, M., Kyriacou, C.P., Dept. of Genetics, Leicester University, England: Analysis of biological timers.

Hoy, R.R.,[1] Hoikkala, A.,[2] Kaneshiro, K.,[2] [1]Section of Neurobiology and Behavior, Cornell University, Ithaca, New York; [1]Hawaiian Evolutionary Biology Program, University of Hawaii, Honoluiu: Auditory signals in the courtship of Hawaiian *Drosophila*.

POSTERS

Artavanis-Tsakonas, S., Bryant, R., Fehon, R., Fleming, R., Flach, J., Herndon, V., Johansen, K.M., Ramos, R., Scott, N., Xu, T., Dept. of Biology, Yale University, New Haven, Connecticut: Molecular biology of neurogenesis.

Baker, N., Moses, K., Rubin, G.M., Dept. of Biochemistry, University of California, Berkeley: Autosomal mutations affecting the *Drosophila* eye.

Banerjee, U., Renfranz, P.J., Hinton, D.R., Rabin, B., Benzer, S., Division of Biology, California Institute of Technology, Pasadena: Localization of expression of the *seven-less* gene protein product.

Bernstein, L., Teng, D., Blake, A., Kolodkin, A., Venkatesh, T., Institutes of Molecular Biology and Neuroscience, University of Oregon, Eugene: Characterization of hybrid dysgenesis-induced mutations that perturb photoreceptor cell structure and function.

Bier, E., Ackerman, L.A., Barbel, S., Jan, L., Jan, Y.M., Howard Hughes Medical Institute and Dept. of Physiology, University of California, San Francisco: Identification and characterization of a neuronal-specific nuclear antigen.

Bloomquist, B.,[1] Shortridge, R.,[1] Montell, C.,[2] Steller, H.,[2] Rubin, G.,[2] Pak, W.L.,[1] [1]Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana; [2]Dept. of Biochemistry, University of California, Berkeley: Isolation and characterization of the *norpA* gene.

Boschert, U., Fischback, K.-F., Institut für Biologie, Universität Freiburg, Federal Republic of Germany: Disorders of the optic chiasms in *D. melanogaster*.

Cagan, R.L., Ready, D.F., Dept. of Biology, Princeton University, New Jersey: Primary pigment cells are lost in *facet-glossy*.

H. Steller, G. Rubin

Campos, A.R., Rosen, D., Robinow, S., White, K., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Molecular genetic analysis of the *elav* locus in *D. melanogaster.*

Campos-Ortega, J.A., Bremer, K.A., de la Concha, A., Dietrich, U., Knust, E., Technau, G.M., Tietze, K., Vässin, H., Ziemer, A., Institut für Entwicklungsphysiologie, Universität Köln, Federal Republic of Germany: Genetics of early neurogenesis in *D. melanogaster.*

Canal, I.,[1] Ferrus, A.,[2] [1]Universidad Autonoma; [2]Instituto Cajal, CSIC, Madrid, Spain: Genetic basis of metamerism in the CNS of *Drosophila.*

Crews, S., Thomas, J., Goodman, C., Dept. of Biological Sciences, Stanford University, California: The *Drosophila S8* gene encodes a nuclear protein with homology to the *per* gene product.

Dambly-Chaudière, C.,[1] Ghysen, A.,[1] Leyns, L.,[1] Jan, L.Y.,[2] Jan, Y.N.,[2] [1]Laboratory of Genetics, Université Libre de Bruxelles, Belgium; [2]Howard Hughes Medical Institute, San Francisco, California: Genetics of peripheral neurogenesis in *Drosophila.*

de Couet, H.G.,[1] Yamamoto, M.,[1] Davies, J.,[1] Pirrotta, V.,[2] Miklos, G.L.G.,[1] [1]Developmental Neurobiology Group, Research School of Biological Sciences, Canberra, Australia; [2]Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Genetic characterization and molecular cloning of a flightless locus at the base of the X chromosome of *D. melanogaster.*

Egger, M.D.[1] Harris, S.,[1] Peng, B.,[1] Schneiderman, A.M.,[2] Wyman, R.J.,[2] [1]Dept. of Anatomy, UMDNJ, Robert Wood Johnson Medical School, Piscataway, New Jersey; [2]Dept. of Biology, Yale University, New Haven, Connecticut: Morphometric analysis of thoracic muscles in wild type and in *bithorax* complex mutants.

Elkins, T., Patel, N., Jacobs, R., Goodman, C., Dept. of Biological Sciences, Stanford University, California: Deletion studies of the *Fasciclin III* gene.

Hackett, R.W., Benzer, S., Division of Biology, California Institute of Technology, Pasadena: Characterization of *eyes absent*, a mutation that eliminates the compound eyes.

Harrelson, A., Zinn, K., Snow, P., Bastiani, M., Goodman, C., Dept. of Biological Sciences, Stanford University, California: Molecular characterization of the Fasciclin II cell-surface glycoprotein.

Hartenstein, V., Posakony, J.W., Dept. of Biology, University of California, San Diego, La Jolla: Sensory-neuron development in *string*, a mutant that inhibits postblastoderm cell proliferation.

Reinke, R., Hess, R., Hart, A., Zipursky, L., Dept. of Biological Chemistry, University of California School of Medicine, Los Angeles: *bride of sevenless* – An autosomal locus required for the development of photoreceptor cell seven.

Hyde, D.R., Palazzolo, M.J., Mecklenburg, K.L., Raghavan, K.V., Meyerowitz, E.M., Benzer, S., Division of Biology, California Institute of Technology, Pasadena: Identification and characterization of 60 optic-pathway-specific cDNAs from a pool of 500 "head"-specific clones.

Palazzolo, M.J., Hyde, D.R., Raghavan, K.V., Mecklenburg, K., Benzer, S., Meyerowitz, E.M., Division of Biology, California Institute of Technology, Pasadena: Isolation and preliminary characterization of 500 noncross-hybridizing *Drosophila* "head-specific" cDNA clones by sorting a subtracted λSWAJ cDNA library.

Isono, K.,[1] Tanimura, T.,[2] Tsukahara, Y.,[1] [1]Research Center for Applied Information Sciences, Tohoku University, Sendai, [2]Dept. of Biology, Fukuoka University, Japan: Biogenesis of 3-hydroxyretinal and visual pigment in the compound eyes of *D. melanogaster.*

Jones, S., Fitzgerald, G., Tan, L., Kankel, D.R., Dept. of Biology, Yale University, New Haven, Connecticut: Genetic and biochemical analysis of the role of glycoproteins in the development of *Drosophila.*

Kiger, J.A., Jr., Bellen, H.J., Dept. of Genetics, University of California, Davis: cAMP phosphodiesterase coded by the *dunce* gene is essential for normal embryogenesis.

Kimura, K.-I.,[1] Tanimura, T.,[2] [1]Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, [2]Dept. of Biol-

B. Ganetzky, C.-F. Wu, M. Stern

S. Benzer

J. Hall

ogy, Faculty of Science, Fukuoka University, Japan: Mutations affecting programmed cell death of head muscles in *Drosophila*.

Knust, E., Tietze, K., Ziemer, A., Campos-Ortega, J.A., Institut für Entwicklungsphysiologie, Köln, Federal Republic of Germany: A genetic and molecular analysis of *Enhancer of split*, a complex of gene functions involved in neurogenesis in *D. melanogaster*.

LaBonne, S.,[1] Mahowald, A.P.,[2] [1]Dept. of Biochemistry, University of Mississippi Medical Center, Jackson; [2]Dept. of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio: Molecular genetics of the maternal-effect neurogenic locus *pecanex*.

Meinertzhagen, I.A., O'Neil, S.D., Life Sciences Centre, Dalhousi University, Halifax, Nova Scotia. Canada: Synaptic organization of the lamina cartridge in *Drosophila*.

Muskavitch, M.A.T., Alton, A.K., Fechtel, K., Kopczynski, C.C., Shepard, S.B., Kooh, P.J., Peterson, K.R., Parks, C.G., Institute for Molecular and Cellular Biology and Dept. of Biology, Indiana University, Bloomington: Molecular genetics of neurogenesis in *Drosophila*.

Nakao, K.[1] Hotta, Y.,[1] Asahara, H.,[1] Mogami, K.,[1] Miyake, T.,[1] [1]Dept. of Physics, University of Tokyo, [2]Mitubishi-Kasei Institute of Life Sciences, Tokyo, Japan: Immunohistological screenings of the second chromosomal embryonic lethals having neural defects.

O'Tousa, J.E., Washburn, T., Dept. of Biological Sciences, University of Notre Dame, Indiana: Molecular defects in *Drosophila* rhodopsin mutants.

Palka, J., Schwaninger, H., Dept. of Zoology, University of Washington, Seattle: Phenotypic interactions between *hairy* and several venation mutations.

Perkins, L.,[1] Zhang, K.,[1] Perrimon, N.,[1] Mahowald, T.,[2] [1]Dept. of Genetics, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio: Genes expressed in cultured neuroblasts.

Pollock, J.A., Benzer, S., Division of Biology, California Institute of Technology, Pasadena: Expression of the *seven-less* gene.

Preiss, A., Harley, D., Artavanis-Tsakonas, S., Dept. of Biology, Yale University, New Haven, Connecticut: Molecular analysis of *Enhancer of split*.

Raghavan, K.V., Celniker, S.E., Lewis, E.B., Meyerowitz, E.M., Division of Biology, California Institute of Technology, Pasadena: Role of the nervous system and epidermis in muscle development – Genetic and molecular analysis of the *Drosophila* locus *stripe*.

Robinow, S., Campos, A.R., White, K., Dept. of Biology, Brandeis University, Waltham, Massachusetts: In situ localization of products of the locus *elav*.

Rothberg, J., Hartley, D., Artavanis-Tsakonas, S., Dept. of Biology, Yale University, New Haven, Connecticut: Molecular genetics of a growth-factor-like gene exhibiting a specific neural pattern of expression.

Rubin, G.M., Baker, N., Bowtell, D., Fortini, M., Heilig, J., Kimmel, B., Mismer, D., Moses, K., Simon, M., Steller, H., Tomlinson, A., Dept. of Biochemistry and Howard Hughes Medical Institute, University of California, Berkeley: Development of the *Drosophila* visual system.

Smouse, D.,[1,2] Goodman, C.,[1] Perrimon, N.,[2] [1]Dept. of Biological Sciences, Stanford University, California; [2]Dept. of Genetics, Harvard Medical School, Boston, Massachusetts: *Polyhomeotic* and *Posterior sex combs* are required for axonal guidance in the embryonic CNS.

Steller, H.,[1] Heilig, J.S.,[1] Fischback, K.-F.[2] Rubin, G.M.,[1] [1]Howard Hughes Medical Institute and Dept. of Biochemistry, University of California, Berkeley; [2]Institut für Biologie, Universität Freiburg, Federal Republic of Germany: Developmental and molecular genetic analyses of *disconnected*, a locus involved in neuronal cell recognition.

Tomlinson, A., Bowtell, D., Hafen, E., Rubin, G., Howard Hughes Medical Institute and Dept. of Biochemistry, University of California, Berkeley: Localization of *sevenless* protein in the eye imaginal disk of *Drosophila*.

Uemura, T., Barbel, S., Jan, L., Jan, Y.N., Howard Hughes Medical Institute and Dept. of Physiology, University of California, San Francisco: A mutation that causes reduction of sensory neurons and duplication of sensory structures.

Vässin, H., Bremer, K.A., Knust, E., Campos-Ortega, J.A., Institut für Entwicklungsphysiologie, Universität Köln, Federal Republic of Germany: The neurogenic gene *Delta of D. melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats.

Watanabe, T., Kankel, D.R., Dept. of Biology, Yale University, New Haven, Connecticut: Molecular genetics studies on the *l(1) ogre* locus.

Weinzierl, R.,[1] Axton, M.,[1] Ghysen, A.,[2] Akam, M.,[1] [1]Dept. of Genetics, University of Oxford, Cambridge, England; [2]Laboratoire de Genetique, Universite de Bruxells, Belgium: An *Ultrabithorax* microexon mutation suggests a CNS-specific RNA processing pathway for *Ubx* transcripts.

Wong, F.,[1,2] Yuh, X.-T.,[1] Schafer, E.L.[1] Roop, B.C.,[1] Depts. of [1]Ophthalmology, [2]Anatomy and Cell Biology, University of Illinois, Chicago: Overlapping transcription units in the *transient receptor potential* locus of *D. melanogaster*.

Woodard, C., Sun, H., Helfand, S., Carlson, J., Dept. of Biology, Yale University, New Haven, Connecticut: Genetic and molecular analysis of olfaction.

Zinn, K., McAllister, L., Snow, P., Elkins, T., Goodman, C., Dept. of Biological Sciences, Stanford University, California: Molecular genetics of grasshopper and *Drosophila* Fasciclin I.

Aldrich, R.W., Zagotta, W.N., Brainard, M.S., Dept. of Neurobiology, Stanford University, California: Single potassium channels in wild-type and *Shaker Drosophila* myotubes.

Atkinson, N., Robertson, G., Ganetzky, B., Laboratory of Genetics, University of Wisconsin, Madison: Isolation and analysis of new mutations at the *slowpoke* locus.

Bargiello, T.A.,[1] Saez, L.,[1] Baylies, M.K.,[1] Young, M.W.[1] Spray, D.C.,[2] [1]Howard Hughes Medical Institute, Rockefeller University, New York, [2]Albert Einstein College of Medicine, Bronx, New York: A biological clock mutation alters intercellular junctional communication.

Colot, H.V., Yu, Q., Wheeler, D., Petersen, G., Hall, J.C., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Interspecific comparisons of the *period* gene.

Drysdale, R., Ganetzky, B., Laboratory of Genetics, University of Wisconsin, Madison: Molecular analysis of *eag* – A gene affecting two potassium channels in *Drosophila*.

Ewer, J., Rosbash, M., Hall, J.C., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Requirement of *per* gene expression during development for the expression of an adult circadian locomotor activity rhythm.

Folkers, E., Quinn, W.G., Dept. of Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge, Massachusetts: Behavioral and genetic analysis of the *Drosophila* mutant *radish*.

Gailey, D.A., Hall, J.C., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Behavioral and cytogenetic analysis of the *fruitless* phenotype.

Loughney, K., Gantezky, B., Laboratory of Genetics, University of Wisconsin, Madison: Homology between *para* and the rat sodium channel.

Germeraad, S., O'Dowd, D.K., Aldrich, R.W., Dept. of Neurobiology, Stanford University, California: A "zero-dose" screen for genes affecting ion channel function.

Gundelfinger, E.D., Hermans-Borgmeyer, I., Schlob, P., Sawruk, E., Betz, H., ZMBH, Heidelberg, Federal Republic of Germany: The neuronal nicotinic acetylcholine receptor of *Drosophila*.

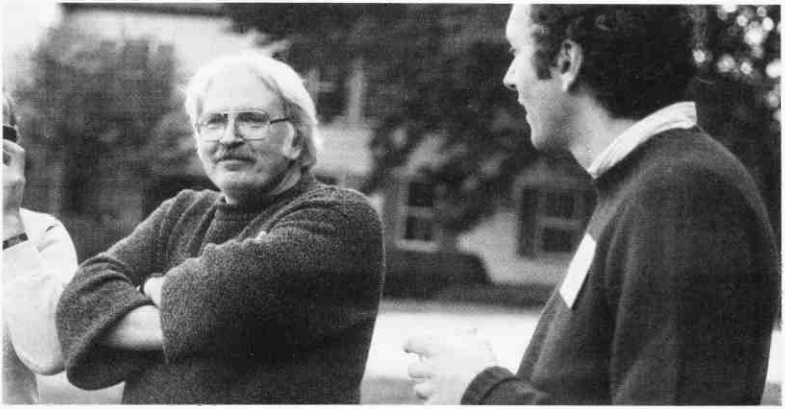
Hall, L.M., Gil, D.W., Kasbekar, D.P., Dept. of Genetics, Albert Einstein College of Medicine , Bronx, New York: The *tip*-E locus affects a subset of sodium channels.

Jackson, F.R., Elliott, K., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Chromosomal walking through the *Andante* clock locus.

Kasbekar, D.P., Hall, L.M., Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Novel phenotypes associated with the *enhancer of seizure*, a membrane excitability mutation.

Komatsu, A., Rathe, P., Gorczyca, M., Wu, C.-F., Dept. of

R. Greenspan, J. Pollack



Biology, University of Iowa, Iowa City: Single-channel recording of Ca^{++}-dependent potassium currents in normal and mutant *Drosophila* muscles.

Kulkarni, S.J., Vargo, M.A., Steinlauf, A.D., Hall, J.C., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Neurogenetic connections between the control of courtship singing and visual system functions in *D. melanogaster*.

Liu, X., Lorenz, L., Yu, Q., Hall, J.C., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Temporal and spatial expression of the *per* gene assayed with β-galactosidase fusions and in situ hybridization.

Lorenz, L., Hall, J.C., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Studies on the cycling 0.9-kb transcript neighboring the *period* gene.

McGuire, T.R., Dept. of Biological Sciences and Bureau of Biological Research, Rutgers University, Piscataway, New Jersey: Mutant analysis of *D. melanogaster* for geotaxis—A preliminary report.

Neckameyer, W.S., Quinn, W.G., Dept. of Brain and Cognitive Sciences and Biology, Massachusetts Institute of Technology, Cambridge: Characterization of tyrosine hydroxylase in *Drosophila*.

Okamoto, H., Takasu-Ishikawa, E., Sakai, K., Goto, S., Nakao, K., Suzuki, A., Hotta, Y., Dept. of Physics, Faculty of Science, University of Tokyo, Japan: Isolation of the *Drosophila* genes homologous to the eel sodium channel gene.

Provost, N., Somers, D., Hurley, J.B., Dept. of Biochemistry and Howard Hughes Medical Institute, University of Washington, Seattle: Isolation and characterization of a *Drosophila* G protein gene.

Quan, F., Liston, D., Forte, M., Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland: Isolation of *Drosophila* sequences homologous to the human amyloid A4 protein gene.

Schaeffer, E., Choi, K.-W., Mardon, G., Quinn, W., Dept. of Brain and Cognitive Science and Biology, Massachusetts Institute of Technology, Cambridge: Isolation and characterization of two *Drosophila* protein kinase C genes.

Schmitt, B., Hermans-Borgmeyer, I., Sawruk, E., Gundelfinger, E.D., Betz, H., ZMBH, Heidelberg, Federal Republic of Germany: Molecular cloning of neurotransmitter receptors from *Drosophila*.

Schneider, L.E., Taghert, P.H., Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri: Isolation of a gene that encodes FMRF-amide-like neuropeptides in *D. melanogaster*.

Siwicki, K.K., Eastman, C., Rosbash, M., Hall, J.C., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Circadian clock probed with antibodies to the *period* gene product.

Bossy, B.,[1] Ballivet, M.,[2] Spierer, P.,[1] Depts. of [1]Molecular Biology, [2]Biochemistry, University of Geneva, Switzerland: An acetylcholine receptor gene of *Drosophila*.

Stern, M., Robertson, G., Ganetzky, B., Laboratory of Genetics, University of Wisconsin, Madison: New mutations affecting neuromuscular function in *Drosophila*.

Sun, Y.A., Wyman, R.J., Dept. of Biology, Yale University, New Haven, Connecticut: Is a current driven through *Drosophila* egg chambers?

Tompkins, L., Dept. of Biology, Temple University, Philadelphia, Pennsylvania: Are male-specific *Sex-lethal* transcripts required for normal sexual behavior?

Whitaker, J., Nash, H., NIMH, National Institutes of Health, Bethesda, Maryland: Anesthesia mutants of *D. melanogaster*.

Yu, Q., Rutila, J., Hall, J.C., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Analysis of *period* gene transcripts.

BANBURY CENTER

BANBURY CENTER DIRECTOR'S REPORT

Banbury Center's tenth year was overshadowed by the tragic death of Steve Prentis in February of 1987. That his program went ahead just as he had planned was due to Terri Grodzicker who became the acting director, and Bea Toliver, the Center's administrative assistant, who took on all responsibility for the day-to-day running of the Center. A total of 14 meetings were held on a wider variety of topics than has been usual in the Banbury program. In addition, we conducted a workshop for science journalists and the second in a series of special meetings arranged in conjunction with Shearson Lehman Brothers. Banbury Center was also host to four advanced lecture courses.

Risk Assessment Program

Three meetings in the risk assessment program were held in 1987, two dealing with the problems of mutations. In the early part of the year, the meeting on Mammalian Cell Mutagenesis reviewed the latest data on the molecular mechanisms of mutational events in mammalian cells, and a session of particular importance dealt with recent advances in methods for detecting mutations. It was agreed that there had been interesting developments in understanding the roles of recombination and repair processes in modulating mutational processes in mammalian cells.

The meeting on Eukaryotic Transposable Elements as Mutagenic Agents brought together investigators in the areas of eukaryotic retroviruses and transposable elements, cancer biology, DNA damage and repair, and human risk assessment. An area of particular interest concerned the mechanisms of retroviral latency and the factors that might influence this state.

In October, there was a meeting that exemplified the nature of Banbury Center conferences, where a group including academic and industrial scientists and representatives of the regulatory agencies came together to review New Directions in the Qualitative and Quantitative Aspects of Carcinogen Risk Assessment. It was clear that risk assessment per se is an extremely complicated and difficult subject, and the Banbury Center will continue to provide a forum for exploring the consequences of the interaction between science and public policy.

Banbury Meeting House

Meetings in the Corporate Sponsor Series

The Corporate Sponsor Program has become the core support of the Banbury Center's activities, providing funds that support meetings concerned with a variety of topics that are of great intrinsic interest and also of importance to those companies that generously contribute to the program.

Two of the meetings dealt with basic techniques in molecular biology. One meeting was concerned with the Development of the Human Lymphocyte Protein Database, a system for analyzing and classifying lymphocytes on the basis of their specific proteins detected by two-dimensional polyacrylamide gel electrophoresis. Antisense RNA has been promoted as a general method for manipulating gene expression, and a meeting was held in December to review the use of antisense

RNA techniques in a variety of systems. The results have been rather variable, depending on the organism and genes being studied, and this was a particularly timely meeting to examine factors that might account for this variation.

The application of molecular techniques to the analysis of the strategies by which parasites thwart the defenses of their hosts promises to yield tremendous benefits, and a meeting to review advances in the Molecular Genetics of Parasitic Protozoa was held at Banbury in November. Another meeting dealing with a topic of great practical importance was that on the Transformation of Agriculturally Important Crops. Genetic engineering of plant crops has progressed well beyond the laboratory stage, and much of the meeting was concerned with the practicalities and consequences of developing large-scale field testing. The meeting continued the Banbury theme of dealing with scientific issues of great public interest.

A very popular meeting was that on Nuclear Oncogenes, and it was notable for bringing together research workers who perhaps spend less time talking with each other than they should. Among the topics covered were *ras*, *fos*, *myc*, and *myb*, and adenovirus E1A, and although much new information was presented, it is not yet clear what relationship, if any, there is between the mechanisms by which these various oncogenes act.

The question of how extracellular signals are transmitted across the plasma membrane was the subject of a meeting on the Role of Inositol Lipids in Signaling. The importance of this mechanism of signal transduction has been recognized increasingly, and as well as dealing with the biochemical pathway involved, the meeting also examined the ways in which increases in free cytoplasmic Ca^{++} concentration come about and how the system is involved in the regulation of cell growth.

Program Meetings

Two other major meetings were held during 1987 at the Banbury Center. In April, a meeting entitled Therapeutic Peptides and Proteins: Assessing the New Technologies was organized with support from the James S. McDonnell Foundation and a number of biotechnology companies. The meeting encompassed a wide range of subjects, from reviews of new methods of producing peptides and proteins using recombinant DNA techniques or chemical or enzymatic syntheses, through their characterization and clinical testing, to the ways in which regulatory agencies in the United States and in other countries are dealing with these new developments.

The largest meeting of the year was that on the Role of the Heat-shock Response in Biology and Disease, supported by the Samuel Freeman Trust. The meeting considered the function of the so-called heat-shock proteins produced in response to noxious stimuli or disease. It was also one of the most wide-ranging of our meetings, bringing together scientists working on the physiological and behavioral responses of desert animals to heat, with scientists cloning genes and analyzing proteins!

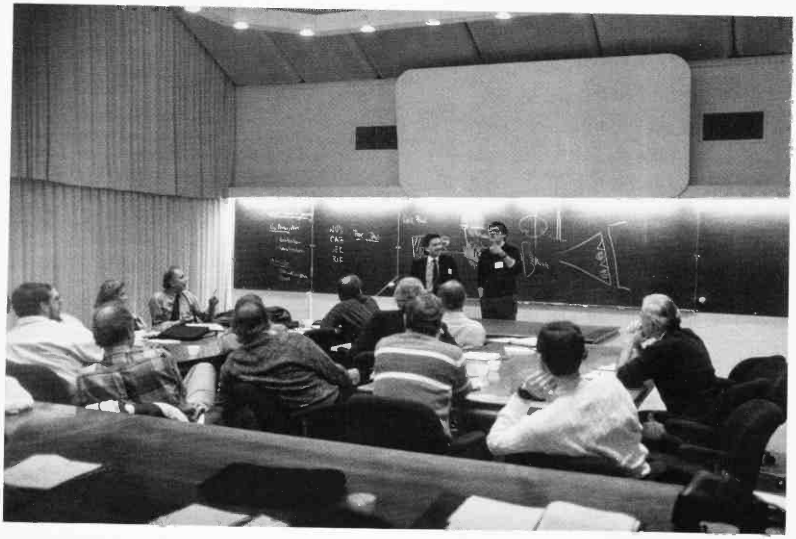
Congressional Staff and Science Journalist Workshops

A growing interest of the Laboratory is the education of the general public in the area of biotechnology, and molecular biology and genetics in particular. In 1980, the Banbury Center, with support from the Alfred P. Sloan Foundation, instituted a

series of small workshops for science journalists and for congressional staffers. The workshops are intended to provide an opportunity for these two groups, who play significant roles in shaping and enacting science policy, to learn in depth about topics of current public concern. In March, a journalists' workshop was held on the **Cellular and Molecular Basis of Normal and Abnormal Development**. An interesting feature of this meeting was that it dealt with development in widely differing animal groups, including *Drosophila*, nematodes, amphibia, and mammals, and with the increasing evidence for the involvement of oncogenes in developmental processes.

Joint Meeting with Shearson Lehman Brothers

Another part of the Banbury Center program for making the scientific basis of biotechnology better understood is a series of meetings for chief executive officers and other senior staff members of companies with an interest in biotechnology. Organized in conjunction with Shearson Lehman Brothers, the meetings are



intended to give the executives who attend an opportunity to learn, at first hand, from scientists who are world authorities in particular topics. The first meeting, **The Genetic Knowledge of Man**, was held in 1986, and the second meeting, entitled **The Human Brain**, was held in October of 1987. The topics covered ranged from the gross anatomy of the brain, through cloning the genes for color vision pigments, to the impact of neurobiology on philosophical problems such as mind-body dualism and free will. An outstanding group of scientists presented talks, and the meeting was a tremendous success.

Robertson House provides housing and dining accommodations at Banbury Center.

Other Meetings

The Banbury Center was also used as a venue to promote the activities of groups concerned with topics of biological or medical importance. A meeting on CCK Antagonists was organized under the auspices of the National Institute of Mental Health. We were pleased to welcome again the Esther A. and Joseph Klingenstein Fund that used the Center to bring together the young neuroscientists supported by the Fund and the Fund's trustees and scientific advisors. The contemplative ambience of Banbury Center meetings was exploited for weekend meetings both by the deans of the Associated Medical Schools of New York and by the Psychiatric Department of Mt. Sinai Hospital Medical School.

Meetings of quite a different kind began in 1987, when the facilities of the Center were used by our neighbors in Lloyd Harbor for a seminar series. Appropriately, the first of these seminars was given by members of the Laboratory: Doug Hanahan spoke on the use of transgenic mice in studying cancer and I spoke on the molecular genetics of Duchenne muscular dystrophy.

Publication of Banbury Center Meetings

Meetings on risk assessment are published as Banbury Reports, and as reviews of volumes in the series show, the Banbury Reports are established as authoritative and timely studies. In 1987, Banbury Report 25, Nongenotoxic Mechanisms in Carcinogenesis, was published. Meetings in the Corporate Sponsor Program held at the Banbury Center are published as Current Communications in Molecular Biology. In 1987, four titles were published in this series: Gene Transfer Vectors for Mammalian Cells; Angiogenesis: Mechanisms and Pathobiology; Inositol Lipids in Cellular Signaling; and Nuclear Oncogenes. These publications are records of the meetings held here at Banbury, but if the subject of the meeting justifies it, then a book based on the meeting, but dealing with the subject in greater detail, may be considered.

The Future

The Banbury Center is firmly established as a unique contributor to the processes of disseminating and promoting knowledge in the international scientific community. As can be seen from this report, Steve Prentis had begun to diversify the topics covered by Banbury Center meetings, and this is a trend that I intend to continue. The Corporate Sponsor Program and generous long-term funding from sources such as the Alfred P. Sloan Foundation and the James S. McDonnell Foundation have helped support the program, but funding is a continuing concern. I should like to establish series of meetings that would have funds assured over three-year periods, giving us long-term stability for planning exciting and topical meetings. For example, we would hold two meetings each on risk assessment, human genetics, neurobiology, cancer, plant molecular biology, AIDS, and biotechnology each year. These are topics that are scientifically challenging and exciting, and they are areas in which there will be increasing interaction between science and society. The consequences of such interactions must be examined if we are to promote the advancement of scientific knowledge and the well being of society. The Banbury Center will continue to contribute to this process by examining important issues involving the biological sciences and public policy.

Jan Witkowski

Publications

Caskey, C.T., R.E. Gibbs, J.A. Witkowski, and J.F. Hejtmancik. 1988. Human inheritable diseases. *Philos. Trans. R. Soc. Lond. B* (in press).

Hejtmancik, J.F., J.A. Witkowski, S. Gunnel, S. Davis, L. Baumbach, and C.T. Caskey. 1988. Prenatal and carrier detection of Duchenne muscular dystrophy using recombinant DNA technology. In *Nucleic acid probes in diagnosis of human genetic diseases* (ed. A.M. Willey). Alan R. Liss Inc., New York. (In press.)

McCabe, E.R.B., J. Towbin, J. Chamberlain, L. Baumbach, J.A. Witkowski, G.J.B. van Ommen, M. Koenig, L.M. Kunkel, and W.K. Seltzer. cDNA for the Duchenne muscular dystrophy locus demonstrate a previously undetectable deletion in a patient with dystrophic myopathy, glycerol kinase deficiency and congenital adrenal hypoplasia. *Lancet* (Submitted.)

Pizzey, J.A., J.A. Witkowski, and G.E. Jones. 1987. Spreading behaviour of cultured fibroblasts from carriers of Duchenne muscular dystrophy. *J. Cell Sci.* **87:** 163–169.

Witkowski, J.A. 1987. Optimistic analysis—Chemical embryology in Cambridge, 1920–1942. *Med Hist.* **31:** 247–268.

———. 1987. Cell aging in vitro: A historical perspective. *Exp. Gerontol.* **22:** 231–248.

———. 1988. The molecular genetics of Duchenne muscular dystrophy: The beginning of the end? *Trends Genet.* (in press).

———. 1988. RNA splicing—A scientific revolution. *Trends Biochem. Sci.* (in press).

———. 1988. Huxley in the laboratory: Embracing inquisitiveness and widespread curiosity. In *Julian Huxley—Biologist and statesman of science* (ed. A. van Helden). (In press.)

Witkowski, J.A. and C.T. Caskey. 1988. Duchenne muscular dystrophy—DNA diagnosis in practice. In *Current neurology* (ed. S.H. Appel). Medical Year Books, Chicago. (In press.)

MEETINGS

Nuclear Oncogenes

March 3–March 6

ARRANGED BY

E. Harlow, Cold Spring Harbor Laboratory, New York
F. Alt, Columbia University, New York, New York
E.B. Ziff, New York University Medical Center, New York

SESSION 1

Chairperson: **E.B. Ziff,** New York University Medical Center, New York

C. Stiles, Dana-Farber Cancer Institute, Boston, Massachusetts: Do inducible nuclear proto-oncogenes play a functional role in growth factor action?
E.B. Ziff, New York University Medical Center, New York: Transcriptional regulation of the c-*fos* gene.
M. Gilman, Cold Spring Harbor Laboratory, New York: Intracellular mediators of c-*fos* induction.
R. Treisman, MRC Laboratory of Molecular Biology, Cambridge, England: Regulation of c-*fos* transcription.
B.H. Cochran, Massachusetts Institute of Technology, Cambridge: Activation of transcription by growth factors.
P. Sassone-Corsi, The Salk Institute, San Diego, California: c-*fos* regulation is mediated by positive and negative cellular factors.
R.G. Roeder, Rockefeller University, New York, New York: Identification and functional analysis of common and gene-specific transcription factors for regulated eukaryotic genes.

B. Stillman, J. Adams

SESSION 2

Chairperson: **A.J. Levine,** Princeton University, New Jersey

B. Vennstrom, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Biochemical and biological functions of the cellular and viral e*rbA* oncogenes.
T. Curran, Roche Institute of Molecular Biology, Nutley, New Jersey: Possible role of c-*fos* in signal transduction.
T. Jenuwein, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Analysis of the molecular and biological function of *fos*.
E. Premkumar Reddy, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Structure, mechanisms of activation, and function of *myb* oncogene.
A.J. Levine. Princeton University, New Jersey: Interaction of p53 with viral and cellular proteins.
D. Beach, Cold Spring Harbor Laboratory, New York: Interaction between products of c*dc2* and *suc1* genes of fission yeast and between the homologs in human cells.

N. Kohl

T. Jenuwein

SESSION 3

Chairperson: E. Harlow, Cold Spring Harbor Laboratory, New York

N.C. Jones, Imperial Cancer Research Fund Laboratories, London, England: Functional and structural characterization of the adenovirus E1A proteins.

E. Moran, Cold Spring Harbor Laboratory, New York: Functional domains in the adenovirus E1A proteins.

M.R. Green, Harvard University, Cambridge, Massachusetts: A mutational analysis of the adenovirus type 5 E1A protein.

B. Wasylyk, Faculté de Medecine, Strasbourg, France; The *ras* oncogene and a tumor promoter stimulate the polyomavirus enhancer.

E. Harlow, Cold Spring Harbor Laboratory, New York: Regions of the adenovirus E1A proteins required for transformation are binding sites for cellular proteins.

J.R. Nevins, The Rockefeller University, New York, New York: Cellular factors involved in E1A gene control.

J. Brady, National Cancer Institute, Bethesda, Maryland: Transcriptional regulatory sequences in the human T-lymphotropic virus type I long terminal repeat.

E. Harlow, B. Vennstrom

SESSION 4

Chairperson: R. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, Washington

F. Alt, Columbia University, New York, New York: Structure and expression of *myc*-family genes.

R. Dalla-Favera, New York University Medical Center, New York: Mechanisms and biological role of c-*myc* oncogene activation in B-cell tumors.

R. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, Washington: Proteins encoded by the c-*myc* oncogene.

W. Lee, University of California, San Francisco: Definition of regions in human c-*myc* involved in transformation and nuclear localization.

R.A. Watt, Smith Kline & French Laboratories, Swedeland, Pennsylvania: Functional characterization of the c-*myc* protein.

K.B. Marcu, State University of New York, Stony Brook: Regulation and biological properties of the c-*myc* proto-oncogene.

M. Groudine, Fred Hutchinson Cancer Research Center, Seattle, Washington: Control of c-*myc* transcription by blocking elongation.

M. Keuhl

F. Alt

B. Moran

SESSION 5

Chairperson: **F. Alt,** Columbia University, New York, New York

M. Cole, Princeton University, New Jersey: c-*myc* and the control of cellular gene expression.

U.R. Rapp, N.C.I.-Frederick Cancer Research Facility, Maryland: Role of *myc* in tumor induction, growth factor abrogation, and control of c-*myc* expression.

N. Kohl, Massachusetts Institute of Technology, Cambridge: Mechanisms of oncogene collaboration.

J.M. Adams, Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia: Consequences of enforced *myc* expression within the B-cell lineage of E_μ-*myc* transgenic mice.

W.M. Kuehl, National Naval Medical Center, Bethesda, Maryland: Structure, expression, and regulation of murine c-*myb*.

T. Roberts, Dana-Farber Cancer Institute, Boston, Massachusetts: Analysis of the effects of polyoma and SV40 large T antigens on differentiation.

D. Hanahan, Cold Spring Harbor Laboratory, New York: Expression of the murine p53 protein in β cells of insulin-SV40 T antigen transgenic mice.

Journalists' Workshop on the Cellular and Molecular Basis of Normal and Abnormal Development

March 11–March 13

ARRANGED BY

S. Prentis, Cold Spring Harbor Laboratory, New York

SESSION 1

R. Pedersen, University of California, San Francisco: Early development of the mammalian embryo.

I.B. Dawid, National Institutes of Health, Bethesda, Maryland: Development of amphibians.

SESSION 2

M.P. Scott, University of Colorado at Boulder: The development of *Drosophila* and the role of homeotic genes.

R. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Oncogenes.

R. W. Cooke

SESSION 3

J. Kimble, University of Wisconsin, Madison: Nematodes and sex determination.

E.J. Lammer, Massachusetts General Hospital, Boston: Developmental toxicity—The case of accutane.

B. Patrusky

D. Zimmerman

Mammalian Cell Mutagenesis

March 22-March 25

ARRANGED BY

M.M. Moore, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina
F.J. de Serres, Research Triangle Institute, Research Triangle Park, North Carolina
D.M. DeMarini, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina
K.R. Tindall, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

SESSION 1: A REVIEW OF GENETIC MARKERS FOR THE STUDY OF MUTATION IN MAMMALIAN CELLS

Chairperson: **R.J. Albertini,** University of Vermont College of Medicine, Burlington

G.M. Adair, University of Texas System Cancer Center, Smithville: The Chinese hamster *aprt* locus.
J.A. Nicklas, University of Vermont, Burlington: Mutation at the human major histocompatibility complex (HLA) as a genotoxicity assay.
D. Clive, Burroughs Wellcome Company, Research Triangle Park, North Carolina: Historical overview of the mouse lymphoma TK$^{+/-}$ mutagenesis assay.
A.W. Hsie, University of Texas Medical Branch, Galveston: The *hyprt* locus as an ideal and cautious choice for studying quantitative mammalian cell mutagenesis.
Panel Discussion (G.M. Adair, R.J. Albertini, D.Clive, A.W. Hsie, J.A. Nicklas)

SESSION 2: DIFFERENTIAL RECOVERY OF MUTANTS AT DIFFERENT LOCI

Chairperson: **D.M. DeMarini,** U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

D.M. DeMarini, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina: Differential recovery of mutants at hemizygous vs. heterozygous loci in mammmalian cell specific locus assays.
F.J. de Serres, Research Triangle Institute, Research Triangle Park, North Carolina: Specific-locus studies in *Neurospora crassa* predict differential recovery of mutants in mammalian cells.
L.F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, Pennsylvania: Detection of clastogens vs. point mutagens at the *gpt* or *hgprt* locus.
H.H. Evans, Case Western Reserve University, Cleveland, Ohio: Differential recovery of mutants in cells heterozygous vs. hemizygous for the *tk* locus.
M.M. Moore, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina: Factors that

J. Hozier, K. Dixon

P. O'Neil, R. Jensen, L. Stankowski

affect the recovery of all classes of induced mutations in mammalian cells.

L.B. Russell, Oak Ridge National Laboratory, Tennessee: Genetic and molecular characterization of chromosomal regions surrounding specific loci of the mouse.

Panel Discussion (H.M. Brockman, D.M. DeMarini. F.J. de Serres, H.H. Evans, M.M. Moore, L:B. Russell, L.F. Stankowski, Jr., K.R. Tindall)

SESSION 3: DIFFERENTIAL RECOVERY OF MUTANTS IN VIVO

Chairperson: M.M. Moore, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

R.J. Albertini, University of Vermont College of Medicine, Burlington: Quantitative considerations in in vivo studies.

R.H Jensen, Lawrence Livermore National Laboratory, California: Use of MN markers in human erythrocytes to quantitate mutation.

Panel Discussion (R.J. Albertini, R.H. Jensen)

SESSION 4: THE MOLECULAR ANALYSIS OF MUTATION

Chairpersons: F. Hutchinson, Yale University, New Haven, Connecticut
K.R. Tindall, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

A. Analysis of Chromosomal Loci

The *aprt* locus

B.W. Glickman, York University, Downsville, Toronto, Ontario, Canada: Studies on mutational specificity in the *aprt* gene of CHO cells.

M. Meuth, Imperial Cancer Research Fund, Herts, England: Molecular basis of genome rearrangements at the hamster *aprt* locus.

Panel Discussion (G.M. Adair, P.J. de Jong, B.W. Glickman, M. Meuth)

Other loci

L.A. Chasin, Columbia University, New York, New York: Mutation at the *dhfr* locus in CHO cells.

E.H.Y. Chu, University of Michigan Medical School, Ann Arbor: Analysis of multilocus mutations in human somatic cells based on protein variants detected by two-dimensional polyacrylamide electrophoresis.

Panel Discussion (L.A. Chasin, E.H.Y. Chu)

SESSION 5: THE MOLECULAR ANALYSIS OF MUTATION (cont'd)

The *tk* and *hgprt* loci

J.C. Hozier, Florida State University, Tallahassee: Cytogenetic and molecular studies of *tk* mutagenesis in mouse L51787 cells.

J.B. Little, Harvard School of Public Health, Boston, Massachusetts: Molecular analyses of *tk* and *hgprt* mutations in human cells.

R.A. Gibbs, Baylor College of Medicine, Houston, Texas: Molecular analysis of HPRT mutations.

J.P. O'Neill, University of Vermont, Burlington: DNA alterations in spontaneous in-vivo- and γ-irradiation-induced in vitro *hgprt* mutants of human T lymphocytes.

Panel Discussion (M. Applegate, R.A. Gibbs, J.C. Hozier, J.B. Little, J.A. Nicklas, J.P. O'Neill)

The *gpt* gene as a chromosomally integrated structure

K.R. Tindall, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Molecular analysis of mutation in the AS 52 cell line.

C.R. Ashman, University of Chicago Medical Center, Illinois: Recovery and analysis of spontaneous and induced mutations from mammalian chromosomal DNA.

Panel Discussion (C.R. Ashman, T.G. Rossman, L.F. Stankowski, Jr., K.R. Tindall)

SESSION 6: THE MOLECULAR ANALYSIS OF MUTATION (cont'd)

B. Shuttle Vectors and SV40
Chairperson: A. Sarasin, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France

M.P. Calos, Stanford University School of Medicine, California: Use of shuttle vectors in human cells.
K. Dixon, National Institute of Child Health and Human Development, Bethesda, Maryland: Analysis of mutation induction in mammalian cells with an SV40-based shuttle vector.
A. Sarasin, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Use of SV40 to analyze point mutations induced by UV-light or chemical carcinogens.
Panel Discussion (M.P. Calos, K. Dixon, A. Sarasin)

SESSION 7: RECOMBINATION AND REPAIR AS MODULATORS OF MUTATIONAL PROCESSES IN MAMMALIAN CELLS

Chairperson: F.J. de Serres, Research Triangle Institute, Research Triangle Park, North Carolina

L.H. Thompson, Lawrence Livermore National Laboratory, Livermore, California: Cloning of human DNA repair genes by functional complementation.
V.M. Maher, Michigan State University, East Lansing: Molecular analysis of recombination and gene conversion in carcinogen-treated mouse cells.
Panel Discussion (V.M. Maher, L.H. Thompson)

Summary

E.H.Y. Chu, University of Michigan Medical School, Ann Arbor

J. Little, M. Meuth

B. Glickman

Inositol Lipids in Cellular Signalling

April 5–April 8

ARRANGED BY

R.H. Michell, University of Birmingham, England
J.W. Putney, Jr., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

SESSION 1: BIOCHEMISTRY OF INOSITOL LIPIDS AND INOSITOL PHOSPHATES

Chairperson: R.J. Haslam, McMaster University, Hamilton, Ontario, Canada

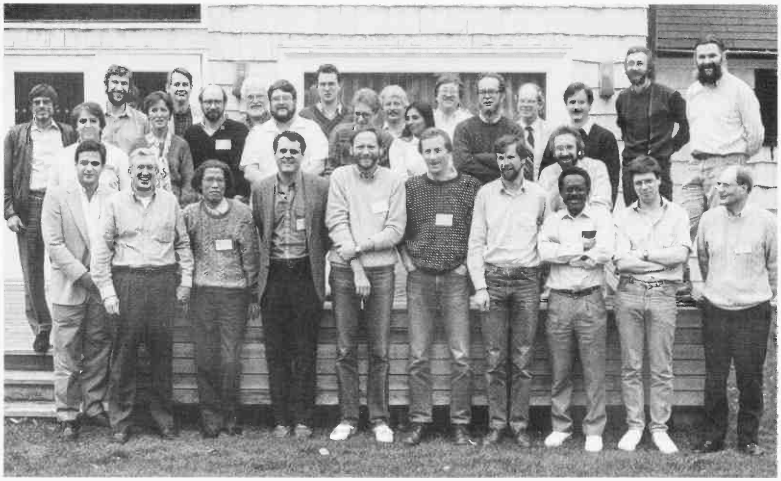
R.H. Michell, University of Birmingham, England: Metabolic pooling of inositol lipids.
C.P. Downes, Smith Kline and French Research Ltd., Welwyn, Herts, England: Specificity of agonist-stimulated

phospholipase C and metabolism of inositol phosphates.

M.C. Gershengorn, Cornell University Medical College, New York, New York: TRH stimulating of inositol lipid metabolism—Evidence for direct hydrolysis of PI as well as PIP_2.

W.R. Sherman, Washington University School of Medicine, St. Louis, Missouri: Evidence that PtdIns and PtdIns-(4,5)P_2 respond independentiy to stimulation.

S.B. Shears, University of Birmingham, England: Hepatic metabolism of inositol polyphosphates.



SESSION 2: RECEPTOR COUPLING TO PHOSPHOLIPASE C

Chairperson: T.F.J. Martin, University of Wisconsin, Madison

M. Ui, University of Tokyo, Japan: Differential roles of G_i and G_o in coupling to multiple receptors in brain.

S. Cockcroft, University College London Medical School, England: Regulation of polyphosphoinositide phosphodiesterase by a G-protein.

R.J. Haslam, McMaster University, Hamilton, Ontario, Canada: Activation of platelet phospholipase C by a guanine-nucleotide-binding protein.

T.K. Harden, University of North Carolina, Chapel Hill: Guanine-nucleotide-binding-protein-mediated regulation of phospholipase C.

C.O. Rock, St. Jude Children's Research Hospital, Memphis, Tennessee: Regulation of phosphatidylinositol 4,5-bisphosphate phospholipase C.

J.N. Fain, Unviersity of Tennessee Center for the Health Sciences, Memphis: Regulation of phosphoinositide-specific phospholipase C in cell-free systems by ligands and guanine nucleotides.

J.C. Cambier, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado: Antigen receptor coupling to inositol lipid hydrolysis in isolated B-lymphocyte membranes: Is GTP required?

C. Rock, M. Feinstein

S. Cockcroft, L. Cantley

SESSION 3: ACTION OF INOSITOL LIPID-DERIVED MEDIATORS

Chairperson: I. Schulz, Max-Planck-Institut für Biophysik, Frankfurt, Federal Republic of Germany

T.J. Rink, Smith Kline and French Research Ltd., Welwyn, Herts, England: Receptor-mediated calcium mobilization.

P.H. Cobbold, University of Liverpool, England: The generation of repetitive free-calcium transients in hormone-stimulated cells.

D.L. Gill, University of Maryland School of Medicine, Baltimore: Calcium release mediated by guanine nucleotides and inositol 1,4,5-trisphosphate.

A.P. Thomas, Thomas Jefferson University, Philadelphia, Pennsylvania: GTP-dependent regulation of the $InsP_3$-activated Ca^{++} channel.

M.B. Feinstein, University of Connecticut Health Center, Farmington: Monoclonal antibodies that block response to IP_3.

A.R. Saltiel, Rockefeller University, New York, New York: A role for novel glycosylated phosphoinositides in insulin action.

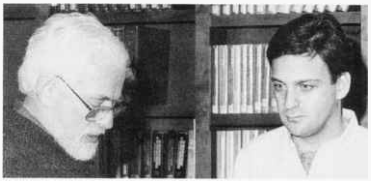
SESSION 4: GROWTH FACTOR AND ONCOGENES

Chairperson: M.D. Houslay, University of Glasgow, Scotland

B.C. Tilly, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands: Signal transduction by growth factors.

L.C. Cantley, Tufts University School of Medicine, Boston, Massachusetts: PI kinases.

I.G. Macara, University of Rochester Medical Center, New York: Down-regulation of protein kinase C by oncogenic transformation.



B. Agranoff, A. Saliel

SESSION 5: PHOSPHOINOSITIDE-LINKED RECEPTORS IN MODEL SYSTEMS

Chairperson: C.P. Downes, Smith Kline and French Research Ltd., Welwyn, Herts, England

J.W. Putney, Jr., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Receptor regulation of phospholipase C in paratid acinar cells.

I. Schulz, Max-Planck-Institut für Biophysik, Frankfurt, Federal Republic of Germany: Effects of inositol-1,4,5-triphosphate and guanosine nucleotides in stimulus-secretion coupling of exocrine pancreas cells.

B.W. Agranoff, University of Michigan, Ann Arbor: Inositol lipids in CNS.

S.E. Rittenhouse, University of Vermont College of Medicine, Burlington: Regulation of platelet phospholipase C activation.

T.F.J. Martin, University of Wisconsin, Madison: Mechanisms underlying stimulus-secretion coupling in GH_3 pituitary cells.

A.H. Drummond, University of London, England: Perturbation by lithium ions of inositol phosphate metabolism in GH_3 pituitary cells.

M.D. Houslay, University of Glasgow, Scotland: Desensitization of glucagon-stimulated adenylate cyclase exposes interactions between the signal transduction mechanism that produces cAMP and that stimulating inositol phospholipid metabolism.

J. Putney, R. Mitchell

I. Schultz

Therapeutic Peptides and Proteins: Assessing the New Technologies

April 12–April 15

ARRANGED BY

D.R. Marshak, Cold Spring Harbor Laboratory, New York
A.N. Schechter, National Institutes of Health, Bethesda, Maryland
D.R. Bangham, National Institute for Biological Standards and Control, London, England

SESSION 1: NEW PRODUCTION TECHNOLOGIES

Chairperson: D.R. Marshak, Cold Spring Harbor Laboratory, New York

R.A. Flavell, Biogen Research Corporation, Cambridge, Massachusetts: Recombinant DNA products from prokaryotes.
J. Obijeski, Genentech, Inc., South San Francisco, California: Recombinant DNA products from eukaryotes.
S.B.H. Kent, California Institute of Technology, Pasadena: The chemical synthesis of therapeutic peptides and proteins.
J.T. Johansen, Carlsberg Biotechnology Ltd., Copenhagen, Denmark: Enzymatic synthesis of peptides.

SESSION 2: CHEMICAL AND BIOLOGICAL EVALUATION OF PRODUCTS

Chairperson: D.T. Liu, Food and Drug Administration, Bethesda, Maryland

R.L. Heinrikson, The Upjohn Company, Kalamazoo, Michigan: Purification and chemical characterization of recombinant proteins—The example of human renin.
D.R. Marshak, Cold Spring Harbor Laboratory, New York: Physiochemical characterization of proteins produced by chemical synthesis.
P.W. Robbins, Massachusetts Institute of Technology, Cambridge: Glycosylations.
J.A. Smith, Massachusetts General Hospital, Boston: Acylation and removal of acetylated amino acids.
A.N. Schechter, National Institutes of Health, Bethesda, Maryland: Disulfide bonds.
W. Hancock, Genentech, Inc., South San Francisco, California: Oxidation and degradation.
M. Wigler, Cold Spring Harbor Laboratory, New York: Possible contaminants, DNA-containing oncogenes.
P.L. Storring, National Institute for Biological Standards and Control, London, England: The role of biological methods in evaluating highly purified peptide and protein products.

E. Esber, D. Bangham

SESSION 3: CLINICAL EVALUATION

Chairperson: A.N. Schechter, National Institutes of Health, Bethesda, Maryland

J.E. Osborn, University of Michigan: Possible contaminants, viruses.
R.A. Houghten, Scripps Clinic and Research Foundation, La Jolla, California: Immunogenicity.
A.M. Breckenridge, University of Liverpool, England: Clinical toxicity.
B.J. Marafino, Jr., Cetus Corporation, Emeryville, California: The appropriate toxicologic testing of recombinant proteins.
N. Stebbing, I.C.I. Pharmaceutical Division, Cheshire, England: Risk assessment.
D. Hanahan, Cold Spring Harbor Laboratory, New York: Transgenic animal models of disease.
G.W.H. Jay, National Cancer Institute, Bethesda, Maryland: Transgenic animal models for the study of human retroviruses.

D. Liu, L. Sjodin

A. Schecter, D. Marshak

P. Storring

B. Fraser, B. Marafino

Y. Y. Chiu, W. Hancock

D. Vapnek

P. Robbins, R. Blacher

J. Johanson, L. Frycklund

SESSION 4: CASE STUDIES

Chairperson: David L. Aronson, Food and Drug Administration, Bethesda, Maryland

Y. Chiu, Food and Drug Administration, Rockville, Maryland: Scientific review on the safety of the peptide hormones: insulin, hGH, LHRH.
G. Poste, Smith Kline and French Laboratories, Philadelphia, Pennsylvania: Characterization and testing of lymphokines and cytokines.
E. Grossbard, Genentech, Inc., South San Francisco, California: TPA.
D. Vapnek, AMGen, Thousand Oaks, California: Characterization of human erythropoietin produced in CHO cells.
L. Fryklund, KabiVitrum Peptide Hormones AB, Stockholm, Sweden: Recombinant IGF-1 produced in yeast.

SESSION 5: REGULATORY CONCEPTS

Chairperson: D.T. Liu, Food and Drug Administration, Bethesda, Maryland

E.C. Esber, Food and Drug Administration, Bethesda, Maryland: U.S. perspective.
D.R. Bangham, National Institute for Biological Standards and Control, London, England: U.K. perspective.
T. Hayakawa, National Institute of Hygienic Sciences, Tokyo, Japan: Control of the quality of biotechnological therapeutic agents intended for human use in Japan.
L. Sjodin, Socialstyrelsen, Lakemedelsavdelningen, Uppsala, Sweden: Swedish perspective.
J. Hsieh, Development Center for Biotechnology, Taipei, Taiwan, Republic of China: Development of HBsAg vaccine processes in Taiwan, Republic of China.

Eukaryotic Transposable Elements as Mutagenic Agents

April 21–April 24

ARRANGED BY

I.B. Weinstein, Columbia University College of Physicians & Surgeons, New York, New York
M.E. Lambert, Cold Spring Harbor Laboratory, New York
J.F. McDonald, University of Athens, Georgia

SESSION 1: INTRODUCTION: OVERVIEW OF PROKARYOTIC TRANSPOSABLE ELEMENTS

J.A. Shapiro, University of Chicago, Illinois: What transposons do in the bacterial genome.
D. Roberts, Massachusetts Institute of Technology, Cambridge: Communication between a bacterial transposable element and its host.
E.M. Witkin, Rutgers–The State University of New Jersey, Piscataway: Prokaryotic models: SOS functions and mutation.

SESSION 2: MUTATIONAL EFFECTS OF TRANSPOSABLE ELEMENT INSERTIONS–1

M.M. Green, University of California, Davis: Overview.
M. Strobel, N.C.I–Frederick Cancer Research Facility, Maryland: Molecular genetic analysis of the murine dilute locus.
N. Fedoroff, Carnegie Institution of Washington, Baltimore, Maryland: Regulation of the expression of the maize suppressor-mutator transposable element.

SESSION 3: MUTATIONAL EFFECTS OF TRANSPOSABLE ELEMENT INSERTIONS–2

M.F. Singer, National Institutes of Health, Bethesda, Maryland: Line-1 sequences in primates.
E.L. Kuff, National Cancer Institute, Bethesda, Maryland: Intracisternal A-particle elements as insertional mutagens.
R. Callahan, National Cancer Institute, Bethesda, Maryland: Endogenous human retrovirus-like elements.

SESSION 4: INDUCERS/REGULATORS OF TRANSPOSABLE ELEMENT EXPRESSION AND TRANSPOSITION

Part 1: Host Effects
P.M. Bingham, State University of New York, Stony Brook: Evidence that suppressor-of-white-apricot is a regulatory gene acting at the level of RNA processing.
V.G. Corces, Johns Hopkins University, Baltimore, Maryland: Retroviral elements and suppressor genes in *Drosophila*.
M.C. Wilson, Research Institute of the Scripps Clinic, La Jolla, California: Expression of endogenous replication-defective retroviral elements as regulated by *trans*-acting genes.

SESSION 5: INDUCERS/REGULATORS OF TRANSPOSABLE ELEMENT EXPRESSION

Part 2: Host Effects
F. Winston, Harvard University School of Medicine, Boston, Massachusetts: Host genes required for Ty-mediated gene expression in yeast.
R. Rothstein, Columbia University College of Physicians & Surgeons, New York, New York: Genetic control of recombination between retrotransposon elements in yeast.
J.D. Boeke, Johns Hopkins University, Baltimore, Maryland: Ty element transposition and the yeast genome.

SESSION 6: INDUCERS/REGULATORS OF TRANSPOSABLE ELEMENT EXPRESSION

Part 3: Genetic Mutator Systems
M.G. Kidwell, University of Arizona, Tucson: Regulatory aspects of expression of P-M hybrid dysgenesis in *Drosophila*.
F.H. Sobels, State University of Leiden, The Netherlands: Mutation induction by MR(P) and its modification by various conditions.
D.J. Finnegan, University of Edinburgh, Scotland: Mutation and chromosome rearrangements stimulated by I-R hybrid dysgenesis in *D. melanogaster*.

P. Bingham, B. McClintock, J. Shapiro, V. Chandler, M. Green

SESSION 7: INDUCERS/REGULATORS OF TRANSPOSABLE ELEMENT EXPRESSION

Part 4: Genomic Stress and Environmental Effects

J.F. McDonald, University of Georgia, Athens: Stress response and other host-mediated effects on retroviral element expression in *Drosophila*.

C. Paquin, University of Cincinnati, Ohio: Effect of temperature on Ty transposition.

V.L. Chandler, University of Oregon, Eugene: Regulation of mutator transposable elements in maize.

SESSION 8: INDUCERS/REGULATORS OF TRANSPOSABLE ELEMENT EXPRESSION

Part 5: Genomic Stress and Environmental Effects

G.R. Anderson, Roswell Park Memorial Institute, Buffalo, New York: Induction of VL30 and endogenous retroviruses in rat by anoxic stress and cyanide.

M.E. Lambert, Cold Spring Harbor Laboratory, New York: Inducible cellular responses to DNA damage.

H. zur Hausen, Deutsches Krebforschungszentrum, Heidelberg, Federal Republic of Germany: Mutations and DNA amplifications induced by DNA viruses.

SESSION 9: INDUCERS/REGULATORS OF RETROVIRAL ELEMENT EXPRESSION

R. Rothstein, M. Lambert

I.B. Weinstein, Columbia University College of Physicians & Surgeons, New York, New York: Constitutive expression of endogenous retrovirus-related sequences during chemical carcinogenesis.

E. Gateff, Johannes Gutenberg-Universität Mainz, Federal Republic of Germany: c-*src* expression, retrovirus-like particles, and reoviruses in tumors of *D. melanogaster*.

W.C. Greene, Duke University, Durham, North Carolina: HTLV-I, HIV, and human T-cell growth.

SESSION 10: SUMMARY: OVERVIEW

K. Sankaranarayanan, State University of Leiden, The Netherlands: Transposable genetic elements, spontaneous mutations, and the assessment of genetic radiation hazards in man.

J. Cairns, Harvard School of Public Health, Boston, Massachusetts: Implications for cancer.

The Development of the Human Lymphocyte Protein Database

May 10–May 12

ARRANGED BY

R. Franza, Cold Spring Harbor Laboratory, New York
L. Hood, California Institute of Technology, Pasadena

SESSION 1

J.D. Watson, Cold Spring Harbor Laboratory, New York. Introduction.

J. Garrels and R. Franza, Cold Spring Harbor Laboratory, New York: The REF52 cellular protein database: A model for database analysis of mammalian cells.

F.C. Neidhardt, University of Michigan Medical School, Ann Arbor: The *E. coli* protein database.

J.R. Warner, Albert Einstein College of Medicine, Bronx, New York: The yeast protein database.

I. Weissman, Stanford University, California: Systems for the study of lymphoblast differentiation.

SESSION 2

P. Jones, Stanford University, California: Potential of database analysis on studies of T and B lymphoblasts.

C.R. Merril, National Institute of Mental Health, Bethesda, Maryland: Strategies for the use of protein databases to examine disease processes.

E.P. Lester, University of Tennessee, Memphis: GELLAB.

S.M. Hanash, University of Michigan Medical School, Ann Arbor: Application of two-dimensional gels to genetic analyses.

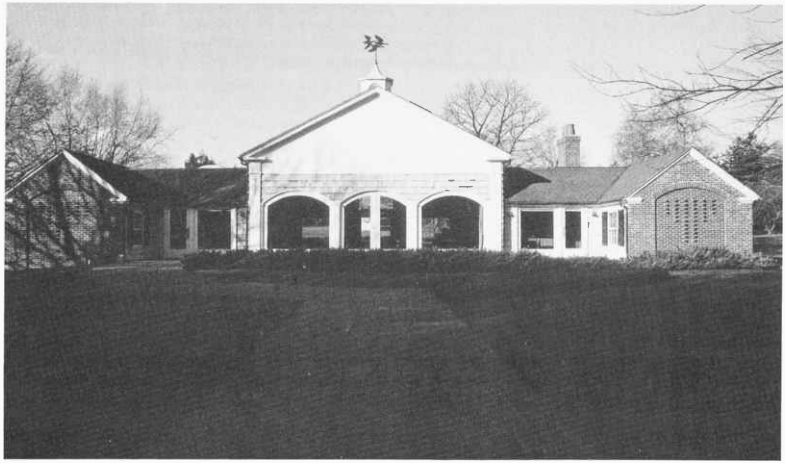
W.R. Parker, Bio Image, Ann Arbor, Michigan: Electrophoretic analysis and data management with machine vision instruments.

N.L. Anderson, Large Scale Biology Corporation, Rockville, Maryland: Studies of gene expression in human lymphoid cells.

I. Lefkovits, Basel Institute for Immunology, Switzerland: Toward an objective classification of the cells in the immune system.

K.E. Willard-Gallo, Catholic University of Louvain, Brussels, Belgium: Two-dimensional gel studies of subpopulations of T lymphoblasts.

S. Blose, Protein Databases, Inc., Huntington Station, New York: The PDQUEST system: Development and construction of protein databases for access and distribution.



Banbury Meeting House

SESSION 3

L. Hood and R. Aebersold, California Institute of Technology, Pasadena: Use of database-directed, two-dimensional gel technology in the purification and biochemical characterization of proteins leading to the isolation of the encoding genes.

L.A. Herzenberg, Stanford University School of Medicine, California: Sorting technologies and gene transfection studies: Impact on the study of lymphoblasts.

R. DeMars, University of Wisconsin, Madison: Two-dimensional gel analysis of MHC-encoded proteins.

D. Goldman, National Institutes of Health, Bethesda, Maryland: Structuring a lymphoblast database to study protein polymorphisms.

R. Franza, Cold Spring Harbor Laboratory, New York: An approach to the study of lymphoblast proteins that interact with control regions in the HIV-LTR.

SESSION 4: DISCUSSION OF POSSIBLE MECHANISMS FOR THE ASSEMBLY OF SPECIFIC LYMPHOCYTE DATABASES

Moderators: **J.D. Watson,** Cold Spring Harbor Laboratory, New York
L. Hood, California Institute of Technology, Pasadena

Discussion of the establishment of a resource to interconnect and distribute such databases.

Discussion of the establishment of a group responsible for the identification of human cellular proteins in specific B- and T-lymphocyte lines for dissemination to all builders and users of lymphocyte databases.

Formation of an internationally recognized coordinating committee to monitor the progress of such an effort.

New Directions in the Qualitative and Quantitative Aspects of Carcinogen Risk Assessment

October 11–October 14

ARRANGED BY

F.D. Hoerger, The Dow Chemical Company, Midland, Michigan
R.W. Hart, National Center for Toxicological Research, Jefferson, Arkansas
J.D. Wilson, Monsanto Company, St. Louis, Missouri

SESSION 1: STRUCTURE ACTIVITY RELATIONSHIP DATA

Chairperson: E.K. Weisburger, National Cancer Institute, Bethesda, Maryland

D. Thakker, National Institutes of Health, Bethesda, Maryland: High-molecular-weight structure.
W. Lijinsky, NCI-Frederick Cancer Research Facility, Maryland: Nitrogen-containing alkylating carcinogens.
Discussants – C.J. Michejda, NCI–Frederick Cancer Research Facility; D.E. Stevenson, Shell Oil Company; H.S. Rosenkranz, Case Western Reserve University; K. Enslein, Health Designs, Inc.

SESSION 2: PHARMACOKINETIC AND METABOLIC ACTIVITY DATA

Chairperson: R.W. Estabrook, University of Texas, Dallas

F.P. Guengerich, Vanderbilt University, Nashville, Tennessee: Interindividual metabolic variation in humans – Mechanisms, methods of assessment, and consequences.
W. Weber, University of Michigan, Ann Arbor: Acetylation pharmacogenetics; acetylator phenotype and assessing susceptibility to aromatic amine carcinogenesis.
W. Farland, U.S. Environmental Protection Agency, Washington, D.C.: Use of pharmacokinetic and metabolism data in quantitative risk assessment.
Discussants: C.C. Travis, Oak Ridge National Laboratory; D.B. Clayson, Health and Welfare Canada; P. Fu, National Center for Toxicological Research; M.C. Poirier, National Cancer Institute

SESSION 3: MOLECULAR BIOLOGICAL DATA

Chairperson: R.B. Setlow, Brookhaven National Laboratory, Upton, New York

B. Singer, University of California, Berkeley: Molecular distortions in DNA.
J.E. Trosko, Michigan State University, East Lansing, Michigan: Nongenotoxic mechanisms in carcinogenesis: Role of inhibited intercellular communication.
W.G. Flamm, U.S. Food and Drug Administration, Washington, D.C.: How molecular data is presently used.
Discussants – M.S. Cohn, U.S. Consumer Product Safety Commission; F.A. Beland, National Center for Toxicological Research; K. Kraemer, National Cancer Institute; R.W. Tennant, National Institute of Environmental Health Sciences

SESSION 4: APPROACHES TOWARD INTEGRATION

Chairperson: E.A. Pfitzer, Hoffmann-La Roche Inc., Nutley, New Jersey

K.S. Crump, Clement Associates, Inc., Ruston, Louisiana: Comparison of estimates from animal and human data.
I.C. Munro, Canadian Centre for Toxicology, Guelph, Ontario, Canada: Qualitative factors in classification.
J.D. Graham, Harvard University, Boston, Massachusetts: Judgmental considerations.
T. Thorslund, Clement Associates, Inc., Washington, D.C.: Framework for a biological model.
D. Krewksi, Environmental Health and Welfare, Vanier, Ontario, Canada: Recent developments in carcinogenic risk assessment.

SESSION 5: IMPLICATIONS FOR POLICY AND RESEARCH

Chairperson: F.D. Hoerger, The Dow Chemical Company, Midland, Michigan

E. Anderson, Clement Associates, Inc., Washington, D.C.: Perspective on risk assessment of carcinogens.
C. St. Hilaire, ILSI Risk Science Institute, Washington, D.C.: Research needs to improve the basis of risk assessment.

Subsession A: Implications for Research Directors
Panel discussion: F.P. Perera, Columbia University School of Public Health; R.W. Hart, National Center for Toxicological Research; R.A. Neal, Chemical Industry Institute of Toxicology

Subsession B: Recommendations for Improving Regulatory Policy for Dealing with Carcinogens
Panel discussion: P.F. Deisler, Jr., University of Houston; A.K. Ahmed, Natural Resources Defense Council

Transformation of Agriculturally Important Crops

October 25–October 28

ARRANGED BY

N.M. Frey, Pioneer Hi-Bred International, Inc., Johnston, Iowa
R.T. Fraley, Monsanto Company, St. Louis, Missouri
J. Schell, Max-Planck-Institut, Koln, Federal Republic of Germany

SESSION 1: PLANT TRANSFORMATION SYSTEMS

J. Schell, Max-Planck-Institut, Koln, Federal Republic of Germany: Plant transformation using *Agrobacterium* and direct DNA uptake methods.
R.B. Horsch, Monsanto Agricultural Company, St. Louis, Missouri: Strategies for practical gene transfer into agriculturally important crops.
A. Weissinger, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Maize transformation via microprojectile bombardment.
D.M. Stalker, Calgene Inc., Davis, California: Development of herbicide resistance in transgenic plants.

SESSION 2: TRANSFORMATION TO IMPROVE TRAITS OF AGRONOMIC IMPORTANCE

C.J. Lamb, The Salk Institute, San Diego, California: Transfer of defense genes.
J.J. Leemans, Plant Genetic Systems N.V., Gent, Belgium: Engineering insect and herbicide resistance in crops.
R.N. Beachy, Washingon University, St. Louis, Missouri: Transformation to produce virus-resistant plants.
C.J. Arntzen, Du Pont Experimental Station, Wilmington, Delaware: Agronomically useful genes for crop plants.
J.B. Mudd, The Plant Cell Research Institute, Inc., Dublin, California: Altering protein and oil quality traits in seeds.
J. Bedbrook, Advanced Genetic Sciences, Oakland, California: Chitinase as a suppressor of fungal diseases.

SESSION 3: FIELD TESTING AND THE DEVELOPMENT OF SEED PRODUCTS

N.M. Frey, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Developing seed products before genetic engineering.

P. Dale, IPSR Cambridge Laboratory, England: Progress toward cereal transformation and field trials of genetically engineered potatoes.

T. Helentjaris, Native Plants, Inc., Salt Lake City, Utah: Identification of agronomically useful genes through RFLP analysis.

S. Rothstein, CIBA-Geigy Corporation, Research Triangle Park, North Carolina: Field testing genetically engineered plants.

R.T. Fraley, Monsanto Company, St. Louis, Missouri: Field testing transgenic plants.

SESSION 4: REGULATORY REQUIREMENTS FOR TESTING AND COMMERCIALLY DEVELOPING GENETICALLY ENGINEERED PLANTS

S. Poe, U.S. Department of Agriculture, Hyattsville, Maryland: U.S.D.A. regulation of genetically engineered plants and microorganisms.

P. Roberts, U.S. Environmental Protection Agency, Washington, D.C.: E.P.A. regulation of genetically engineered plants and microorganisms.

J.H. Maryanski, U.S. Food and Drug Administration, Washington, D.C.: Genetically modified agricultural crops—An F.D.A. perspective.

P.B. Moses, National Research Council, Washington, D.C.: The N.A.S. policy process—Examples in biological control and organism introductions.

SESSION 5: THE ISSUES OF SCIENCE, ECONOMICS AND PRODUCT DEVELOPMENT FOR AGRONOMIC CROPS

R.T. Fraley, Monsanto Company, St. Louis, Missouri—Discussion Leader

V.W. Ruttan, Department of Economics, University of Minnesota, St. Paul

N. Federoff, Carnegie Institution of Washington, Baltimore, Maryland

M.M. Simpson, Congressional Research Service, Washington, D.C.

Recent Advances in the Molecular Genetics of the Parasitic Protozoa

November 3–November 6

ARRANGED BY

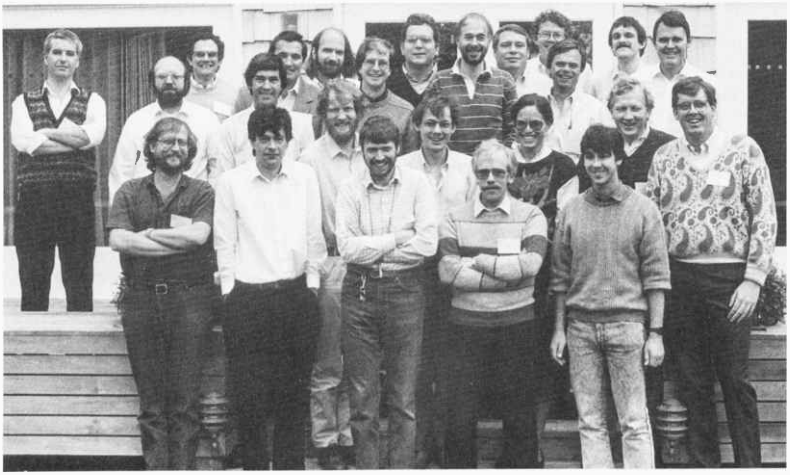
M.J. Turner, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey
D. Arnot, New York University Medical Center, New York

SESSION 1: MALARIA

V. Enea and D. Arnot, New York University Medical Center, New York: The circumsporozoite gene of *Plasmodia*.
M. Walgren, University of Stockholm, Sweden: Asparagine-rich molecules of *P. falciparum*.
J.V. Ravetch, Sloan-Kettering Institute, New York, New York: Molecular biology of parasite/host interactions.
D. Walliker, University of Edinburgh, Scotland: Genetic recombination in *P. falciparum*.
M. Lockyer, Wellcome Research Laboratories, Beckenham, Kent, England: Variation in *P. falciparum* gene structure.

SESSION 2: MALARIA, THEILERIA AND LEISHMANIA

T.E. Wellems, National Institutes of Health, Bethesda, Maryland: Structural analysis of chromosome-length polymorphisms in *P. falciparum*.
T.F. McCutchan, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: Stage-specific ribosomes in *Plasmodium*.
A. Tait, Wellcome Unit of Molecular Parasitology, Glasgow, Scotland: Sporozoite and infection-specific antigen genes in *Theileria*.
R. Williams, University of Karlsruhe, Federal Republic of Germany: Autocrine loop immortalization of lymphocytes by *T. parva*.
S.M. Beverley, Harvard Medical School, Boston, Massachusetts: Chromosomal basis of gene amplification in *Leishmania*.
J.M. Kooter, The Netherlands Cancer Institute, Amsterdam: H. Circler in Leishmania.
B. Ullman, Oregon Health Sciences University, Portland: Genetic analysis of purine metabolism in *L. donoyani*.



SESSION 3: TRYPANOSOMES

A. Tait, Wellcome Unit of Molecular Parisitology, Glasgow, Scotland: Genetic exchange in *T. brucei*: Allelic segregation and reassortment.

J.M. Wells, University of Cambridge, England: Genetic analysis of hybrid *T. brucei* clones.

B. Sollner-Webb, Johns Hopkins University School of Medicine, Baltimore, Maryland: Expression of DNA transfected in *T. brucei*.

G.A.M. Cross, Rockefeller University, New York, New York: Mapping the 117 VSG gene-expression site.

L. Van der Ploeg, Columbia University College of Physicians & Surgeons, New York, New York: Role of chromosomal rearrangements in antigenic variation.

J.M. Kooter, The Netherlands Cancer Institute, Amsterdam: Structure and expression of a telomeric VSG gene expression site in *T. brucei*.

E. Pays, Free University of Brussels, Belgium: Transcription in *T. brucei*.

SESSION 4: TRYPANOSOMES

J. Boothroyd, Stanford University School of Medicine, California: *trans*-Splicing and polycistronic transcripts in trypanosomes.

R. Nelson, University of California, San Francisco: RNA processing and trypanosome gene expression.

S.L. Hajduk, University of Alabama, Birmingham: Structure and transcription of k-DNA of trypanosomes.

R. Benne, University of Amsterdam, The Netherlands: Mitrochondrial genes in trypanosomes.

K. Stuart, Seattle Biomedical Research Institute, Washington: Regulation of gene expression in trypanosomes by mRNA editing.

R. Layden, Fred Hutchinson Cancer Research Center, Seattle, Washington: Ubiquitin in trypanosomatids.

J. Manning, University of California at Irvine: Characterization of the 85-kD surface antigen gene of *T. cruzi*.

SESSION 5: GIARDIA AND OTHERS

T.E. Nash, National Institutes of Health, Bethesda, Maryland: Antigenic variation in *Giardia*.

D. Wirth, Harvard School of Public Health, Boston, Massachusetts: Divergence of k-DNA minicircle sequences in *Leishmania mexicana*.

K. Stuart, Seattle Biomedical Research Institute, Washington: Multicopy virus-like nucleic acids in *Leishmania*.

The Role of the Heat-Shock (Stress) Response in Biology and Human Disease

November 9–November 13

ARRANGED BY

R. Morimoto, Northwestern University, Evanston, Illinois
W. Welch, Cold Spring Harbor Laboratory, New York

A. Tissieres, University of Geneva, Switzerland: History and perspective of heat shock.

SESSION 1: THERMOBIOLOGY

Chairperson: **M.J. Schlesinger,** Washington University, St. Louis, Missouri

A.F. Bennett, University of California, Irvine: Thermal dependence of physiological function.

R.B. Huey, University of Washington, Seattle: Physiological adjustments to fluctuating environments: An ecological perspective.

W.P. Porter, University of Wisconsin, Madison: Multiple low-level stressors affecting growth and reproduction potential in small mammals.

M.J. Kluger, University of Michigan, Ann Arbor: Effects of febrile temperatures on host defense responses.

H.C. Heller, Stanford University, California: Central nervous control of body temperature in health and disease.

SESSION 2: CELLULAR AND MOLECULAR ASPECTS OF THE HEAT–SHOCK RESPONSE. I

Chairperson: C. Georgopoulos, University of Utah School of Medicine, Salt Lake City

S. Lindquist, University of Chicago, Illinois: Expression and function.

J.J. Bonner, Indiana University, Bloomington: Regulation of the heat-shock response.

J. Lis, Cornell University, Ithaca, New York: Engaged RNA polymerase II at the start of uninduced heat-shock genes.

C. Wu, National Cancer Institute, Bethesda, Maryland: Purification and properties of heat-shock activator proteins.

M.L. Pardue, Massachusetts Institute of Technology, Cambridge: 93D-, a different sort of heat-shock locus.

E. Craig, University of Wisconsin, Madison: Genetics and regulation of *hsp70* genes in yeast.

R. Voellmy, University of Miami, Florida: Studies of heat-shock promoters; abnormal protein induction of heat-shock genes.

Evening Informal Session, "Nomenclature of Stress Proteins"

SESSION 3: THERMOTOLERANCE

Chairperson: F. C. Neidhardt, University of Michigan, Ann Arbor

G.M. Hahn, Stanford University, California: RIF-1 and RIF-TR cells: A novel system for investigation of heat resistance, adaptation to heat, and thermotolerance.

E.W. Gerner, University of Arizona, Tucson: Factors affecting thermotolerance expression in mammalian cells.

W.C. Dewey, University of California, San Francisco: Thermotolerance for heat killing and heat radiosensitization of CHO cells.

G.C. Li, University of California, San Francisco: hsp70 as an indicator of thermotolerance: Clinical relevance.

R. Hallberg, Iowa State University, Ames: The eukaryotic homolog of the product of the *E. coli* heat-shock gene, *groEL*, is a mitochondrial protein.

K.N. Prasad, University of Colorado, Denver: Modification of heat sensitivity in neural tumors by cAMP and prostaglandin A_2.

SESSION 4: CELLULAR AND MOLECULAR ASPECTS OF THE HEAT SHOCK RESPONSE. II

Chairperson: S. Lindquist, University of Chicago, Illinois

W. Welch, Cold Spring Harbor Laboratory, New York: Properties and possible functions of mammalian stress proteins.

P. Arrigo, Cold Spring Harbor Laboratory, New York: Characterization of the low-molecular-weight heat-shock proteins.

P.K. Sorger and Hugh R. B. Pelham, M.R.C. Laboratory of Molecular Biology, Cambridge, England: Regulation of the yeast heat-shock transcription factor.

J.R. Subjeck, Roswell Park Memorial Institute, Buffalo, New York: Glucose-regulated response and resistance to chemotherapeutic agents.

M.J. Schlesinger, Washington University, St. Louis, Missouri: Events in heat-shocked chicken fibroblasts.

R. Morimoto, Northwestern University, Evanston, Illinois: Cell cycle and viral oncogene activation of human heat-shock gene expression.

B. Wu, Genetics Institute, Cambridge, Massachusetts: Transcriptional regulation of the human *hsp70* gene.

L. Nover, Institute of Plant Biochemistry, Academy of Sciences of German Democratic Republic: Cytoplasmic heat-shock granules.

Evening Informal Session, "Are Stress Proteins Involved in Thermotolerance?"

SESSION 5: CLINICAL APPLICATIONS OF HYPERTHERMIA

Chairperson: W.C. Dewey, University of California, San Francisco

T. Herman, Dana Farber Cancer Institute, Boston, Massachusetts: Systemic cisplatin (CDDP), local hyperthermia, and radiation for treatment of locally advanced human malignancies.

C. Vernon, Hammersmith Hospital, London, England: Thermal dose.

M.W. Dewhirst, Duke University, Durham, North Carolina: Feasibility of incorporation of spatially varying modifiers of hyperthermic effect in the clinical setting.

J.R. Oleson, Duke University, Durham, North Carolina: Is there evidence of thermal tolerance in clinical hyperthermic results?

J.M.C. Bull, University of Texas, Houston: Systematic hyperthermia combined with chemotherapy.

M. Abe, Kyoto University, Japan: RF capacitive hyperthermia for deep-seated tumors.

SESSION 6: STRESS PROTEIN EXPRESSION DURING DEVELOPMENT

Chairperson: M.L. Pardue, Massachusetts Institute of Technology, Cambridge

N.S. Petersen, University of Wyoming, Laramie: Phenocopy induction and thermotolerance.

L. Hightower, University of Connecticut, Storrs: Expression of mammalian heat-shock genes in early embryos, cultured rat cells, and *E. coli*.

D.J. Wolgemuth, Columbia University College of Physicians & Surgeons, New York, New York: Expression of *hsp70* gene family members in the male mammalian germ cell.

E. Baulieu, INSERM, Bicetre, France: Antisteroid hormones, receptor structure, and heat-shock protein MW 90,000 (hsp90).

D. Pauli and C. H. Tonka, University of Geneva, Switzerland: Structure and expression during development of heat-shock genes 2 and 3 from *Drosophila* locus 67B.

M. Tytell, Wake Forest University, Winston-Salem, North Carolina: Significance of heat-shock protein production in nervous system trauma.

I.R. Brown, University of Toronto, Canada: Analysis of heat-shock gene expression in the mammalian brain using in situ hybridization.

T.S. Nowak, Jr., National Institute of Neurological, Communicative Disorders and Stroke, Bethesda, Maryland: Stress response in brain following transient cerebral ischemia.

D. Young, Hammersmith Hospital, London, England: Stress proteins are antigens in leprosy and tuberculosis.

SESSION 7: CONTROL AND FUNCTION OF STRESS REGULONS

Chairperson: E. Craig, University of Wisconsin, Madison

F.C. Neidhardt, University of Michigan, Ann Arbor: Functions of bacterial heat-shock response.

C. Georgopoulos, University of Utah, Salt Lake City: Role of *E. coli* heat-shock proteins in bacteriophage λ growth.

T. Yura, Kyoto University, Japan: On the roles of σ32 and heat-shock proteins in *E. coli*.

C. Gross, University of Wisconsin, Madison: Regulation of the heat-shock response in *E. coli*.

K. McEntee, University of California, Los Angeles: Stress responsive genes in yeast; transcription regulation of DDR genes by heat shock and DNA damage.

A. Varshavsky, Massachusetts Institute of Technology, Cambridge: Ubiquitin system, selective protein turnover, and the stress response.

A.L. Goldberg, Harvard Medical School, Boston, Massachusetts: Protein breakdown and the heat-shock response.

Antisense RNA

December 1–December 3

ARRANGED BY

D.A. Melton, Harvard University, Cambridge, Massachusetts

SESSION 1: NATURAL EXAMPLES OF ANTISENSE REGULATION

R.W. Simons, University of California, Los Angeles: Antisense RNA control of IS*10* gene expression.

N. Kleckner, Harvard University, Cambridge, Massachusetts: Mechanism of IS*10*'s antisense RNA regulation in vitro.

W.R. McClure, Carnegie Mellon University, Pittsburgh, Pennsylvania: Roles for antisense RNA in bacteriophage gene regulation.

M.M. Susskind, University of Southern California, Los Angeles: Control of the phage P22 antirepressor gene by a small antisense RNA.

M. Inouye, UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey: Antisense RNA as a tool in viral immune systems.

SESSION 2: ANTISENSE OLIGONUCLEOTIDES

J. Walder, University of Iowa, Iowa City: Mechanism of hybrid-arrested translation and applications of antisense oligonucleotides.

A. Weiner, Yale University School of Medicine, New Haven, Connecticut: Inhibition of mRNA splicing by U1 snRNPs with altered specificity.

P.S. Miller, Johns Hopkins University, Baltimore, Maryland: "Antisense" oligonucleotide methylphosphonates.

C. Helene, Museum National d'Histoire Naturelle, INSERM, Paris, France: Regulation of gene expression by oligodeoxynucleotides covalently linked to intercalating agents.

J.J. Toulme, Museum National d'Histoire Naturelle, INSERM, Paris, France: Antisense oligodeoxynucleotides as regulatory agents for parasitic genes.

SESSION 3: ANTISENSE RNAs REGULATING GENE FUNCTION IN VIVO. I

R.A. Firtel, University of California, San Diego, La Jolla: Use of antisense to examine gene function in *Dictyostelium*.

S. Cohen, Max-Planck-Institut, Tubingen, Federal Republic of Germany: Phenocopies produced by antisense RNA in *Drosophila* embryos.

S. Lundquist, University of Chicago, Illinois: Use of antisense RNA in investigating the function and regulation of heat-shock proteins.

M. Jacobs-Lorena, Case Western Reserve University, Cleveland, Ohio: Disruption of ribosomal protein gene

expression in *Drosophila* by the conditional expression of an integrated antisense gene.

R.G. Oshima, La Jolla Cancer Research Foundation, California: Suppressive effects of antisense Endo B cytokeratin RNA on embryonal carcinoma differentiation.

SESSION 4: ANTISENSE RNAs REGULATING GENE FUNCTION IN VIVO. II

H. Weintraub, Fred Hutchinson Cancer Research Center, Seattle, Washington: Teflon RNA.

R. Moon, University of Washington School of Medicine, Seattle: Antisense RNA inhibits expression of membrane skeleton protein 4.1 during *Xenopus* development.

S. Strickland, State University of New York at Stony Brook: Antisense injections into the mouse oocyte.

C.T. Caskey, Baylor College of Medicine, Houston, Texas: Antisense inhibition of HPRT in cultured cells and transgenic mice.

R.W. Wagner, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Expression of an RNA duplex unwindase activity in mammalian cells.

SESSION 5: ANTISENSE RNAs REGULATING GENE FUNCTION IN VIVO. III

P.E. Neiman, Fred Hutchinson Cancer Research Center, Seattle, Washington: Antisense RNA effects in avian retroviral vector systems: Viral replication and target gene expression.

M. Kindy, The Salk Institute, San Diego, California: Inhibition of c-*fos* gene expression does not alter the differentiation pattern of PC12 cells.

J.G. Izant, Yale University School of Medicine, New Haven, Connecticut: Enhancement of antisense RNA activity: Antisense RNA to study cell organization.

J. Goodchild, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Antisense oligodeoxynucleotides as potential inhibitors of HIV replication in tissue culture.

E.W. Holmes, Duke University Medical Center, Durham, North Carolina: Inhibition of translation by antisense RNA complementary to 3′-coding and 3′-noncoding sequences.

D.A. Melton, Harvard University, Cambridge, Massachusetts: Summary.

EDUCATIONAL ACTIVITIES

Postgraduate Courses

The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Molecular Approaches to Ion Channel Function and Expression

June 5–June 25

INSTRUCTORS

Dionne, Vincent E., Ph.D., University of California, San Diego
Snutch, Terry, Ph.D., California Institute of Technology, Pasadena
White, Michael, Ph.D., University of Pennsylvania, Philadelphia
Margiota, Joseph, Ph.D., University of California, San Diego
Beam, Kurt, Ph.D., Colorado State University, Fort Collins
Corey, David, Ph.D., Harvard University, Cambridge, Massachusetts

ASSISTANT

Stevens, Meg, B.A., Harvard University, Cambridge, Massachusetts

The technologies of molecular biology and patch-clamping promise major revisions and novel approaches for the examination of many neurobiological problems. Applications of these methods to the study of ion channels are taught in this intensive laboratory/lecture course. Students concentrate initially on the basic aspects of these two methods and then proceed to more integrated studies. *Single Channel Recording:* cell-attached, excised patch and whole cell recording; design and implementation of recording equipment; theory and analysis of single-channel currents. *Ion channel expression:* mRNA isolation and handling; the use of *Xenopus* oocytes as an expression system; characterization of newly expressed ion-channels in oocytes using voltage- and patch-clamp methods; monitoring changes in ion channel expression during differentiation using northern blot analysis. There are opportunities during the final week for students to undertake special projects of their own design which utilize the methods taught in the course.

PARTICIPANTS

Avery, Leon, Ph.D., Massachusetts Institute of Technology, Cambridge
Boland, Linda M., M.S., University of North Carolina, Durham
Chalmers, Alison E., Ph.D., American Cyanamid, Princeton, New Jersey
Dixon, Donald B., M.S., University of Toronto, Canada
Enyeart, John J., Ph.D., Ohio State University, Columbus
Getchell, Thomas V., Ph.D., Wayne State University, Detroit, Michigan
Karpen, Jeffrey W., Ph.D., Stanford University, California
Nedergaard, Maiken, M.D., Cornell Medical Center, New York, New York
Surmeier, D. James, Ph.D., University of Tennessee, Memphis
Wong, Linda, B.S., University of Texas, Galveston

SEMINARS

Sahley, C., Yale University. Membrane properties in learning and memory.
Kinnamon, S. C., Colorado State University. Mechanisms of taste transduction.
Belardetti, F., University of Texas. Modulation of ion channels in Aplysia.
Stevens, C. F., Yale University. Glutamate receptors.
Brehm, P., Tufts University. Electrophysiology of sodium and acetylcholine receptor channels.
Mandel, G. Tufts University. Molecular biology of the sodium channel.

Molecular Embryology of the Mouse

June 5–June 25

INSTRUCTORS

Beddington, Rosa, Ph.D., ICRF, Oxford, England
Robetson, Liz, Ph.D., Cambridge University, England
Rossant, Janet, Ph.D., Mount Sinai Hospital, Toronto, Canada
Pedersen, Roger, Ph.D., University of California, San Francisco

ASSISTANTS

Calof, Anne, Ph.D., Tufts University, Medford, Massachusetts
Fox, Niles, Ph.D., Wistar Institute, Philadelphia, Pennsylvania
Mahon, Kathy, Ph.D., National Institutes of Health, Bethesda, Maryland

This course is designed for molecular biologists, biochemists and cell biologists interested in applying their expertise to the study of mouse embryonic development. Lecture and laboratory components of the course emphasize both the practical aspects of working with mouse embryos and the conceptual basis of current embryonic research. The following procedures and their potential applications are described: isolation and culture of germ cells and preimplantation and postimplantation embryos, embryo transfer, establishment of embryo-derived stem

cell lines, germ layer separation, chimera formation, nuclear transplantation, microinjection of DNA into eggs, retroviral infection of embryos, microinjection of cell lineage tracers, *in situ* hybridization using immunofluorescence and immunoperoxidase techniques.

PARTICIPANTS

Avraham, Karen B., B.S., Weizmann Institute, Rehovot, Israel
Barker, Douglas D., Ph.D., University of California, Los Angeles
Boisseau, Sylvie, M.S., C.N.R.S., Gif-sur-Yvette, France
Ciejek, Elena M., Ph.D., University of Rochester, New York
Dodd, Jane, Ph.D., Columbia University, New York, New York
Harvey, Richard, Ph.D., Harvard University, Cambridge, Massachusetts
Karlsson, Stefan, Ph.D., National Institutes of Health, Bethesda, Maryland
Narasimhan, Nalini, Ph.D., California Institute of Technology, Pasadena
Nguyen-Huu, Chi, Ph.D., Columbia University, New York, New York
Overbeek, Paul A., Ph.D., Baylor College of Medicine, Waco, Texas
Parvari, Ruti, M.S., Weizmann Institute, Rehovot, Israel
Poirier, Francoise, Ph.D., National Institute for Medical Research, London, England
Weaver, Steven, Ph.D., University of Illinois, Urbana
Williams, David A., M.D., Childrens Hospital, Boston, Massachusetts

SEMINARS

Chalfie, M., Columbia University. Cell lineages in *C. elegans*.
Chapman, V., Roswell Park Memorial Institute. Mouse genetic resources.
Copeland, N., Frederick Cancer Research Institute. Ecotropic viruses and insertional mutagenesis.
Costantinz, F., Columbia University. Analysis of B. globin expression in transgenic mice.
Darling, S., Mount Sinai Hospital. Sex determination in mammals.
Gardner, R., ICRF. Analysis of extraembryonic lineages.
Hanahan, D., Cold Spring Harbor Laboratory. Expression of oncogene constructs in transgenic mice.
Hastie, N., MRC Clinical and Population Cytogenetics Unit. Organization of the mouse genome.
Herrup, K., Yale University. Retrospective analysis of cell lineages in the central nervous system.
Jaenisch, R., Whitehead Institute. Retroviruses and insertional antigenesis.
Jenkins, N., Frederick Cancer Research Institute. Genetics of mouse coat colors.
Lacy, E., Memorial-Sloan Kettering Cancer Center. Insertional mutations in transgenic mice.
Papaoannou, G., Tufts University. Developmental mutants.
Petersen, A., Ludwig Institute, Montreal. Development of the nervous system and neurological mutants.
Ruddle, F., Yale University. Mouse homeobox genes.
Sargent T., National Institutes of Health. Induction and determination in the early *Xenopus* embryo.
Silver, L., Princeton University. Developmental genetics of the T-complex.
Solter, D., Wistar Institute. Nuclear transfer and genomic imprinting.
Struhl, G., Columbia University. Developmental genetics of *Drosophila*.
Wassarman, P., Roche Institute of Molecular Biology. Gametogenesis and fertilization.

Advanced Bacterial Genetics

June 5–June 25

INSTRUCTORS

Berget, Peter, Ph.D., Carnegie Mellon University, Pittsburgh, Pennsylvania
Maurer, Russell, Ph.D., Case Western Reserve University, Cleveland, Ohio
Weinstock, George, Ph.D., University of Texas, Houston

ASSISTANTS

Eraso, Jesus, B.S., University of Texas, Houston
Mjoumdar, Mona Lisa, Ph.D., Carnegie Mellon University, Pittsburgh, Pennsylvania
Shrimankar, Paresh, M.S., Case Western Reserve University, Pittsburgh, Pennsylvania

This laboratory course demonstrates genetic approaches that can be used to analyze biological processes and their regulation as well as detailed structure/function relationships of genes. Techniques that are covered include: isolation, complementation and mapping of mutations, use of transposable genetic elements, construction of gene fusions, cloning and manipulation of DNA, and DNA sequencing. The course consists of a set of experiments incorporating most of these techniques supplemented with lectures and discussions. The aim is to develop in students the ability to design a successful genetic approach to any biological problem.

PARTICIPANTS

Butler, Charles A., M.S., University of Tennessee, Nashville
Dunlap, Paul V., Ph.D., New Mexico State University, Las Cruces
Giroux, Sylvie, B.S., University of Montreal, Canada
Harriman, Philip D., Ph.D., National Science Foundation, Washington, D.C.
Kalpana, Ganjam V., M.S., Albert Einstein College, Bronx, New York
Kobiashi, Ichizo, Ph.D., National Medical Research Center, Tokyo, Japan
Lin-Chao, Sue D., Ph.D., University of Texas, Dallas
McCarty, Shawn C., B.A., University of Louisville, Kentucky
Michiels, Christiaan W., M.S., Catholic University of Leuven, Belgium
Reilly, Pamela M., M.S., University of Minnesota, Duluth
Richter, Agneta A., B.S. University of Uppsala, Sweden
Rohlman, Christopher E., M.S., University of Michigan, Ann Arbor
Stoltzfus, Arlin B., B.A., University of Iowa, Iowa City
Thaler, David S., Ph.D., University of Oregon, Eugene
Vezina, Guy, M.S., Laval University, Quebec, Canada
Willey, Joanne M., B.S., Woods Hole Oceanographic, Massachusetts

SEMINARS

Sauer, R., Massachusetts Institute of Technology. Genetic dissection of protein structure.

Taylor, R., University of Tennessee. The use of phoA fusions to study secreted virulence determinants.

Hillyard, R., Howard Hughes Medical Institute. Macrorestriction of the salmonella chromosome.

Figurski, D., Columbia University College of Physicians & Surgeons. Replication control in promiscuous plasmid RK2.

Craig, N., University of California, San Francisco. Transposition of Tn7.

Smith, G., University of Missouri. Using felamentous phages in bacterial genetics.

Silhavey, T., Princeton University. Genetic analysis of protein export.

Molecular Biology of the Nervous System

June 5–June 19

INSTRUCTORS

Evans, Ronald, Ph.D., Salk Institute, San Diego, California
McKay, Ronald, Ph.D., Massachusetts Institute of Technology, Cambridge
Reichardt, Louis, Ph.D., University of California, San Francisco
Zipursky, Larry, Ph.D., University of California, Los Angeles

This lecture course is designed for neuroscientists who wish to understand the concepts and methods of molecular biology and their application to problems in neuroscience. The participants are drawn from a wide range of backgrounds. The methods of recombinant DNA technology are introduced in a series of lectures. These are followed by lectures from visiting faculty. The lectures cover a variety of topics to give an overview of the molecular mechanisms underlying the development and function of the nervous system.

PARTICIPANTS

Augenbraun, Eliene, B.A., Columbia University, New York, New York
Condic, Maureen L., B.A., University of California, Berkeley
Giza, Christopher C., B.A., West Virginia University, Morgantown
Hall, Garth F., Ph.D., University of California, Berkeley
Khrestchatisky, Michel, Ph.D., University of California, Los Angeles
Lagrutta, Armando A., B.S., University of Connecticut, Storrs
Martin, Kathleen, M.S., Yale University Medical School, New Haven, Connecticut
Ottiger, Hans Peter, M.D., Scripps Clinic and Research Institute, La Jolla, California
Padgett, Janet L., B.S., Johns Hopkins University, Baltimore, Maryland
Penn, Anna A., B.S., Harvard University, Cambridge, Massachusetts
Petrucci, Tamara C., Ph.D., Yale University, New Haven, Connecticut
Redies, Christoph, M.D., Montreal Neurological Institute, Canada
Saccomano, Nicholas A., Ph.D., Pfizer Central Research, Groton, Connecticut
Szigethy, Eva M., B.A., Montreal Neurological Institute, Canada
Thormodsson, Finnbogi R., B.S., Cornell University, Ithaca, New York
Valtorta, Flavia, M.D., Rockefeller University, New York, New York
Wang, Samuel, B.S., Stanford University, California
Wolswijk, Guus, M.S., Institute of Neurology, London, England

SEMINARS

Struhl, K., Harvard University. Controlling gene expression in bacteria and yeast.
Green, M., Harvard University. RNA splicing mechanisms.
Myers, R., University of California, San Francisco. Long range structure of the human genome.
Zuker, C., University of California, San Diego. Structure and function of *Drosophila* opsins.
Mitchison, T., University of California, San Francisco. Microtubule function.
Reese, T., Woods Hole Oceanographic Institute. The structure and function of axons.
Kelly, R., University of California, San Francisco. Cellular mechanisms of secretion and uptake.
Jessel, T., Columbia University. Cell adhesion systems in early CNS development.
Phillips, D., University of California, San Francisco. The cell and molecular biology of platelets.
Wigler, M., Cold Spring Harbor Laboratory. Oncogenesis.
Hall, J., Brandeis University. *Drosophila* behavioral neurogenetics.
Sternberg, P., University of California, San Francisco. Nematode development.
Levine, M., Columbia University. Genetic control of *Drosophila* development.
Anderson, D., California Institute of Technology. The differentiation of chromafin cells.
Bronner-Fraser, Marianne, University of California, Irvine. The development of the peripheral nervous system.
Aldrich, R., Stanford University. The biophysics of ion flux.
Makowski, L., Columbia University. The structure and function of gap junctions.
Partick, J., Salk Institute. The molecular analysis of acetylcholine receptors.

Molecular Neurobiology of Human Disease

June 21–July 1

INSTRUCTORS

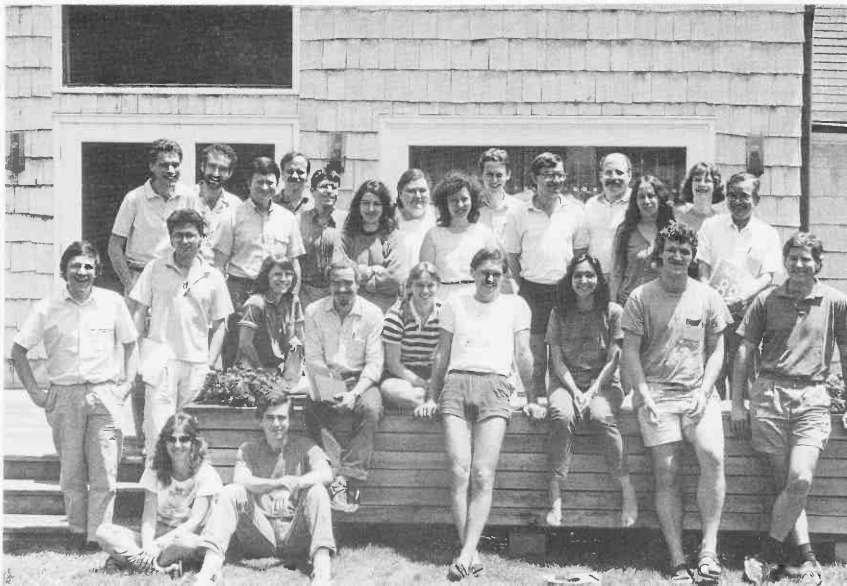
Black, Ira B., M.D., Cornell University Medical College, New York, New York
Breakefield, Xandra, Ph.D., E.K. Shriver Center, Harvard Medical School, Boston
Gusella, James, Ph.D., Massachusetts General Hospital, Boston

This intensive seminar course explores the molecular and cellular basis of abnormal neural function. It focuses on basic scientific studies that have provided insight into the etiology and pathogenesis of neurologic and psychiatric diseases. Emphasis is also placed on new techniques in neuroscience and molecular genetics that should provide additional insights. Topics include: Molecular pathology of neurotransmitter derangement; Developmental plasticity and choice of neurotransmitter phenotype; Synthesis and regulation of neuropeptides; Cellular

events in neural regeneration and brain transplantation; Neural pathways involved in pain syndromes; Genetic linkage analysis using DNA polymorphisms; Defects in DNA repair; Activation of *onc* genes and genetic homozygosity in neural tumors; Mutations causing the Lesch-Nyhan syndrome and possible means of gene therapy; Biochemistry of the lipidoses; Autoimmune diseases; Brain imaging and metabolism; Epilepsy and seizure disorders; Cell death in degenerative disorders; Viral infections of the nervous system; Experimental models of learning and memory.

PARTICIPANTS

Acsay, Vilmos, M.S., Hoffmann-La Roche, Basel, Switzerland
Amano, Takehiko, M.D., Mitsubishi-Kasei Institute, Tokyo, Japan
Bahro, Marcel, M.D., University of Zurich, Switzerland
Baron, Miron, M.D., New York State Psychiatric Institute, New York
Boni, Claudette, Ph.D., CNRS, Gif-Sur-Yvette, France
Campos, Ana R., M.A., Brandeis University, Waltham, Massachusetts
Chneiweiss, Herve, Ph.D., INSERM, Paris, France
Dorkins, Huw R., B.A., University of Oxford, England
Eshhar, Nomi, M.S., Weizmann Institute, Rehovot, Israel
Gheuens, Jan, M.D., Universitair Hospitaal, Antwerpen, Belgium
Gottlieb, Jacqueline, B.S., Yale University, New Haven, Connecticut
Jacob, William F., Ph.D., National Institutes of Health, Bethesda, Maryland
Kaufman, Charles, M.D., Columbia University, New York, New York
Lieman-Hurwitz, Judy, Ph.D., Hebrew University of Jerusalem, Israel
Lombroso, Paul, M.D., St. Vincents Hospital, New York, New York
Lynch, Sue A., M.D., North Carolina Memorial Hospital, Chapel Hill
Nance, Martha, M.D., University of Minnesota, Minneapolis
Naujoks, K., Ph.D., Boehringer Mannheim GmbH, Tutzing, Federal Republic of Germany
Pandolfo, Massimo, M.D., University of California, Irvine
Schlaug, Gottfried, M.D., Louisiana State Medical Center, New Orleans
Spigelman, Igor, M.S., University of British Columbia, Vancouver
Tashayyod, Dara, B.A., Medical College of Pennsylvania, Philadelphia
Turetsky, Dorothy, B.S., University of California, San Francisco



SEMINARS

Gravel, R., Hospital for Sick Children. Cloning of lysosomal enzymes.
Davies, P., Albert Einstein College of Medicine. Neuropathological and neurochemical features of Alzheimer disease.
Caskey, T., Baylor College of Medicine. Molecular defects of HPRT in the Lesch-Nyhan syndrome.
Dingledine, R., University of North Carolina Medical School. Epilepsy, kinddomg and ion channels.
McNamara, J.O., Durham VA Hospital–Duke University Medical Center. Epilepsy, kindling and ion channels.
Fields, H., University of California School of Medicine. Pain and neuropeptides.
Jessell, T.J., Harvard Medical School. Pain and neuropeptides.
Aguayo, A., Montreal General Hospital. Nerve regeneration.
Ray, P., Hospital for Sick Children. Duchenne's muscular dystrophy gene locus.
Milner, R., Research Institute of Scripps Clinic. Proteolipoprotein gene defect in *jimpy* mice.
Hood, L., California Institute of Technology. Correction of cyelin basic protein defect in transgenic *shiverer* mice and sequencing the human genome.
Sly, W., St. Louis University School of Medicine. Processing of lysosomal enzymes and role in lipidoses.
Cavenee, W., University of Cincinnati Medical School. Retinoblastoma.
Gallie, B., Hospital for Sick Children. Retinoblastoma.
Clements, J., Johns Hopkins University. Visna virus.
Gage, F., University of California, San Diego. Brain transplantation.
Schwarcz, R., Maryland Psychiatric Research Center. Huntington disease: Molecular and human aspects.
Wexler, N., Hereditary Disease Foundation. Huntington disease: Molecular and human aspects.
Gurney, M., University of Chicago. Motor neuron growth factor.
Caviness, V., Massachusetts General Hospital. Nuclear magnetic resonance.
Sejnowski, T., Johns Hopkins University. Speech.
Mishkin, M., National Institute of Mental Health. Memory.

Neurobiology of *Drosophila*

June 28–July 18

INSTRUCTORS

Bate, Michael, Ph.D., University of Cambridge, England
Jan, Lily Yeh, Ph.D., University of California, San Francisco
Jan, Yuh Nung, Ph.D., University of California, San Francisco

ASSISTANT

Hay, Bruce, University of California, San Francisco

Genetics has been used to approach questions in neurobiology that are otherwise difficult to address. *Drosophila* is an ideal organism for such studies. This laboratory/lecture course provides an introduction to current research in neuronal function and development in *Drosophila*. It is intended for researchers at all levels who may want to use *Drosophila* as an experimental system for studying neurobiology.

The course begins with a crash course on *Drosophila* genetics and other techniques which make *Drosophila* research distinctive, such as cytogenetics and DNA transformation. The main emphasis, however, is on studies of the nervous system.

The course covers basic electrophysiological techniques, as applied to mutants with altered ion channels or sensory physiology. It also includes mutant analysis of complex behaviors, such as courtship, circadian rhythm, learning and memory.

In the developmental section, processes of neurogenesis including determination and pathway formation are examined. The course familiarizes students with various preparations, including the embryonic nervous system, imaginal discs, and adult nervous system. It also reviews the different approaches being used in attempts to unravel the molecular basis of neural development.

PARTICIPANTS

Bastia, Deepak, Ph.D., Duke University, Durham, North Carolina
Drain, Peter F., B.A., Massachusetts Institute of Technology, Cambridge
Heberlein, Ulrike A., M.S., University of California, Berkeley
Hortsch, Michael, M.S., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany
Krantz, David E., B.S., University of California School of Medicine, Los Angeles
Nedivi, Elly, B.S., Stanford University, California
O'Kane, Cahir, Ph.D., University of Basel, Switzerland
Siegler, Melody, Ph.D., Emory University, Atlanta, Georgia
Singh, Satpal, Ph.D., University of Iowa, Iowa City
Yovell, Yoram, B.S., Weizmann Institute, Rehovot, Israel

SEMINARS

Ganetzky, B., University of Wisconsin. *Drosophila* genetics.
Wu, C.-F., University of Iowa. Ionic channels in *Drosophila*.
Rubin, G., University of California, Berkeley. Molecular studies of neural development.
Heisenberg, M., Institut fur Genetik und Mikrobiologie. Structural brain mutants and behavior.
Weischaus, E., Princeton University. Genes involved in cellularization and gastrulation in *Drosophila*.
Ready, D., Princeton University. Eye development and mutants of eye development.
Campos-Ortega, J., Universitat Koln. Early neurogenesis in *Drosophila*.
Technau, G., Universitat Koln. Lineage analysis of transplanted individual cells in *Drosophila* embryos.
Quinn, W., Massachusetts Institute of Technology. Learning mutants.
Goodman, C., Stanford University. Cellular and molecular studies of axon guidance.
Hall, J., Brandeis University. Circadian rhythm and courtship mutants.
Murphy, R.K., State University of New York, Albany. Sensory neuron projections in *Drosophila*.

Advanced Techniques in Molecular Cloning

June 28–July 17

INSTRUCTORS

Zoller, Mark, Ph.D., Cold Spring Harbor Laboratory, New York
Brosius, Jurgen, Ph.D., Columbia University, New York
Cate, Richard, Ph.D., Biogen, Cambridge, Massachusetts
Gubler, Ueil, Ph.D., Hoffmann La-Roche, Nutley, New Jersey
Henikoff, Steve, Ph.D., Fred Hutchinson Cancer Center, Seattle, Washington
MacGillivry, Ross, Ph.D., University of British Columbia, Vancouver
Schatzman, Allan, Ph.D., Smith, Kline & Beckman, King of Prussia, Pennsylvania
Urdea, Micky, Ph.D., Chiron Corp., Emoryville, California
Wallner, Barbara, Ph.D., Biogen, Cambridge, Massachusetts

This is a laboratory and lecture course on important aspects of molecular cloning. The primary focus is aimed at the use of oligonucleotides and antibodies to isolate the gene coding for a specific protein and the subsequent characterization and manipulation of the cloned gene. The experiments consist of construction of cDNA libraries, screening of libraries with oligonucleotides and antibodies, DNA sequencing, probing Northern and Southern blots with oligonucleotides, expression of foreign genes in E. coli, gene synthesis, oligonucleotide directed mutagenesis using phagemid vectors, and the use of computers for DNA and protein sequence analysis. Instructors present the application of these techniques to the analysis of cloned genes and discuss alternative approaches not covered in the laboratory section.

PARTICIPANTS

Carmack, Condie E., Ph.D., Institute for Cancer Research, Philadelphia, Pennsylvania
Dinsmore, Jonathan H., B.S., Dartmouth College, Hanover, New Hampshire
Eng, John, M.D., Veterans Hospital, Bronx, New York
Ennulat, David J., Ph.D., New York University Medical Center, New York
Hasemann, Charles, B.S., University of Texas Health Science Center, Dallas
Hogarth, P. Mark, Ph.D., University of Melbourne, Australia
Jahn, Reinhard, Ph.D., Max-Planck-Institute, Martinsried, Federal Republic of Germany
Lannigan, Deborah A., Ph.D., University of Rochester, New York
Larsson, Gunilla K., M.S., Chemical Center, Sweden
Lauerman, Tod W., B.A., Johns Hopkins University, Baltimore, Maryland
Parnell, Laurence D., B.S., University of Wisconsin, Madison
Pouyssegur, Jacques, Ph.D., CNRS, Nice, France
Qian, Su Wen, B.S., Shanghai Institute, Republic of China
Schwander, Juerg C., M.D., Kantonsspital, Basel, Switzerland
Yellen, Gary I., Ph.D., Johns Hopkins University, Baltimore, Maryland
Yonamoto, Wes, Ph.D., University of California, San Diego

SEMINARS

Helfman, D., Cold Spring Harbor Laboratory. Molecular aspects of muscle and non-muscle tropmyosins.
Pielak, G., University of Oxford. Analysis of mutated proteins by two-dimensional NMR.
Summers, M., Texas A&M University. Baculovirus cloning system.
Schatzman, A., Smith, Kline & Beckman. Cloning and expression of human alpha-1 antitrypsin in *E. coli*.
Cate, R., Biogen. Mullerian inhibiting substance.
Henikoff, S., Fred Hutchinson Cancer Research Center. Peculiar organization of *Drosophila* genes.
Arasu, P., New England Biolabs. Lymphatic filariasis: Molecular cloning and characterization of antigen.

Molecular and Developmental Biology of Plants

June 28–July 17

INSTRUCTORS

Sussex, Ian, Ph.D., Yale University, New Haven, Connecticut
Messing, Joachim, Ph.D., Waksman Institute, Rutgers University, Piscataway, New Jersey
Horsch, Robert, Ph.D., Monsanto Company, St. Louis, Missouri

ASSISTANTS

Chereskin, Barbara, Ph.D., Rutgers University, Piscataway, New Jersey
Das, Prem, Rutgers University, Piscataway, New Jersey
Hinchee, Maud, Monsanto Company, St. Louis, Missouri
Kerk, Nancy, B.S., Yale University, New Haven, Connecticut
Solberg, Lorraine, Yale University, New Haven, Connecticut
Syzmkowaik, Eugene, Yale University, New Haven, Connecticut

This course provides an intensive overview of current topics and techniques in plant biology, with emphasis on molecular and developmental biology and genetics. It is designed for scientists with working knowledge of molecular techniques who are either working with plant systems or wish to. The course consists of a rigorous lecture series, a hands-on laboratory, and informal discussions. Different guest speakers provide both an in-depth discussion of their work and an overview of their specialty as well as informal discussions after their seminars. The laboratory covers established and novel techniques in plant biology, including plant structure and development, nucleic acid manipulations, gene transfer techniques, tissue and cell culture techniques, photosynthesis, and genetics and cytogenetics of maize.

PARTICIPANTS

Bachmair, Andreas, Ph.D., Massachusetts Institute of Technology, Cambridge
Caelles, M. Carme, B.A., University of Barcelona, Spain
Cardarelli, Maura, Ph.D., University "La Sapienza," Rome, Italy
DeMoor, Janice M., M.S., Agriculture Canada, Ottawa
Foley, Kevin P., B.A., Wallenberg Traditional High School, San Francisco, California
Gousseau, Helene D. M., Ph.D., University of Calgary, Canada
Holwerda, Barry C., M.S., University of Saskatchewan, Canada
Hsu, Francis, Ph.D., E. I. DuPont de Nemours & Co., Wilmington, Delaware
Keierleber, Carolyn, B.S., University of California, Irvine
Luehrsen, Kenneth R., Ph.D., Stanford University, California
Niblett, Charles L., Ph.D., University of Florida, Tallahassee
San Francisco, Michael J., Ph.D., University of Maryland, College Park
Schalk, Joachim, B.S., Max-Planck-Institut, Koln, Federal Republic of Germany
Stout, Richard G., Ph.D., Montana State University, Bozeman
Weigel, Detlef, B.S., Max-Planck-Institut, Tubingen, Federal Republic of Germany
Wright, Rhonda L., M.S., University of Missouri, Columbia

SEMINARS

Horsch, R., Monsanto Company. The leaf disk transformation system.
Sommerville, C., Michigan State University. Biochemical mutants of Arabidopsis.
Nasrallah, J., Cornell University. Self incompatibility in Brassica.
Beachy, R., Washington University. Studies of TMV coat protein in transgenic plants.
Lamb, C., Salk Institute. Pathways in plant pathogens interaction.
Rogers, J., Washington University. Differential gene expression in barley aleurone tissue.
Ho, D., Washington University. Plant hormones and growth regulators.
Staskawizc, B., University of California, Berkeley. Microbial genes involved in plant pathogenesis.
Wessler, S., University of Georgia. Transposable elements in maize.
Tanksley, S., Cornell University. Mapping plant genomes with RFLP.
Bevan, M., Plant Breeding Institute. Potatin gene expression using glucoronidase gene fusions.
Gronenborn, B., Max-Planck Institute. Wheat dwarf virus replication.
Ahlquist, P., University of Wisconsin. In vitro genetics of brome mosaic virus.
Messing, J., Rutgers University. High methionine corn.
Fink, G., Whitehead Institute. Mutants in amine acid biosynthesis.
Bogorad, L., Harvard University. Structure and function of the chloroplast genome.
Hanson, M., Cornell University. Mitochondrial genes in plants.

Cell and Molecular Biology of Learning and Memory

July 10–July 24

INSTRUCTORS

Byrne, Jack, Ph.D., University of Texas Medical School, Houston
Kandel, Eric, M.D., Columbia University, New York, New York
Nottebohm, Fernando, Ph.D., Rockefeller University, New York, New York
Pearson, Keir, Ph.D., University of Alberta, Canada

This lecture course provides an introduction to cell and molecular biological approaches to learning and memory. It is suited for graduate students in molecular biology, neurobiology and psychology as well as research workers who are interested in an introduction to this new field. The course covers topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. Rather than an extensive survey, the lectures provide an intensive coverage of four selected areas: 1) an introduction to modern behavioral studies of learning and memory; 2) the cell biology of neuronal plasticity and the regulation of gene expression by experience; 3) cellular and molecular mechanisms of simple forms of learning and memory in invertebrates and vertebrates; and 4) neural approaches to cognition as evident in studies of communication and language. To put the cellular work on learning into perspective, selected examples are taken from human behavior and its abnormalities.

PARTICIPANTS

Amano, Takehiko, Ph.D., Mitsubishi-Kasei Institute, Tokyo, Japan
Aztely, F., University of Göteborg, Sweden
Beers, David Robert, B.S., University of Utah, Salt Lake City
Bonhoeffer, Tobias, M.S., Max-Planck-Institute, Tuebingen, Federal Republic of Germany
Brown, Glen D., B.A., University of Iowa, Iowa City
Burrous, Mary R., B.A., Rockefeller University, New York, New York
Christenson, Johan, B.A., Karolinska Institute, Stockholm, Sweden
Cooper, Lee D., Ph.D., New York State Psychiatric Institute, New York
Eliot, Lise S., B.A., New York State Psychiatric Institute, New York
Gomez, Hortensia Gonzalez, M.S., CILVESTAV-IPN, Mexico City
Hanse, Eric L., B.S., University of Göteborg, Sweden
Jacobs, Kimberle M., B.A., Brown University, Providence, Rhode Island
Kano, Masanobu, Ph.D., Jichi Medical School, Tochigi-Ken, Japan
Keller, Hansjorg, B.S., University of Zurich, Switzerland
Lockery, Shawn R., B.A., University of California, San Diego
Marcus, Emilie A., B.A., Yale University, New Haven, Connecticut
Middleton, Pamela, M.A., Columbia University, New York, New York
Nilsson, Ola G., B.S., University of Lund, Sweden
O'Malley, Edward K., B.S., Cornell University, New York, New York
Sato, Thomas, B.S., Georgetown University, Washington, D.C.
Sinclair, Alison, Ph.D., Children's Hospital, Boston, Massachusetts
Taylor, Anne J., B.S., Boston University, Massachusetts
Terlau, Heinrich, M.S., Max-Planck-Institute, Gottingen, Federal Republic of Germany
Yun, Yungdae, B.S., Michigan State University, East Lansing

SEMINARS

Rescorla, R., University of Pennsylvania. Introduction to learning theory.
Gould, J., Princeton University. Ethological approaches to learning.
Wyman, R., Yale University. Introduction to the study of genes and behavior.
Knapp, M., Columbia University. Cloning of genes important for learning.
Schwartz, J., Columbia University. Overview of second messenger systems and their role in learning and memory.
Adler, J., University of Wisconsin. Genes and the behavior of bacteria.
Kung, C., University of Wisconsin. Genes, ion channels, and behavior of paramecium, yeast, and *E. coli*.
Quinn, J., Massachusetts Institute of Technology. Genetic approaches to study associative learning in *Drosophila*.

Thompson, R., Stanford University. Cellular locus and correlates of the classical conditioning of the nictitating membrane response.
Lisberger, S., University of California, San Francisco. Plasticity in the vestibulo-occular reflex.
Thach, T., Washington University. Neuronal correlates of motor learning.
Bliss, T., National Institute for Medical Research. Presynaptic mechanisms in long-term potentiation.
Stevens, C., Yale University. Postsynaptic mechanisms in long-term potentiation.
Berger, T., University of Pittsburgh. Role of long-term potentiation in learning.
Black, I., Cornell University. Modulation of gene expression in the vertebrate nervous system.
Squire, L., University of California, San Diego. Memory in humans and non-human primates.
Price, D., Johns Hopkins University. Alzheimer's diseases.

Immunoglobulins: Molecular Probes of the Nervous System

July 20–August 9

INSTRUCTORS

Levitt, Pat, Ph.D., Medical College of Pennsylvania, Philadelphia
Evans, Christopher, Ph.D., Stanford University, California

PART-TIME INSTRUCTORS

Lafenaur, Carl, Ph.D., University of Pittsburgh School of Medicine, Pennsylvania
Pintar, John, Ph.D., Columbia University College of Physicians & Surgeons, New York, New York
Carlson, Steve, Ph.D., University of Washington, Seattle
Hockfield, Susan, Ph.D., Yale University School of Medicine, New Haven, Connecticut

ASSISTANT

Barbe, Mary, Ph.D., Medical College of Pennsylvania, Philadelphia

Antibodies are invaluable probes used extensively in neurobiological research. This course is designed to provide an advanced understanding of the power and pitfalls of immunoglobuiins as biochemical and anatomical reagents. Through laboratory work participants explore many immunotechniques including generation of monoclonals, preparation and characterization of antibodies to synthetic peptides and complex antigens, immunohistochemistry, immunoassays, affinity purification, western blotting, antibody conjugation interfacing immunoassays with HPLC, *In Vitro* education and *in situ* hybridization.

PARTICIPANTS

Benfenati, Fabio, M.D., Rockefeller University, New York, New York
Born, Donald E., Ph.D., University of Washington, Seattle
Cisse, Soriba, M.S., INRS-Sante, Montreal, Canada
Cserr, Helen F., Ph.D., Brown University, Providence, Rhode Island
Hevner, Robert F., B.S., Medical College of Wisconsin, Milwaukee
Hsu, Hsiao-Lan, B.A., Johns Hopkins University, Baltimore, Maryland
Koroshetz, Walter J., M.D., Massachusetts General Hospital, Boston, Massachusetts
Rizzo, Joseph, M.D., Massachusetts Eye and Ear Infirmary, Boston, Massachusetts
Taylor, Marianne, B.S., University of Calgary, Canada
Teixeira, Gerlinda, B.S., Universidade Federal, Niterio, Brazil
Tiffany Tsui, Ho-Ching, Ph.D., Washington University Medical School, Seattle
Volpe, Bruce T., M.D., New York Hospital-Cornell Medical Center, New York

SEMINARS

Steiner, L., Massachusetts Institute of Technology. Introduction to Immunoglobulins.
Woodland, R., University of Massachusetts Medical Center. B cells and cellular immunology.
Janeway, C., Yale University School of Medicine. T-cells and the immune response.
Scharff, M., Albert Einstein College of somatic mutation and the generation of better hybridomas.
Alt, F., Columbia University College of Physicians & Surgeons. Ig gene rearrangements.
Levitt, D., Hoffman-La Roche. Interleukins and the immune response.
Lindstrom, J., Salk Institute. Monoclonal antibodies as probes of the acetylcholine receptor.
Lagenaur, C., University of Pittsburgh School of Medicine. Cell surface molecules and nerve cell development.
Barald, K., University of Michigan Medical School. No-flow cytometry as a tool to study neural crest development.
Milner, T., Cornell University Medical College. EM immunocytochemistry.

Molecular Cloning of Eukaryotic Genes

July 20–August 9

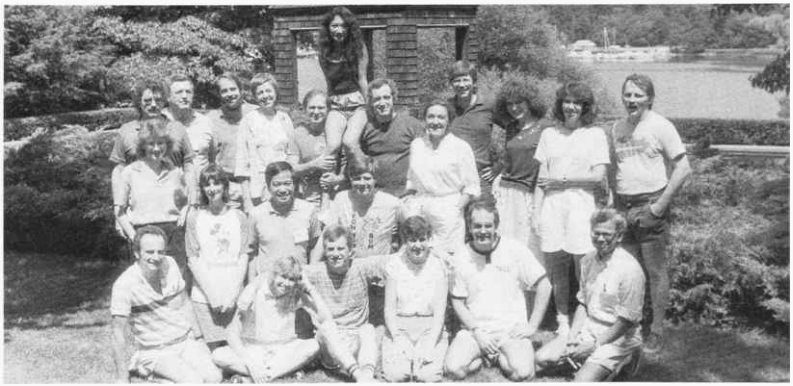
INSTRUCTORS

Alt, Fred, Ph.D., Columbia University, New York, New York
Bothwell, Al, Ph.D., Yale University, New Haven, Connecticut
Roberts, Thomas, Ph.D., Harvard University, Cambridge, Massachusetts

ASSISTANTS

Okada, Ami, B.S., Columbia University, New York, New York
Callum, Robert, B.S., Columbia University, New York, New York
Carbleg, Michael, B.S., Harvard University, Cambridge, Massachusetts

This laboratory and lecture course covers 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 are emphasized. Among the topics covered are: construction of cDNA libraries in plasmid or bacteriophage λ vectors, construction of bacteriophage λ and cosmid libraries of high-molecular-weight eukaryotic DNA, screening DNA libraries with gene-specific hybridization probes, purification and characterization of recombinant clones using restriction endonuclease and blot hybridization analyses, and reintroduction and expression of cloned genes in heterologous systems. Strategies for isolating genes that encode rare mRNA sequences are discussed. Guest lecturers discuss the application of molecular cloning procedures to the study of specific eukaryotic gene systems.



PARTICIPANTS

Cass, Carol E., Ph.D., University of Alberta, Canada
Cavanaugh, Colleen M., Ph.D., Harvard University, Cambridge, Massachusetts
Cerni, Christa M., M.D., University of Vienna, Austria
Chaganti, Raju S., Ph.D., Memorial Sloan Kettering Cancer Center, New York, New York
D'Amore, Patricia A., Ph.D., The Childrens Hospital, Boston, Massachusetts
Dennert, Gunther, Ph.D., University of Southern California, Los Angeles
Ernberg, Ingmar T., Ph.D., Karolinska Institutet, Stockholm, Sweden

Fu, Shu Man, Ph.D., Oklahoma Medical Research Foundation
Glukhova, Marina, Ph.D., Institute of Cardiology, Moscow, USSR
Kaplan, Jerry, Ph.D., University of Utah, Salt Lake City
Kincade, Paul W., Ph.D., Oklahoma Medical Research Foundation, Oklahoma City
Latt, Samuel A., Ph.D., Harvard Medical School, Boston, Massachusetts
Pratt, Melanie M., Ph.D., University of Miami, Florida
Seaman, William E., M.D., VA Medical Center, San Francisco, California
Smith, Frances I., Ph.D., Mt. Sinai School of Medicine, New York, New York
Thompson, Linda F., Ph.D., Scripps Clinic & Research Foundation, La Jolla, California

SEMINARS

Hood, L., California Institute of Technology. Structure and function of antigen receptor genes.
——. Biotechnology
Lutzker, S., and P. Ferrier, Columbia University. Gene transfer into mammalian cells.
Mulligan, R., Whitehead Institute. Use of retrovirus vectors to analyze Hematopoietic differentiation.
Cantor, C., Columbia University. New techniques for human rnolecular genetics.
Hanahan, D., Cold Spring Harbor Laboratory. Oncogene function in transgenic mice.
Moss, B., National Institutes of Health. Vaccinia virus: Regulation of transcription and use as an expression vector.
Alt, F., and G. Yancopoulos, Columbia University. Hybridization techniques.
Goff, S., Columbia University. Definition of retrovirus genes by in vitro mutagenesis.
Schimke, R., Stanford University. Gene amplification, Part 1.
——. Gene amplification, Part 2.
Snider, M., Yale University. Use of gt11 to clone and study yeast gene products: Centromere binding proteins.
Wigler, M., Cold Spring Harbor Laboratory. Structure and function of cellular oncogenes.
Davis, M., Stanford University. T-cell receptor and XLR gene families.
Tucker, P., Southwestern Medical School. Regulation of Ig isotype expression.
McNight, S., Carnegie Institute. Regulation of transcription.
Pintar, J., Columbia University. Use of in situ hybridization to study mammalian development.
Kellems, R., Baylor University. The adenosine deaminase gene and gene therapy.
Kaufman, R., Genetics Institute. Molecular genetics of Factor VIII.
Rosenberg, M., Smith, Kline and French. Expression of proteins in *E. coli*.

Yeast Genetics

July 20–August 9

INSTRUCTORS

Sherman, Fred, Ph.D., University of Rochester, New York
Winston, Fred, Ph.D., Harvard Medical School, Boston, Massachusetts
Rose, Mark, Ph.D., Princeton University, New Jersey
Fink, Gerald, Ph.D., Whitehead Institute, Cambridge, Massachusetts

PART-TIME INSTRUCTOR

Hicks, James, Ph.D., Research Institute of Scripps Clinic, La Jolla, California

ASSISTANTS

Rose, Alan, M.S., Princeton University, New Jersey
Swanson, Michele, B.A., Harvard Medical School, Boston, Massachusetts

The major laboratory techniques used in the genetic analysis of yeast are 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 is carried out by all students. Recombinant DNA techniques, including yeast transformation, filter hybridization, and gel electrophoresis are applied to cloning and genetic

analysis of yeast DNA. Lectures on fundamental aspects of yeast genetics are presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Adair, W. Lee, Ph.D., University of South Florida, Tampa
Alfano, Christine L., B.S., Johns Hopkins University, Baltimore, Maryland
Cellini, Allessandra, Ph.D., Cell Biology Institute, Rome, Italy
Dranginis, Anne M., Ph.D., NIDDK, National Institutes of Health, Bethesda, Maryland
Goyer, Charles, M.S., McGill University, Montreal, Canada
Jakubowski, Heironim, Ph.D., UMDNJ–New Jersey Medical School, Piscataway
Kallal, Lorena A., B.S., University of Notre Dame, Indiana
Kambouris, Nicholas G., B.A., University of Virginia, Charlottesville
Kosman, Daniel J., Ph.D., State University of New York, Buffalo
Lieberman, Howard B., Ph.D., Columbia University College of Physicians & Surgeons, New York, New York
Morgan, Edward A., Ph.D., Roswell Park Memorial Institute, Buffalo, New York
Perfect, John R., M.D., Duke University, Durham, North Carolina
Picksley, Steven, Ph.D., Potters Bar, Imperial Cancer Research Fund, England
Ruggieri, Rosamaria, M.S., Weizmann Institute of Science, Rehovot, Israel
Thoma, Fritz, Ph.D., Institut for Zellbiologie, Zurich, Switzerland
Tien, Ming, Ph.D., Pennsylvania State University, University Park

SEMINARS

Petes, T., University of Chicago. Meiotic recombination in yeast: The rules of the game.
Warner, J., Albert Einstein College of Medicine. Ribosomal genes: Structure and regulation.
Guarente, L., Massachusetts Institute of Technology. DNA binding and transcriptional activation.
Hinnebusch, A., National Institutes of Health. Translational control of GCN4.
Wigler, M., Cold Spring Harbor Laboratory. Control of cell proliferation in yeast.
Carlson, M., Columbia University. Glucose repression involves a protein kinase.
Sternglanz, R., State University of New York, Stony Brook. DNA topoisomerase and histone acetyltransferase.
Klar, A., Cold Spring Harbor Laboratory. The yeast sex.
Pringle, J., University of Michigan. Cellular morphogenesis in yeast.
Broach, J., Princeton University. Plasmid propagation in yeast.
Fox, T., Cornell University. Nuclear control of translation of mitochondrial mRNAs.
Herskowitz, I., University of California, San Francisco. Cell specialization in yeast.
Douglas, M., University of Texas Health Science Center. Mitochondrial import in yeast.
Schekman, R., University of California, Berkeley. Protein localization in yeast.

Computational Neurosciences: Vision

July 26–August 9

INSTRUCTORS

Movshon, J. Anthony, Ph.D., New York University, New York
Hildreth, Ellen C. Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANTS

Clemens, David, M.S. Massachusetts Institute of Technology, Cambridge
Horswill, Ian, B.S., Massachusetts Institute of Technology, Cambridge

Computational approaches to problems in neuroscience have produced important advances in our understanding of neural processing. The most prominent successes have come in areas where strong inputs from neurobiological, behavioral and computational approaches can interact. Through a combination of lectures and hands-on experience with a computer laboratory, this intensive course examines several areas of vision including motion analysis, binocular stereo and color. The theme is that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience. Students should have experience in neurobiological or computational approaches to visual processing. A strong background in mathematics will be beneficial.

PARTICIPANTS

Bornstein, Yael, M.S., Weizmann Institute, Rehovot, Israel
Bracewell, Robert M., B.A., University of California, San Diego
Brainard, David H., B.A., Stanford University, California
Britten, Kenneth H., Ph.D., State University of New York, Stony Brook
Deno, Curtis D., M.D., Smith Kettlewell Eye Research Foundation, San Francisco, California
Downing, Cathryn J., Ph.D., New York University, New York
Edelman, Shimon, M.S., Weizmann Institute, Rehovot, Israel
Ferrera, Vincent P., B.A., University of Chicago, Illinois
Gallant, Jack, M.S., Yale University, New Haven, Connecticut
Johnston, Elizabeth, M.A., Oxford University, England
Kaley, Ainat, M.S., Hebrew University Givat-Ram, Jerusalem, Israel
Kiorpes, Lynne, Ph.D., New York University, New York
Krauzlis, Richard J., A.B., University of California, San Francisco
McLean, Judith, B.A., University of Pennsylvania, Philadelphia
Mowafy, Lyn K., M.A., University of Arizona, Tucson
Noble, Alison J., B.A., Robotics Research Group, Oxford, England
Poirson, Allen B., B.A., Stanford University, California

Rensink, Ron A., M.S., University of British Columbia, Vancouver
Royden, Constance S., B.S., University of California, San Francisco
Snowden, Robert J., B.S., University of Cambridge, England
Thoreson, Wallace B., B.A., University of Minnesota, Minneapolis
Van Hateren, Hans J. H., M.D., Groningen University, Federal Republic of Germany
Zimmerman, George L., B.S., University of Minnesota, Minneapolis
Zohary, Ehud, B.S., Hebrew University, Jerusalem, Israel

SEMINARS

Andersen, R., Massachusetts Institute of Technology. Physiological studies of motion analysis.
Lennie, P., University of Rochester. Psychophysical and physiological studies of color vision.
Maunsell, John, University of Rochester. Task dependent neural behavior. Neural correlates of visual attention and recognition.
Medioni, G. University of Southern California. Computational models of binocular stereo.
Morgan, M., University College London. The psychophysical study of early visual processing.
Nakayama, K., Smith-Kettlewell Eye Research Foundation. The measurement of motion in human vision.
——. Integrating multiple visual cues and the role of visual attention.
Poggio, T., Massachusetts Institute of Technology. The neural implementation of visual computations.
——. A unified theoretical framework for early vision and the integration of multiple visual processes.
Sejnowski, T., Johns Hopkins University. Neural networks for visual computation.
Thompson, W., University of Minnesota. Computational models of motion measurement.
Ullman, S., Massachusetts Institute of Technology and Weizmann Institute of Science. Higher-lever vision: Computing spatial relation, visual routine and object recognition.
Wandell, B., Stanford University. The analysis of color.

Molecular Cloning of Neural Genes

August 11–August 30

INSTRUCTORS

Eberwine, James, Ph.D., Stanford University, California
Evinger, Marian, Ph.D., Cornell University Medical College, New York, New York

PART-TIME INSTRUCTORS

Margolskee, Robert, Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey
Schachter, Beth, Ph.D., Mt. Sinai Medical School, New York, New York

ASSISTANTS

Fox, Susan, Ph.D., Rockefeller University, New York, New York
Inman, Irene, B.S., Stanford Medical Center, California

This intensive laboratory/lecture course is intended to provide scientists with a basic knowledge of the use of the techniques of molecular biology in examining questions of neurobiological significance. Particular emphasis is placed on using and discussing methods for circumventing the special problems posed in the study of the nervous system, for example, examination of low abundance mRNAs in extremely heterogeneous cell populations.

The laboratory work includes mRNA quantitation techniques, preparation of hybridization probes, cloning techniques (plasmid, λgt11 and Okayama-Berg), colony screening techniques (probe hybridization, antibody interaction and activity assays), DNA sequencing, eukaryotic cell transfection and *in situ* hybridization.

The lecture series, by invited speakers, focuses on how molecular biology techniques can be used to supplement more standard neurobiological tools in examining the nervous system. Topics include: use of transgenic mice in neurobiological studies, cloning of neurotransmitter receptors and use of simple model systems in the study of the mammalian nervous system.

PARTICIPANTS

Bendotti, Caterina, Ph.D., Johns Hopkins University, Baltimore, Maryland
Boyle, Mary B., Ph.D., Yale University, New Haven, Connecticut
Coleman, Paul D., Ph.D., University of Rochester, New York
D'Ambrosio, Steven M., Ph.D., Ohio State University, Columbus
Douville, Philippe, B.S., Montreal General Hospital, Canada
Elzinga, Marshall, Ph.D., Brookhaven National Laboratory, Upton, New York
Erdos, Joseph J., Ph.D., Connecticut Mental Health Center, New Haven
Girault, Jean-Antoine, Ph.D., Rockefeller University, New York, New York
Horton, Heather L., B.S., Medical College of Pennsylvania, Philadelphia
Jones, Kathryn J., Ph.D., Chicago Medical School, Illinois
McKinney, Michael, Ph.D., Abbott Laboratories, Abbott Park, Illinois
Melrose, Patricia A., Ph.D., University of Rochester, New York, New York
Schafer, Martin K-H., M.D., Mental Health Research Institute, Ann Arbor, Michigan
Scotting, Paul J., Ph.D., University of Cambridge, England
Soumoff, Cynthia, Ph.D., University of Tennessee, Oak Ridge
Weber, Michel J., Ph.D., CNRS, Toulouse, France

SEMINARS

Chao, M., Cornell University Medical College. Gene transfer and molecular cloning of mammalian cell receptors.
Hahn, W., University of Colorado Medical Center. Perspectives and targets useful in developing strategies for the establishment of the structure and function of brain specific proteins.
Knapp, M., Columbia University College of Physicians & Surgeons. Approaches to the cloning of genes important for the establishment or maintenance of brain specific proteins.
Helfman, D., Cold Spring Harbor Laboratory. Immunological screening methods for expression libraries.
Comb, M., Massachusetts General Hospital. Mechanisms of transsynaptic regulation of gene expression.
Chikaraishi, D., Tufts University School of Medicine. Expression of catecholamine genes.
Watson, S., University of Michigan School of Medicine. Applications of in situ hybridization in the study of the CNS.
Tobin, A., University of California, Los Angeles. Structural and developmental regulation of brain glutamate decarboxylase.
Draft, C., VA Medical Center, Dallas. Isolation and characterization of retinal/pineal cDNA encoding S antigen.
Higgins, G., Scripps Clinic and Research Foundation. In situ hybridization shows regulated expression of amyloid beta protein mRNA in Alzheimer's Disease.
Carroll, J., Cornell University Medical College. DNA sequencing methodologies.
Claudia, T., Yale University. Gene transfer techniques.
Hanahan, D., Cold Spring Harbor Laboratory. Use of transgenic mice in neurobiological studies.
Hoffman, A., Stanford University School of Medicine. Hormonal control of pituitary peptide hormone gene expression.

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.

1987

January

Richard Selinfreund, Los Alamos National Laboratories, New Mexico: A possible role for calmodulin in the transduction of the mitogenic signal.

Wai-Kit Chan, Baylor College of Medicine, Waco, Texas: Membrane insertion and topography of the nonstructural glycoprotein of simian rotavirus SA11.

Robert Eisenman, Fred Hutchinson Research Center, Seattle, Washington: Proteins encoded by the c-*myc* oncogene.

Nigel Crawford, Stanford University Medical Center, California: Molecular biology of plant nitrate reductase.

Carol St. Angelo, Memorial Sloan-Kettering Cancer Center, New York, New York: Influenza virus polymerase protein complexes in baculovirus expression vectors and in influenza-virus-infected cells.

February

David Thaler, University of Oregon, Eugene: Red recombination pathway in bacteriophage λ.

Susan Rosenberg, University of Oregon, Eugene: Chi recombination hot spots: New thoughts on old sequence.

Brian Cullen, Hoffman-La Roche, Nutley, New Jersey: *Trans*-activation of HIV LTR is mediated by nuclear events.

Morris Birnbaum, Memorial Sloan-Kettering Cancer Center, New York, New York: Molecular regulation of glucose transport.

Michael David Marks, Zoecon Research Institute, Palo Alto, California: Development of a novel transformation procedure for *Arabidopsis phaliana*: A model plant for the study of plant molecular biology.

Ed Ziff, New York University Medical Center, New York: Gene regulation by nerve growth factor.

Ursula Vogel, University of Cambridge, England: Molecular cloning of CNPase: A myelin-specific protein.

Maynard Olson, Washington University School of Medicine, St. Louis, Missouri: Physical mapping of yeast chromosomes.

Darryl Rideout, Research Institute of Scripps Clinic, La Jolla, California: Self-assembling cytotoxins.

March

Joyce Fox, Long Island Jewish Medical Center, New Hyde Park, New York: Molecular diagnosis of ornithine transcarbamylase deficiency.

Jean-Francois Brunet, Centre de Immunologie, Marseille, France: Cytotoxic T-lymphocyte-associated transcripts.

Charles Cantor, Columbia University College of Physicians & Surgeons, New York, New York: Determining the structure of the human genome.

Gary Struhl, Columbia University College of Physicians & Surgeons, New York, New York: Genes controlling segment determination in *Drosophila*.

Cori Bargmann, Whitehead Institute, Cambridge, Massachusetts: Activation of the *NEU* oncogene.

Jenine LeBlanc, Wesleyan University, Middletown, Connecticut: Characterization and localization of the 7SL RNA and signal recognition particle in the developing sea urchin.

Joan Brugge, State University of New York, Stony Brook: Expression of the cellular *src* proto-oncogene in normal cells.

Tom Schneider, University of Colorado, Boulder: The information content of binding sites on nucleotide sequences.

April

Cecile Butor, EMBL, Heidelberg, Federal Republic of Germany: Glycolipid polarity in MDCK cells.

Moshe Shani, Weizmann Institute, Rehovot, Israel: Expression of rat muscle specific genes introduced into pluripotent stem cells and mouse germ cells.

Nikki Harter, New Jersey Medical School, Piscataway: Purification and biological characterization of an adenovirus type-2 E1A protein expressed in *E. coli*.

Fred Goldberg, Harvard Medical School, Boston, Massachusetts: Selective degradation of abnormal proteins in animal and bacterial cells.

Keith Joho, State University of New York Downstate Medical School, New York: Identification of a region in the T3 and T7 RNA polymerases that is required for specific promoter recognition.

Carlo Tacchetti, National Institute for Cancer Research, Genova, Italy: In vitro morphogenesis of chick embryo cartilage.

Mark Ptashne, Harvard University, Cambridge, Massachusetts: Gene control in bacteria and yeast.

Manuel Ares, Yale University, New Haven, Connecticut: U2 genes of man and yeast.

Don Rio, Whitehead Institute, Cambridge, Massachusetts: Regulation of *Drosophila* P-element transposition

May

Dan DiMaio, Yale University, New Haven, Connecticut: Genetic analysis of bovine papilloma virus transforming genes.

Benjamin Rich, Yale University, New Haven, Connecticut: Characterization of genes encoding human ribosomal phosphoproteins.

Florence Tsui, Toronto Western Hospital, Canada: Molecular studies on the gene for mammalian histidyl tRNA synthetase.

Fritz Melchers, Basel Institute, Switzerland: B-cell-cycle control.

Greg Conway, Vanderbilt University, Nashville, Tennessee: In vitro RNP assembly suggests a ribonucleosomal architecture for pre-mRNA.

June

Seth Stern, University of California, Santa Cruz: Protein-RNA interactions in the 30S subunit form a basis for the three-dimensional structure of 16S RNA.

Jean Thierry, NCI, Bethesda, Maryland: Control of cytoplasmic Ca^{++} concentration by inositol phosphates and guanine nucleotides in eukaryotic cells.

July

Ulrike Heberlein, University of California, Berkeley: In vitro transcription of developmentally regulated genes in *Drosophila*.

Jergen Behreus, Max-Planck Institut, Tubingern, Federal Republic of Germany: Epithelial cells acquire invasive properties following disturbance of cell-cell adhesion.

September

Paul Lambert, NCI, Bethesda, Maryland: Negative regulation of BPV I transcription.

Frank McCormick, Cetus Corporation, Emoryville, California: Structure and function of RAS proteins.

Mike McClelland, University of Chicago: Restriction endonucleases for pulsed-field gel electrophoresis.

October

Mike Fried, ICRF, London, England: The use of expression-selection for the study of gene regulation in mammalian cells.

Arne Stenlund, University of California, Berkeley: The role of transcriptional elements in the replication of BPV I.

Dominique Belin, University of Geneva, Switzerland: Molecular control of proteolytic activity in mouse male and female genetics.

Julian Downward, Whitehead Institute, Cambridge, Massachusetts: G proteins, $p21^{ras}$ and signal transduction mechanism.

November

Mario Rebecchi, Memorial Sloan-Kettering Cancer Center, New York, New York: Phosphoinositide-specific phospholipase C: Regulation and mechanism.

Peter Sorger, MRC Laboratory of Molecular Biology, Cambridge, England: Regulation of the heat-shock transcription factor by stress.

Michael Greenberg, Harvard Medical School, Boston, Massachusetts: Regulation of the *c-fos* proto-oncogene during cell growth and differentiation.

Joe Nadeau, Jackson Laboratory, Bar Harbor, Maine: Gene mapping and genome organization in mammals.

Pierpaolo Di Fiore, NCI, Bethesda, Maryland: Transforming potential of growth factor receptors: EGF and *erb*B-2.

Michael Mendenhall, Scripps Clinic and Research Laboratory, La Jolla, California: Control of the yeast mitotic cell cycle: Regulation of the *cdc28* protein kinase.

Steinunn Baekkeskov, Hagedorn Research Laboratory, Copenhagen, Denmark: Characterization of a target for autoimmunity in diabetes: A 64K β-cell membrane protein.

December

Earl Ruley, Massachusetts Institute of Technology, Cambridge: Oncogene-induced lipogenesis: In vitro atherosclerosis.

Alan Smith, Monsanto Corporation, St. Louis, Missouri: Isolation and characterization of genes having preferential expression in tomato flowers.

Fred Cross, Fred Hutchinson Cancer Research Facility, Seattle, Washington: A dominant mutation affecting pheromone arrest cell size and cell-cycle control in *S. cerevisiae*.

George Scangos, Molecular Therapeutics, Inc., West Haven, Connecticut: Dysmyelination in transgenic mice expressing the early region of JC virus.

Nancy Reich, Rockefeller Unviersity, New York, New York: Transcriptional regulation of interferon-induced genes.

Joseph Schlessinger, Rorer Biotechnology, Inc., Rockville, Maryland and Weizmann Institute, Rehovot, Israel: The EGF receptor proto-oncogene: Structure, evolution, and role in oncogenesis.

David Levin, University of California, San Francisco: The *Kin* protein kinase genes of yeast.

Frans deBruijn, Max-Planck Institut, Cologne, Federal Republic of Germany: Structure and regulation of Leghemoglobin genes: Conserved *cis*-acting elements and *trans*-acting factors.

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, 310 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 well over 100 applicants, took part in the program, which was supported by Alfred P. Sloan Foundation, Burroughs Wellcome Fund, Pfizer Inc., and Robertson Research Fund

Struan Coleman, Harvard University
Research Advisor: **Daniel Marshak**
Phosphorylation by casein kinase II.

Michelle Dziejman, University of Rochester
Research Advisor: **William Welch**
The mammalian heat shock response.

Malek Faham, University of Maryland
Research Advisor: **Fevzi Daldal**
Genetic analysis of the structure of the quinol oxidization site of the cytochrome bc1 complex.

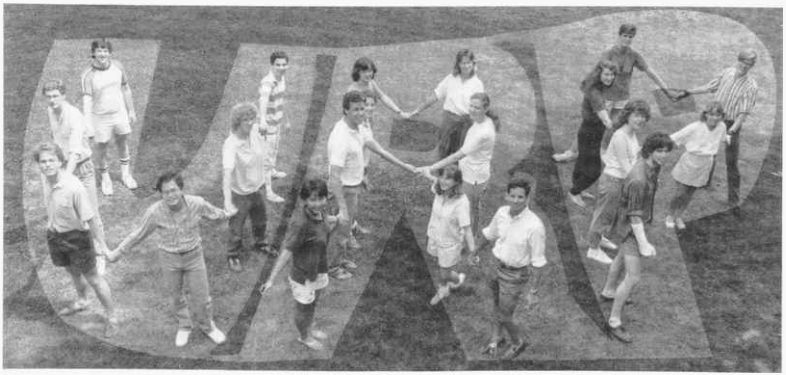
Lisa M. Gloss, Michigan State University
Research Advisor: **Winship Herr**
Transcriptional control in SV40.

Joshua Gordon, Washington University
Research Advisor: **Loren Field**
Construction of an albumin-ANF fusion gene.

Diane Harvey, Cornell University
Research Advisor: **Eileen White**
Expression of the adenovirus E1B gene products.

Adam Kaplin, Yale University
Research Advisor: **David Spector**
Preliminary investigations into the functional significance of the Sm antigen distribution pattern *in situ*.

Elena Levine, Yale University
Research Advisors: **Terri Grodzicker,** and **Margaret Quinlan**
The effect of adenovirus E1A on the SV40 enhancer in CV-1 cells.



Rong Li, Yale University
Research Advisor: **David Frendewey**
Pre-mRNA splicing in *S. pombe*.

John M. Logsdon, Jr., Iowa State University
Research Advisor: **Steve Briggs**
Gene expression in response to a fungal toxin in maize.

Tobe Mellman, Cornell University
Research Advisor: **David Beach**
Isolating suppressors of a cdc13 mutation in *S. pombe*.

Andrew Millar, University of Cambridge
Research Advisor: **Bruce Stillman**
In vitro mutagenesis of the ARS1 replication element of yeast.

Alice Paquette, Massachusetts Institute of Technology
Research Advisor: **Mike Gilman**
Investigation of *c-fos* regulation.

Pam Reinagal, Carnegie Mellon University
Research Advisor: **Nouria Hernandez**
Small nuclear RNA U1 3′ end formation

Barbara A. Sampson, Princeton University
Research Advisor: **Amar Klar**
Mating type switching in *S. pombe*.

Elizabeth Sowell, College of Charleston
Research Advisor: **VenKatesan Sundaresan**
Mapping of the Mu transposon in the *bronze Gene*.

Fiona Stewart, University of Glasgow
Research Advisor: **Betty Moran**
Characterization of conserved regions of adenovirus E1A gene.

Jonathan Tropp, Harvard College
Research Advisor: **James Pflugrath**
Purification of yeast *Ras2*.

Johannes Walter, University of California. Berkeley
Research Advisor: **John Anderson**
DNA-protein interactions at the molecular level.

DNA Literacy Program

David A. Micklos, Director
Mark V. Bloom, Assistant Director

The techniques of "gene splicing" have revolutionized biology over the last 15 years and have spawned the new biotechnology industry. The manifold economic and social implications of the increasing ability to manipulate the DNA blueprint of life appear regularly on the front pages of the nations' newspapers. Even so, there is abundant evidence that we are a nation of biotechnological illiterates. Recent studies show that a majority of Americans have little understanding of DNA and claim not to have heard of biotechnology!

The DNA Literacy Program was founded in 1985 to improve this situation by retraining science teachers nationwide so that they can better prepare students for the biotechnological world they will inherit. We see our mission as threefold:

1. To serve as a direct interface between science and society by translating current research into learning experiences for the general public.
2. To directly adapt research protocols so that they can be safely performed by students at the college and advanced high school levels.
3. To serve as a national clearinghouse for distribution of information about genetics and biotechnology to the general public.

A Japanese exchange student (left) and his lab partner from East Hampton High School participate in a student laboratory on bacterial transformation at the DNA Learning Center.

DNA Literacy Program Director David Micklos (left), summer intern Jeff Diamond, and Dr. Greg Freyer of Columbia University College of Physicians & Surgeons with the Vector Mobile DNA Laboratories in front of Jones Laboratory.

DNA Science Workshops

The most visible aspect of our education program has been the Vector DNA Science Workshop, which has now been taken by more than 600 high school and college educators from New York to California, and from Wisconsin to Alabama. The week-long course gives teachers practical experience with recombinant-DNA techniques, bolstering confidence to integrate more discussion of biotechnology into their classrooms and addressing the practical aspects of implementing DNA labs. All equipment and supplies needed to teach the course are carried in a specially designed Vector Van, which was provided through a 1986 grant from Citibank, N.A. In summers 1985 and 1986, a total of 250 educators attended eight workshops. Demand for courses in summer 1987 was great enough to justify the purchase of a second Vector Van that enables us to teach two workshops simultaneously in different parts of the country. In summer 1987, 370 educators attended 14 Vector Workshops around the country. Two additional workshops were conducted for technicians and researchers at the Cleveland Clinic. In summer 1988, the Vector Vans will visit 12 new venues, including a Navajo Indian reservation at Tuba City, Arizona.

Major Three-Year Support

Receipt in 1987 of major three-year grants from the Josiah Macy, Jr. Foundation ($490,850) and the National Science Foundation ($451,928) has lent legitimacy to the proposition that it is possible to "backpack" a DNA laboratory to essentially anywhere in the nation. These grants provide key support for our teaching staff, as well as stipend and travel expenses for workshop participants. They also provide funds to hold weekend follow-ups during the fall and winter to introduce new innovations that make it easier and more cost-effective to teach biotechnology in the high school classroom. More than 225 teachers attended the 12 follow-up workshops, and the response of participants was overwhelmingly favorable.

Participants who successfully complete both a summer workshop and a weekend follow-up receive an intense 50 hours of instruction. Through a collaboration with the State University of New York at Stony Brook, these individuals are eligible to receive three hours of graduate credit from the Continuing Education Department.

Collaboration with Carolina Biological Supply Company

As our teaching program grew and teachers in many areas began to adopt our laboratory methods, it became clear that we needed to identify a company with the capability to distribute equipment and reagents to teachers who wished to implement lab-teaching programs. Thus, in late 1986, we signed a cooperative agreement with Carolina Biological Supply Company, one of the nation's oldest and most respected science suppliers. In 1987, we collaborated with their biotechnology department to develop reagents and kits that articulate fully with our laboratory text, *DNA Science: A First Laboratory Course in Recombinant-DNA Technology*. We also assisted in the design and evaluation of an inexpensive power supply and an electrophoresis chamber. These apparatuses, which are used to produce DNA "fingerprints," are key to introducing DNA science in the classroom.

DNA Learning Center

The Macy and NSF support, along with continuing grants from a consortium of private foundations and biotechnology companies, emboldened us to put into action our dreams for a "museum" devoted entirely to helping the general public understand the DNA blueprint that determines the life and health of all living things. Thus, we concluded negotiations with the Cold Spring Harbor Central School District for the long-term lease, with purchase option, of the former grade school building at 334 Main Street in Cold Spring Harbor Village.

Considering the magnitude of the Laboratory's commitment, a trustee advisory committee was formed to assume fiduciary responsibility for the Learning Center. Chaired by William Everdell, the committee includes Mrs. Henry U. Harris, Jr., Mrs. George N. Lindsay, Dr. David Botstein, Dr. David D. Sabatini, Byam K. Stevens, Jr., and Mrs. George G. Montgomery, Jr.

The trustees of the Laboratory showed strong support for the Center by approving a capital budget of $158,000 for Phase I renovations. Offices were prepared for a permanent staff of four, and computer, telephone, and fire/security systems were installed. We are most pleased with the teaching laboratory, which we consider the heart of our institution. Designed to accommodate 24–27 participants, the laboratory provides an environment where young people and the lay public can learn science in the same way as scientists, by asking questions and performing experiments.

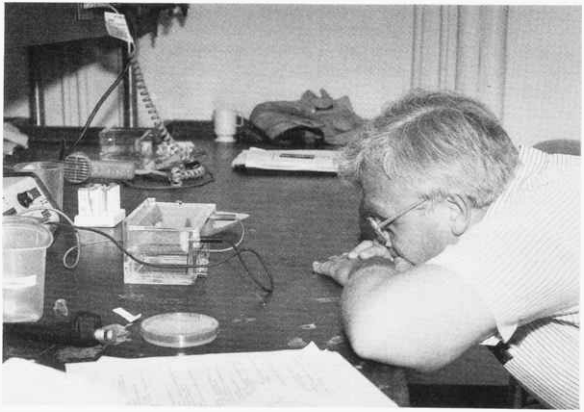
Dr. Mark Bloom instructs high school students at the DNA Learning Center.

Student Laboratory Field Trips

With the completion of the teaching laboratory, we initiated a schedule of six hands-on laboratories per week for high school students from the Metropolitan New York area. Two laboratory experiences introduce the key techniques of gene manipulation:

1. *Bacterial Transformation.* This experiment illustrates the direct link between an organism's genetic complement (genotype) and its observable characteristics (phenotype). Students introduce a new gene into the bacterium *Escherichia coli*, giving it the ability to grow in the presence of the antibiotic ampicillin.
2. *DNA Fingerprinting.* This experiment demonstrates that DNA can be precisely manipulated and that it behaves as predicted by the structure determined by James Watson and Francis Crick in 1953. Students use restriction enzymes to cut purified DNA, and the resulting DNA fragments are analyzed by gel electrophoresis. Students take home Polaroid snapshots of their results.

Response to this new program has been overwhelming. Within three weeks following the announcement of the opening of the teaching lab, our entire spring schedule was booked with 50 class visits.



Raymond Fleischmann (right) from Walt Whitman High School separates DNA fragments using electrophoresis at a week-long teacher workshop at SUNY, Stony Brook. Dr. Jan Witkowski (below) of Cold Spring Harbor Laboratory explains the use of gene probes in diagnosing muscular dystrophy at an honors lecture for local students.

Local Curriculum Study Grows

The DNA Literacy Program arose out of an initial collaboration with science educators in eight neighboring school districts. Using Long Island schools as a proving ground, we developed and tested our lab-teaching programs and workshop methods. The program has now grown to include 20 school districts, which benefit from enhancement activities that include teacher in-service training, honors lectures for students, student research symposia, free and reduced-rate student visits to the Learning Center, and lab-teaching consultation.

The Smithsonian Institution's *Search for Life* exhibit highlights the accomplishments of four Cold Spring Harbor scientists, including Laboratory Director James D. Watson (far left) and Dr. Barbara McClintock (below).

The Search for Life Exhibit

During the past year, the Laboratory cooperated with The National Museum of American History of the Smithsonian Institution and the Maryland Agricultural Experiment Station in the development of their new exhibit, *The Search for Life*, by supplying historical artifacts, photographs, and video footage. The largest and most up-to-date museum exhibit on modern biology in the United States, *The Search for Life* traces the quest to understand the genetic and molecular basis of life, which culminated in the recombinant-DNA revolution. The exhibit also confronts the visitor with the promise and the controversy of our increasing ability to manipulate the genetic code. Key discoveries of four Cold Spring Harbor staff members are specifically highlighted.

We were thus delighted when, in early 1988, we concluded an agreement to have the DNA Learning Center as the first site of a national tour of the exhibit. *The Search for Life* will open at Cold Spring Harbor in September 1988 and run through September 1989. The Smithsonian exhibit represents a rare opportunity to accelerate the development of the DNA Learning Center, allowing it to burst into existence with a powerful education program. The prospect of learning the museum business from the nation's preeminent museum is most exciting.

The 2500-square-foot exhibit was developed in collaboration with New York City theater designers, who utilized historical artifacts, video monitors, interactive displays, and a three-screen slide show within a futuristic "set" composed of aluminum girders whose latticework suggests the DNA helix. The entire program, including narration and theatrical lighting used to guide the visitor through the exhibit, is computer-controlled.

The exhibit will be situated adjacent to the teaching laboratory, where visitors can perform hands-on experiments or view daily demonstrations. Since Smithsonian curators had originally hoped to include live demonstrations in the exhibit, the Learning Center staff has agreed to train subsequent exhibit hosts in appropriate hands-on lab activities. In addition, our staff has committed to develop educational materials to expand and interpret the information in *The Search for Life* exhibit. Pre- and post-trip packages and interpretive materials will be shared with subsequent exhibit hosts.

The exhibit will be seen by a significant number of research biologists who attend meetings and workshops at Cold Spring Harbor Laboratory. Their suggestions and help will be incorporated into a renovation of the exhibit prior to its shipment to subsequent tour sites. Input from visiting scientists will also be of help in developing our own exhibit, *DNA at Work*, to follow on the heels of the Smithsonian exhibit.

Staff Members and Interns

We were most lucky to coax Dr. Mark Bloom to take on responsibility for the lab-training aspects of the DNA Literacy Program. With graduate training in biology and experience as a research molecular biologist at the Roche Institute and Michigan State University, he provides authority to our advice to teachers and insurance that our programs will continue to focus on real science. Joining us in June, he was thrown into the fray of summer activity, teaching 8 of 14 workshops.

Dr. Greg Freyer, who helped found the program and who worked on development of our protocols while a postdoctoral fellow at Cold Spring Harbor Laboratory, is currently an assistant professor at Columbia University College of Physicians and Surgeons. Greg continues to provide research support to our program and has just completed development of plasmid vectors used in our workshop program. To our knowledge, these are the first DNA molecules specifically developed for teaching purposes.

The DNA Literacy Program staff (from the left) Henri Dold, David Micklos, Nancy Baldwin, Mark Bloom, Cortney Armstrong, and John Kruper; (below) summer intern Jeff Mondshein.

Our summer interns, who lead the life of DNA gypsies traveling from workshop to workshop, continued to provide critical support for our teaching staff. Two were students from the neighboring high schools of Jericho and Sachem: Jeff Mondschein, now in his first year of pre-medical training at New York University, completed his second summer with the program, and Thomas Hyland, now a freshman at Rensselaer Polytechnic Institute. Undergraduate intern Jeff Diamond is now completing his junior year in biomedical engineering at Duke University.

Since we consider ourselves an academic unit, we were especially pleased to take on graduate student John Kruper, now working on a D.A. in molecular biology at the University of Illinois at Chicago. With previous training in virology, John is basing his thesis on evaluative data on teachers who have participated in our training workshops. During the winter, one Vector Van was stationed in Chicago with John, who taught four weekend follow-ups in the midwest.

Joining the permanent staff of the Learning Center in 1988, from other positions at the Laboratory, were Nancy Baldwin and Cortney Armstrong. Program Associate Henri Dold, who was among the first teachers in the nation to introduce DNA analysis at the high school level, assumed major responsibility for organizing our summer 1988 schedule of 12 Vector Workshops.

Recalling our Heritage as a Teaching Center

The DNA Literacy Program recalls the founding of the Biological Laboratory at Cold Spring Harbor in 1890 as a summer field station where high school teachers could experience experimental aspects of biology that could not be learned from a textbook. With the opening of the DNA Learning Center, we are now well into the lead of DNA education, but there are few road maps to follow.

As keepers of the nation's first exploratorium of DNA, we are sometimes daunted by the operating costs of our rapidly expanding programs—approximately $50,000 per month, including capital development of the Center. Facing similar needs when he assumed directorship of the Laboratory in 1968, Jim Watson said, "The Lab badly needs a real benefactor, but with much love it will probably survive without one. Of course, I dream an angel will appear soon and make me free of any serious worries for at least a month."

There is no time to tarry in working toward our goal to foster public understanding of the DNA revolution. As a small institution, we are prepared to accept a disproportionate share of the cost of educating the first generation of Americans who already dwell in a brave new world. It is a huge task, but the stakes of biotechnological illiteracy are too high.

Graduate intern John Kruper (left) interprets a DNA fingerprint for teachers at a local Curriculum Study workshop at Cold Spring Harbor High School.

Vector Workshop Sites, 1985–88

Alabama	University of Alabama, Tuscaloosa	1987, 1988
Arizona	Tuba City High School, Tuba City	1988
California	University of California, Davis	1986
Connecticut	Choate Rosemary Hall, Wallingford	1987
Illinois	Argonne National Laboratory, Argonne	1986, 1987
Indiana	Butler University, Indianapolis	1987
Iowa	Drake University, Des Moines	1987
Kentucky	Murray State University, Murray	1988
Maryland	McDonough School, Baltimore	1988
Massachusetts	Beverly High School, Beverly	1986
	Randolph High School, Boston	1988
	Winsor School, Boston	1987
New Hampshire	St. Paul's School, Concord	1986, 1987
New York	Albany High School, Albany	1987
	Bronx High School of Science, The Bronx	1987
	Cold Spring Harbor High School, Cold Spring Harbor	1985, 1987
	DNA Learning Center, Cold Spring Harbor	1988
	Huntington High School, Huntington	1986
	Irvington High School, Irvington	1986
	S.U.N.Y., Stony Brook	1987, 1988
	Wheatley School, Old Westbury	1985
North Carolina	North Carolina School of Science and Math, Durham	1987
Ohio	Cleveland Clinic, Cleveland	1987
Pennsylvania	Dusquesne University, Pittsburgh	1988
	Germantown Academy, Philadelphia	1988
South Carolina	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	1988
Virginia	Jefferson High School for Science, Alexandria	1987
Wisconsin	Marquette University, Milwaukee	1986, 1987

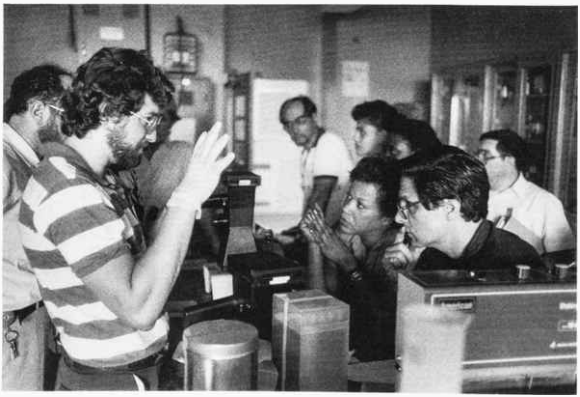
Teachers at a week-long workshop at SUNY, Stony Brook prepare a reaction to cut DNA with restriction enzymes.

Curriculum Study Membership

*Cold Spring Harbor Central School District
Commack Union Free School District
*East Williston Union Free School District
*Great Neck Public Schools
Half Hollow Hills Central School District
Harborfields Central School District
*Herricks Union Free School District
Huntington Union Free School District
*Jericho Union Free School District
Lawrence Public Schools
Locust Valley Central School District
Manhasset Public Schools
*Northport-East Northport Union Free School District
North Shore Central School District
*Oyster Bay-East Norwich Central School District
Plainview-Old Bethpage Central School District
Portledge School
Port Washington Union Free School District
Sachem Central School District at Holbrook
*Syosset Central School District

*Founding Member

Dr. Greg Freyer (left) with workshop participants at the Bronx High School of Science.



Nature Study Program

The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, students can engage in such introductory programs as Nature Detectives, Seashore Life, and Pebble Pups, as well as more advanced programs such as Marine Biology and Nature Photography.

During the summer of 1987 a total of 490 students participated in the Nature Study Program. Most classes were held outdoors, when weather permitted, or at the Uplands Farm Nature Preserve, the headquarters of the Long Island Chapter of the Nature Conservancy. The Laboratory has equipped and maintains classrooms/laboratories as well as a darkroom at Uplands Farm. This facility is used as a base for the student's exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, Caumsett State Park, the Cold Spring Harbor Fish Hatchery, and the Muttontown Preserve, as well as other local preserves and sanctuaries. Students in Marine Biology participated in a whale watch aboard the Finback II, operated by the Okeanos Ocean Research Foundation Inc. Hampton Bays, New York.

In addition to the four-week courses, a series of one-day marine biology workshops was offered to students. Studies on the marine ecology of L.I. Sound were conducted aboard the 66 foot Ketch J.N. Carter chartered from Schooner Inc. of New Haven, Connecticut. Students were able to study the Sound chemically, physically, and biologically using the ship's instrumentation. The three-day Adventure Education class took students on an 18 mile bike hike to Caumsett State Park, a 12-mile canoe trip on the Nissequogue River, and a day of sailing on the Ketch J.N. Carter.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor,
Nassau Community College

INSTRUCTORS

Ruth Burgess, B.A., teacher, Cold Spring Harbor Nurse
Terry Crowley, B.A., science teacher candidate, Longwood School District
Steven Lander, M.S., science teacher, Port Washington School District
Fred Maasch, M.S., science teacher, Islip High School
Linda Payoski, B.A., science teacher, Uniondale High School
Marjorie Pizza, B.A., science teacher, Glen Cove School District

COURSES

Nature Bugs
Nature Detectives
Advanced Nature Study
Introduction to Ecology
Frogs, Flippers, and Fins
Pebble Pups
Bird Study
Fresh Water Life
Seashore Life
Marine Biology
Nature Photography
Adventure Education
Marine Biology Workshops

FINANCIAL STATEMENT

FINANCIAL STATEMENT

CONSOLIDATED BALANCE SHEET
year ended December 31, 1987
with comparative figures for year ended December 31, 1986

ASSETS

	1987	1986
Cash and Cash Equivalents:		
Unrestricted Funds	$2,010,250	$1,882,075
Precollections of Restricted Grants	$400,217	$1,574,992
Restricted for Neuroscience Center Project	$3,333,394	$0
Restricted for Loan Interest and Principal Payments	$6,885,065	$8,263,233
Total Cash and Cash Equivalents	$12,628,926	$11,720,300
Investments and Marketable Securities—Restricted for Capital Projects	$179,000	$98,750
Accounts Receivable and Other Assets	$921,522	$1,104,065
Grants Receivable	$6,568,125	$3,651,596
Inventory of Publications	$425,832	$309,058
Endowment Funds—(at lower of Cost or Market Value)		
Robertson Research Fund (quoted Market 1987—$25,522,567; 1986—$25,757,275)	$24,103,554	$21,603,638
Robertson Maintenance Fund (quoted Market 1987—$4,148,900; 1986—$4,163,713)	$3,939,978	$3,526,426
Other Endowment Funds (quoted Market 1987—$1,937,311; 1986—$1,615,309)	$1,937,311	$332,042
Total Endowment Funds	$29,980,843	$25,462,106
Land, Buildings and Equipment, net of accumulated Depreciation	$21,810,816	$20,038,249
Total Assets	$72,515,064	$62,384,124

LIABILITIES AND FUND BALANCES

	1987	1986
Accounts Payable and Accrued Expenses	$1,884,836	$1,028,268
Deferred Income	$312,553	$228,211
Loan Payable ($2,500,000 due 8/1/88, $4,000,000 due 8/1/89)	$6,500,000	$8,000,000
Unexpended Portion of Restricted Grants	$6,558,028	$5,036,887
Fund Balances:		
Unrestricted Funds	$4,415,391	$4,376,243
Endowment Funds	$29,959,234	$25,422,968
Land, Building and Equipment Funds	$22,885,022	$18,291,547
Total Fund Balances	$57,259,647	$48,090,758
Total Liabilities and Fund Balances	$72,515,064	$62,384,124

CONSOLIDATED STATEMENT OF OPERATING REVENUES AND EXPENSES
year ended December 31, 1987
with comparative figures for year ended December 31, 1986

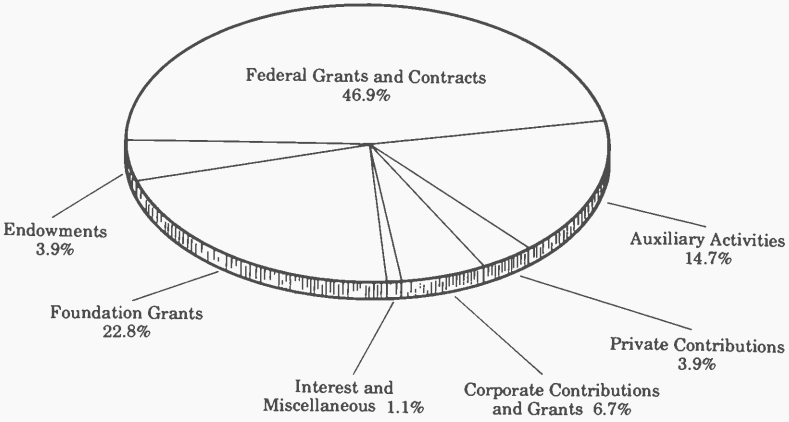
	1987	1986
REVENUES		
Restricted Grant Income	$ 9,566,026	$ 9,111,010
Indirect Cost Allowances	4,544,846	4,491,480
Contributions & Gifts		
Annual Giving	294,108	323,591
Corporate Sponsors	435,000	300,000
Endowment Funds Distributions	995,500	770,000
Royalty & Licensing Fees	41,297	72,334
Summer Programs Fees	793,476	721,951
Marina Rental Income	65,769	63,423
Carnegie Institute of Washington Rental Income	20,732	20,732
Publications	1,646,913	1,499,228
Dining Hall	990,389	855,510
Housing Rentals (Net)	420,387	426,104
Investment Income	340,967	280,187
Miscellaneous	40,062	19,716
Total Revenues	20,195,472	18,955,266
EXPENSES		
Research, Conferences & Educational Programs	10,437,633	9,845,407
Direct Research Overhead	504,153	359,542
Summer Programs Administration	420,930	320,292
Nature Study	27,032	24,202
Publications	1,449,178	1,193,741
Library Services	287,905	285,024
Operation & Maintenance of Plant	2,945,532	2,602,993
General & Administrative	2,184,763	2,135,621
Information Services	221,367	142,478
Dining Hall	1,034,927	919,497
Total Expenses, Before Depreciation	19,513,420	17,828,797
Excess, Before Depreciation	682,052	1,126,469
Depreciation Expense	1,126,349	1,093,084
Net Excess (Deficit) of Revenues over Expenses	$ (444,297)	$ 33,385

NOTE: Copies of our complete, audited financial statements, certified by the independent auditing firm of Peat, Marwick, Main & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory

COLD SPRING HARBOR LABORATORY

SOURCES OF REVENUE

YEAR ENDED DECEMBER 31, 1987



GRANTS AND CONTRIBUTIONS

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of the State of New York. Contributions are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory has been designated a "public charity" and, therefore, may receive funds resulting from the termination of "private" foundations.

Because its endowment is limited and the uses of research grants are formally restricted, the Laboratory depends on generous contributions from private foundations, sponsors, and friends for central institutional needs and capital improvements.

Over the years, the Laboratory has won a reputation for innovative research and high-level science education. By training young scientists in the latest experimental techniques, it has helped spur the development of many research fields, including tumor virology, cancer genes, gene regulation, moveable genetic elements, yeast genetics, and molecular neurobiology. However, the continued development of new research programs and training courses requires substantial support from private sources.

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities You can generally deduct the full amount of the gift on your income tax return, and, of course, you need pay no capital gains tax on the stock's appreciation. We recommend any of the following methods:

(1) Have your broker sell the stock and remit the proceeds to Cold Spring Harbor Laboratory.

(2) Deliver the stock certificates to your broker with instructions to him to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.

(3) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724. In a separate envelope send an exec*uted* stock power.

Bequests Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

Appreciated real estate or personal property Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Conversion of private foundation to "public" status on termination This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a "supporting organization of Cold Spring Harbor Laboratory."

For additional information, please contact the Director of Development, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8455.

GRANTS

January 1, 1987–December 31, 1987

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*Total Award*
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Cancer Research Center, Dr. Mathews	1/87–12/91	$16,787,416*
	Cancer Center Support, Dr. Watson	7/87– 6/90	5,346,907*
Research Support	Dr. Beach	12/84–11/87	361,511
	Dr. Beach	9/86– 8/89	578,929
	Dr. Blose	12/78–11/87	1,094,594
	Dr. Daldal	3/87–11/87	172,385*
	Dr. Feramisco	4/85– 3/88	535,534
	Dr. L. Field	4/87– 3/92	568,521*
	Dr. Franza	9/85– 7/88	478,689
	Dr. Frendewey	4/87– 3/92	775,814*
	Dr. Garrels	1/85–12/89	2,028,833
	Dr. Gilman	9/87– 8/92	608,291*
	Dr. Hanahan	8/87– 7/92	1,587,267*
	Dr. Helfman	9/85– 8/88	572,760
	Dr. Hernandez	7/87– 6/92	1,293,754*
	Dr. Klar	7/81– 6/92	3,302,677
	Dr. Rice	9/87– 8/90	528,037*
	Dr. Roberts	6/87– 5/88	38,991*
	Dr. Roberts	9/86– 8/88	216,446
	Dr. Spector	2/87– 2/88	165,000*
	Dr. Stillman	7/83– 6/92	1,869,678
	Dr. Watson	4/87– 3/88	117,413*
	Dr. Welch	4/84– 3/92	1,575,365
	Dr. Wigler	7/85– 6/92	8,426,929
	Dr. Zoller	9/85– 8/88	545,752
Fellowships	Dr. Bar-Sagi	9/85– 8/87	47,000
	Dr. Cheley	10/86– 3/88	37,494
	Dr. J. Field	10/86– 9/89	63,996
	Dr. McLeod	2/85– 1/88	63,996
	Dr. Morris	11/86–10/89	82,008
	Dr. Munroe	10/87– 4/88	28,000*
	Dr. Potashkin	10/85– 9/88	64,886
	Dr. Quinlan	6/84– 5/87	57,244
Training	Institutional, Dr. Grodzicker	7/78– 8/89	1,291,646
Course Support	Advanced Bacterial Genetics, Dr. Klar	5/80– 4/88	302,523
	Cancer Research Center Workshops, Dr. Grodzicker	1/83– 3/92	1,010,057
	Neurobiology Short Term Training, Dr. Hockfield	5/82– 4/90	723,939
	Neurobiology Short Term Training, Dr. Hockfield	6/79– 3/89	922,087

* New grants awarded in 1987

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Meeting Support	*C. elegans*	1987 & 1989	61,874*
	Eukaryotic DNA Replication	1987	4,600*
	Hepatitis B	1987	4,500*
	Regulation of Liver Gene Expression	1987	16,000*
	Molecular Biology of Mitochondria and Chloroplasts	1987	8,000*
	RNA Processing	1986–1987	3,500
	52nd Symposium: Evolution of Catalytic Function	1987	26,202*
NATIONAL SCIENCE FOUNDATION			
Research Support	Dr. Hiatt	4/87– 6/87	20,000*
	Dr. Klar	8/86– 7/89	330,000
	Dr. Marshak	7/87–12/90	190,000*
	Dr. Roberts	1/83– 5/90	640,000
	Dr. Roberts	2/87– 1/89	359,262*
	Dr. Roberts	8/87– 1/90	20,925*
	Dr. Spector	3/87– 2/89	95,000*
	Dr. Sundaresan	5/87–10/90	270,000*
Course Support	Plant Molecular Biology, Dr. Klar	8/86– 1/90	137,490
Meeting Support	*C. elegans*	1987–1989	17,819*
	Eukaryotic DNA Replication	1987	2,500*
	Hepatitis B	1987	3,000*
	Molecular Biology of Mitochondria and Chloroplasts	1987	8,000*
	RNA Processing	1986–1987	12,400
	52nd Symposium: Evolution of Catalytic Function	1987	3,000*
	Undergraduate Research Program, Dr. Herr	6/87–11/88	32,000*
DEPARTMENT OF AGRICULTURE			
Research Support	Dr. Hiatt	8/86– 6/87	53,347
Meeting Support	Molecular Biology of Mitochondria and Chloroplasts	1987	12,000*
DEPARTMENT OF ENERGY			
Meeting Support	52nd Symposium: Evolution of Catalytic Function	1987	9,000*
	Molecular Biology of Mitochondria and Chloroplasts	1987	3,000*
NONFEDERAL GRANTS			
Research Support			
A.B.C. Foundation	Dr. Wigler	5/82– 4/87	600,000

* New grants awarded in 1987

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*Total Award*
American Cancer Society	Dr. Hanahan	7/87– 6/89	160,000*
	Dr. Spector	7/87– 6/89	162,000*
	Drs. Stillman, Gluzman, Welch – Institute Award	7/82– 6/88	220,000
	Dr. Wigler	4/87– 3/88	10,000*
	Dr. Wigler, Professorship	1986–2012	1,333,333
American Foundation for AIDS Research	Dr. Franza	12/87–11/88	60,000*
Amersham International plc	Dr. Welch	10/85– 9/87	50,000
	Dr. Harlow	11/86–10/87	148,780
Anonymous	Dr. Mathews	1987	10,000*
Cancer Research Institute	Dr. Franza	4/86– 3/87	25,000
Diabetes Research & Education Foundation	Dr. Hanahan	5/86– 4/87	19,960
Fannie E. Rippel Foundation	Dr. Watson	3/87–12/88	125,000*
Juvenile Diabetes Foundation	Dr. Hanahan	9/86– 9/88	66,000
LIBA	Dr. Hernandez	7/87– 6/88	20,000*
LIBA	Dr. Spector	4/87– 3/88	27,000*
Muscular Dystrophy Association	Dr. Feramisco	1/87–12/88	83,742*
	Dr. Helfman	7/86– 6/89	94,500
	Dr. Mathews	1/87–12/89	100,718*
Monsanto Company	Cooperative Research	10/84– 9/90	2,089,200
Pfizer, Inc.	Dr. Wigler	1985–1990	500,000
Pioneer Hi-Bred International, Inc.	Cooperative Research	8/85– 4/91	2,500,000
Rita Allen Foundation	Dr. Herr	9/85– 8/90	150,000
	Dr. Stillman	1/83–12/87	150,000
Scripps Clinic	Dr. Hanahan (subcontract)	1/86– 3/88	377,029
Fellowships			
Albert Heritage Foundation for Medical Research	Dr. Mizzen	7/86– 6/87	19,730
American Heart Association	Dr. Lamb	7/87–11/87	6,750*
Bristol-Myers Company	Fellowship Support	6/86– 5/91	500,000
Cancer Research Institute	Dr. Cone	10/85– 9/87	48,000
	Dr. Young	8/85– 8/87	46,000
	Dr. Sturm	9/86– 8/88	51,000
	Dr. Efrat	9/86– 8/88	51,000
Damon Runyon–Walter Winchell Cancer Fund	Dr. Michaeli	3/87– 2/90	69,000*
Esther and Joseph A. Klingenstein Fund, Inc.	Dr. Marshak	7/87– 6/90	100,000*
J.N. Pew, Jr. Charitable Trust	Dr. Watson	4/87– 4/90	260,000*
Juvenile Diabetes Foundation International	Dr. Grant	7/87– 6/88	25,000*
LIBA	Four Fellowships	1987	100,000*
Life Science Research Foundation	Dr. Colicelli	9/86– 8/89	105,000
Medical Research Council of Canada	Dr. Mizzen	6/87– 5/88	38,052*
NYS Health Research Council	Dr. Bautch	9/86– 8/88	39,000
Oncogene Science Fellowship Fund	Dr. Watson	1987	12,500*
Training			
Alfred P. Sloan Foundation	Undergraduate Research Program	1985–1988	74,000
Burroughs Wellcome Fund	Undergraduate Research Program	1987	16,560*
Grass Foundation	Neurobiology Scholarship Support	1980–1987	126,670
Lucille P. Markey Charitable Trust	Scholarship Support	1985–1988	150,000
Robert H.P. Olney Memorial Fund	Undergraduate Research Program	1987	3,854*

* New grants awarded in 1987

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Pfizer, Inc.	Undergraduate Research Program	1987	8,280*
Samuel Freeman Charitable Trust	Undergraduate Research Program	1987	2,500*
Course Support			
Alfred P. Sloan Foundation	Computational Neuroscience Course	1986–1989	140,000
The Esther A. and Joseph Klingenstein Fund	Neurobiology Support	1982–1988	300,000
Meeting Support			
American Hoechst Corp.	Regulation of Liver Gene Expression	1987	100*
Amoco Corporation	Molecular Biology of Mitochondria and Chloroplasts	1987	1,000*
Anheuser Busch Co.	Yeast Cell Biology	1987	500*
Beckman Instruments	Regulation of Liver Gene Expression	1987	300*
Bethesda Research Laboratories	Regulation of Liver Gene Expression	1987	300*
Bio-Rad, Chemical Division	Regulation of Liver Gene Expression	1987	500*
Ciba-Geigy Corp.	Molecular Biology of Mitochondria and Chloroplasts	1987	500*
Codon	Regulation of Liver Gene Expression	1987	500*
DelMonte, Inc.	Molecular Biology of Mitochondria and Chloroplasts	1987	2,000*
E.I. du Pont de Nemours, & Company	Molecular Biology of Mitochondria and Chloroplasts	1987	1,000*
Gist Brocades	Yeast Cell Biology	1987	500*
Haake Buchler Instruments, Inc.	Regulation of Liver Gene Expression	1987	100*
ICN Biomedicals	Translational Control	1987	20,000*
Labatts Brewing Co.	Yeast Cell Biology	1987	500*
Lucille P. Markey Charitable Trust	52nd Symposium: Evolution of Catalytic Function	1987	15,000*
March of Dimes Birth Defects Foundation	Regulation of Liver Gene Expression	1987	2,000*
Merck & Co., Inc.	Regulation of Liver Gene Expression	1987	500*
Miller Brewing Co.	Yeast Cell Biology	1987	500*
Monsanto Company	Molecular Biology of Mitochondria and Chloroplasts	1987	2,000*
Nestle S.A.	Regulation of Liver Gene Expression	1987	1,000*
Phillips Petroleum	Molecular Biology of Mitochondria and Chloroplasts	1987	1,000*
Pioneer HiBred International, Inc.	Molecular Biology of Mitochondria and Chloroplasts	1987	1,000*
Promega Biotech	Regulation of Liver Gene Expression	1987	500*
Rockefeller Foundation	Vaccines	1986–1988	45,000
Alfred P. Sloan Foundation	52nd Symposium: Evolution of Catalytic Function	1987	25,000*
SmithKline Beckman Corporation	Molecular Biology of Mitochondria and Chloroplasts	1987	500*
Stratagene Cloning System	Regulation of Liver Gene Expression	1987	500*
Stroh Brewing Co.	Yeast Cell Biology	1987	500*
University of Alabama, Birmingham	Regulation of Liver Gene Expression	1987	2,500*
Whatman Inc.	Regulation of Liver Gene Expression	1987	100*
Zymo Genetics, Inc.	Yeast Cell Biology	1987	500*
Zoecon Research Institute/ Sandoz Crop Protection Corp.	Molecular Biology of Mitochondria and Chloroplasts	1987	1,000*

* New grants awarded in 1987

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
DNA Literacy Program			
GIBCO/BRL Research Products division of Life Technologies Inc.	Core Program Support	1987	$10,000*
J.M. Foundation	Core Program Support	1986–1987	60,000
Richard Lounsbery Foundation	Core Program Support	1987	15,000*
Josiah Macy, Jr. Foundation	Core Program Support	7/87– 6/90	490,850*
National Science Foundation	Core Program Support	6/87–11/90	451,928*
Brinkmann Instruments, Inc.	DNA Learning Center	1987	10,000*
Argonne National Laboratory	Vector Workshop Program	1987	1,421*
Center for Biotechnology, SUNY Stony Brook	Vector Workshop Program	1987	11,130*
Cleveland Clinic Foundation	Vector Workshop Program	1987	16,784*
Fotodyne Incorporated	Vector Workshop Program	1987	9,000*
Eli Lilly and Company	Vector Workshop Program	1987	10,000*
Miller Brewing Company	Vector Workshop Program	1987	1,000*
North Carolina Biotechnology Center	Vector Workshop Program	1987	500*
Pioneer Hi-Bred International, Inc.	Vector Workshop Program	1987	9,038*
Cold Spring Harbor School District	Curriculum Study	1987	500*
Commack School District	Curriculum Study	1987	1,500*
East Williston School District	Curriculum Study	1987	500*
Great Neck School District	Curriculum Study	1987	500*
Half Hollow Hills School District	Curriculum Study	1987	3,000*
Harborfields School District	Curriculum Study	1987	4,000*
Herricks School District	Curriculum Study	1987	1,000*
Huntington School District	Curriculum Study	1987	1,500*
Jericho School District	Curriculum Study	1987	2,250*
Lawrence School District	Curriculum Study	1987	1,500*
Locust Valley School District	Curriculum Study	1987	2,000*
Manhasset School District	Curriculum Study	1987	1,500*
Northport-East Northport School District	Curriculum Study	1987	500*
North Shore School District	Curriculum Study	1987	1,500*
Oyster Bay–East Norwich School District	Curriculum Study	1987	500*
Plainview–Old Bethpage School District	Curriculum Study	1987	1,500*
Portledge School	Curriculum Study	1987	1,500*
Port Washington School District	Curriculum Study	1987	1,500*
Sachem School District	Curriculum Study	1987	1,500*
Syosset School District	Curriculum Study	1987	500*

* New grants awarded in 1987

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
FEDERAL GRANTS			
Meeting Support			
Department of Energy	Transposable Elements as Mutagenic Agents	1987	5,000*
Environmental Protection Agency	Transposable Elements as Mutagenic Agents	1987	5,000*
	Mammalian Cell Mutagenesis	1987	46,600*
Food and Drug Administration	Approaches to Carcinogenesis	1987	40,000*
National Institutes of Health	Transposable Elements as Mutagenic Agents	1987	10,000*
NONFEDERAL GRANTS			
Meeting Support			
ABI Biotechnology, Inc.	Therapeutic Peptides and Proteins	1987	2,500*
Alfred P. Sloan Foundation	Journalists and Congressional Workshops	1985–1987	162,000
American Industrial Health Council	New Directions in the Qualitative and Quantitative Aspects of Carcinogen Risk Assessment	1987	10,000*
AMGEN	Therapeutic Peptides and Proteins	1987	1,000*
Applied Biosystems	Therapeutic Peptides and Proteins	1987	1,000*
Development Center for Biotechnology, Republic of China	Therapeutic Peptides and Proteins	1987	2,500*
Dow Chemical	New Directions in the Qualitative and Quantitative Aspects of Carcinogen Risk Assessment	1987	5,000*
International Commission for Protection Against Environmental Mutagens and Carcinogens	Transposable Elements as Mutagenic Agents	1987	6,000*
James C. McDonnell Foundation	Conference Support	1985–1987	300,000
Kabivitrum	Therapeutic Peptides and Proteins	1987	2,000*
Molecular Devices Corp.	Therapeutic Peptides and Proteins	1987	1,000*
Samuel Freeman Charitable Trust	Stress Response in Biology and Disease	1987	30,000*

* New grants awarded in 1987

ENDOWMENTS AND GIFTS FOR FACILITIES AND EQUIPMENT

ENDOWMENTS

	Market Value*
Cold Spring Harbor Laboratory	
Cold Spring Harbor Laboratory Unrestricted Endowment Fund	$ 402,353
1987 Contributors: Anonymous	
Dr. Bayard Clarkson	
Mr. and Mrs. Miner D. Crary, Jr.	
Mr. Robert L. Cummings	
Mr. and Mrs. George W. Cutting, Jr.	
Mr. and Mrs. Norris Darrell	
Mr. William Everdell	
Mr. David L. Luke III	
Dr. David B. Pall	
William and Maude Pritchard Charitable Trust	
The Doubleday Professorship for Advanced Cancer Research	1,359,388
Robert H.P. Olney Memorial Fund	45,961
1987 Contributors: The Rev. Linda Peyton Hancock	
Mr. Peter B. Olney, Jr.	
Mrs. Pamela Peyton Post	
Mrs. Robert W. Tilney, Jr.	
Robertson Research Fund	25,435,105
Robertson Maintenance Fund	4,134,407
Posy White Memorial Fund	26,173†
1987 Contributor: Mrs. George G. Montgomery, Jr.	
Total Cold Spring Harbor	$31,403,387
Long Island Biological Association (LIBA)	
Dorcas Cummings Memorial Lecture Fund	47,499
1987 Contributor: Mr. Robert L. Cummings	
Other Endowments	35,618
Total LIBA	$83,117

FACILITIES AND EQUIPMENT

Arthur and Walter Page Laboratory of Plant Genetics			
Charles E. Culpepper Foundation	Construction	1985–1987	$ 75,000
Ira W. DeCamp Foundation	Construction	1985–1987	200,000
Morgan Guaranty Trust Company of New York	Construction	1987–1988	200,000†
National Science Foundation	Construction	1983–1988	690,715
William and Maude Pritchard Charitable Trust	Construction	1985–1987	220,000
Individual Contributors	Construction	1987	50,471†
Neuroscience Education and Research Facility			
Samuel S. Freeman Charitable Trust	Construction	1987–1988	200,000†
Lita Annenberg Hazen Charitable Trust	Construction	1987	25,000†
Howard Hughes Medical Institute	Construction/ Equipment	1987–1990	5,000,000†#
Esther A. and Joseph Klingenstein Fund, Inc.	Construction	1987–1990	1,000,000†
James S. McDonnell Foundation	Construction	1987	1,000,000
Individual Contributors	Construction	1987	3,047†
X-ray Crystallography Laboratory			
Lucille P. Markey Charitable Trust	Equipment	1986–1988	863,500

* As of December 31, 1987
† New grants awarded in 1987
# Also see research and course support in the non-Federal grants listing

Corporate Sponsor Program

Cold Spring Harbor Laboratory has become the "schoolhouse for modern biology," offering the most comprehensive series of biological conferences available anywhere in the United States. Access to current research presented at these high-level professional meetings is an important resource for the biotechnology industry.

The annual $15,000 membership commitment from each Corporate Sponsor has enabled the Laboratory to significantly expand this role as a clearinghouse for biotechnical information. In 1987, more than 3,700 scientists from around the world attended 26 conferences and 14 advanced training courses at Cold Spring Harbor and Banbury Center.

As the meetings program has expanded, so too has the Laboratory's commitment to create an atmosphere at Cold Spring Harbor that encourages increased interaction between academic and industrial scientists. Since establishment of the Corporate Sponsor program in 1984, corporate attendance has risen 73 percent, while overall attendance has increased 46 percent. Among those attending 1987 meetings were 360 scientists representing more than 100 companies.

Proceeds from the Corporate Sponsor Program allowed initiation of an ongoing series of Special Banbury Conferences that focus on emerging areas of research especially germane to industrial biotechnology. These high-level meetings are the basis for the Laboratory's popular book series *Current Communications in Molecular Biology*. Topics of 1987 conferences were "Nuclear Oncogenes," "Inositol Lipids in Signalling," "Molecular Biology and Genetic Engineering of Crops," "Molecular Genetics of Protozoa," and "Antisense RNA."

Benefits to Sponsor companies include waiver of all fees for six representatives at Cold Spring Harbor meetings and Special Banbury Conferences; gratis Cold Spring Harbor and Banbury publications, including the journal *Genes & Development*; and recognition in meeting abstracts and publications.

Since 1984, more than 30 companies have participated in the Corporate Sponsor Program. The membership renewal rate is 90 percent. 1987 members of the Corporate Sponsor Program—world leaders in the application of biotechnology to health care, agriculture, and manufacturing—were:

Abbott Laboratories
American Cyanamid Company
Amersham International plc
AMGen
Becton Dickinson and Company
Boehringer Mannheim GmbH
Bristol-Myers Company
Cetus Corporation
Ciba-Geigy Corporation
Diagnostic Products Corporation
E.I. du Pont de Nemours & Company
Eastman Kodak Company
Genentech, Inc.
Genetics Institute
Hoffmann-La Roche Inc.
Johnson & Johnson
Eli Lilly and Company
Millipore Corporation
Monsanto Company
Oncogene Science, Inc.
Pall Corporation
Pfizer Inc.
The Procter & Gamble Company
Schering-Plough Corporation
Smith Kline & French Laboratories
Tambrands Inc.
The Upjohn Company
The Wellcome Research Laboratories, Burroughs Wellcome Co.
Wyeth Laboratories

Alumni Cabin Campaign

Since the first Symposium on Quantitative Biology in 1933, Cold Spring Harbor has been a major site for discussion of serious science. Many scientists from around the world who attend Cold Spring Harbor meetings each year remember a time when the whole molecular biology community could be comfortably seated in Vannevar Bush Hall. The spectacular growth of molecular biology research worldwide is reflected in attendance at Cold Spring Harbor meetings. Over the last five years, the number of meetings participants has increased 46 percent, to a record 3,700 scientists in 1987.

The caliber of science discussed at Cold Spring Harbor has remained consistently excellent, but facilities once considered rustic are now just plain inadequate. Last year, as part of a major program to update visitor facilities prior to the Laboratory's centennial year in 1990, meetings attendees, course instructors, and former staff were asked to recall how their careers had benefited from the Cold Spring Harbor experience by contributing to "Alumni Cabin," one of six four-bedroom cabins planned to replace existing, dilapidated structures.

By the end of 1986, nearly 200 biologists from around the world had donated almost $34,000. In late 1987, as ground was broken for construction of the cabins, the fund for "Alumni Cabin" had reached nearly $42,000. In response to a new appeal in early 1988, Cold Spring Harbor alumni continue to make donations, and it is hoped the goal of $100,000 can be reached by the time the cabins come into service later in 1988.

Donations were received prior to December 31, 1987, from:

Dr. Judy Abraham
Dr. and Mrs. Sankar Adhya
Dr. Bruce N. Ames
Dr. Carl Anderson
Anonymous
Dr. E.G. Bade
Dr. Jonathan Beckwith
Dr. and Mrs. Seymour Benzer
Dr. Robert M. Blumentahl
Dr. Herbert Boyer
Dr. Fred Brown
Dr. Janet S. Butel
Ms. Edith Calzolari
Dr. John M. Coffin
Dr. Donald M. Crothers
Ms. Katya Davey
Dr. Norman Davidson
Dr. Henry Drexler
Dr. Harrison Echols
Dr. Robert Eisenman
Dr. Ellen Fanning
Dr. C.R. Fuerst
Dr. William M. Gelbart
Dr. Günter Gerisch
Gesellschaft zur Förderung der molekularbiologischen Forschung in Heidelberg e.V.
Dr. Philip Harriman
Dr. James Hicks
Dr. Bernhard Hirt
Dr. Mahlon Hoagland
Dr. P.H. Hofschneider
Dr. H. Robert Horvitz
Dr. Judith Jaehning
Dr. Rudolf Jaenisch
Dr. Tohru Kataoka
Dr. Yuriko Kataoka
Dr. Yoshiki Katoh
Dr. Daniel E. Koshland
Dr. Charles Laird
Dr. Michael J. Lenardo
Dr. Andrew Lewis
Dr. David M. Livingston
Dr. Elisabeth Ljungquist
Dr. John Mapoles
Dr. Kenichi Matsubara
Dr. Shigeko Matsumura
Dr. William McClure
Dr. Ronald McKay
Dr. Daniel Nathans
Dr. Mary Osborn
Dr. Barry Polisky
Dr. Keith R. Porter
Dr. Jeffrey W. Roberts
Dr. Keith Roberts
Dr. Gerald M. Rubin
Dr. Earl Ruley
Dr. A.J. Shatkin
Dr. David Stacy
Dr. and Mrs. A.R. Srinivasan
Dr. Rolf Sternglanz
Dr. Elizabeth J. Taparowsky
Dr. A.J. Van der Eb
Dr. Volker M. Vogt
Dr. James D. Watson
Dr. Klaus Weber
Dr. Robin A. Weiss
Dr. James F. Williams
Dr. Moshe Yaniv
Dr. Sayeeda Zain

Individual Contributions and Memorial Gifts

American Institute of Architects, Long Island Chapter
Mrs. Donald Arthur
Miss Christine Bartels
Dr. and Mrs. Paul Berg
Dr. Jonathan M. Borkum
Dr. Thomas Broker
Mr. G. Morgan Browne, Jr.
Dr. C. Thomas Caskey
Dr. Louise Chow
Mr. and Mrs. John P. Cleary
Mr. and Mrs. Miner D. Crary, Jr.
Mr. and Mrs. Henry B. Cross
Mr. and Mrs. George W. Cutting, Jr.
Dr. and Mrs. F.E. Donoghue
Mr. and Mrs. James Eisenman
Elsevier Science Publishers
Elsevier Science Publishing Co., Inc.
Mr. Alan Fortunoff
Dr. Don Ganem
Ms. Helena Gaviola
GEICO
Dr. and Mrs. Martin Gellert
Dr. and Mrs. H. Bentley Glass
Dr. and Mrs. Richard L. Golden
Mr. Oliver P. Grace
Miss Maxine Harrison
Dr. Stanley Hattman
Dr. and Mrs. Alfred D. Hershey
Dr. Ira Herskowitz
Mrs. Elsie Herzog
Dr. Bernhard Hirt
Mrs. Warren C. Hutchins
Mr. and Mrs. Reginald L. Jones
Mrs. Robert J. Jones
Dr. Walter Keller
Mr. Arthur Landy
Mr. and Mrs. Richard E. Leckerling
Mr. William Lehrfeld
Dr. and Mrs. Monroe L. Levin
Mr. Henry Luce III
Drs. Fumio and Shigeko Matsumura
Ms. Leila Laughlin McKnight
Mrs. Florence L. Meader and Family
Dr. Jeffrey H. Miller
Mrs. Martha M. Osterhus
Mr. and Mrs. Richard Neuendorffer
Pederson-Krag Center
Mr. J. Gardiner Pieper
Mrs. Andrew R. Povill
Mr. Richardson Pratt, Jr.
Mrs. Sherman Pratt
Dr. and Mrs. John R. Ray III
Ms. Joan R. Read
The Sanibel Writers Group
Ms. Marilyn Louise Schmitt
Dr. and Mrs. Walter Shropshire
Lt.Col. and Mrs. Jesse S. Skaggs III
Dr. David Stacy
Mr. Colton P. Wagner
Dr. and Mrs. James D. Watson
Dr. Robert A. Weisberg
Mr. James F. Wellington
Dr. and Mrs. Heiner Westphal
Mr. and Mrs. Duane N. Williams

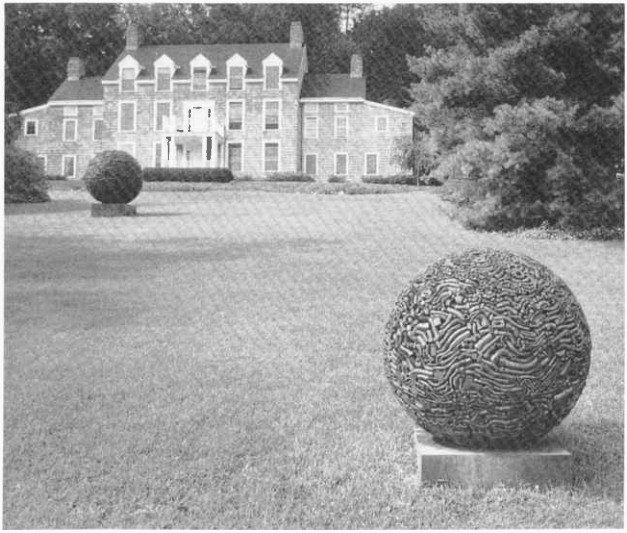
In memory of . . .

Jean Bardsley
Frederich Barnes
Dudley R. Barr
Donald Braid
Grace Eisenberg
R.B. Hintenach
Helen H. Leckerling
Jeanne R. Leckerling
Laurie Lowman
Margaret M. McCarthy
Patricia McDonald
David C. Noyes III
Robert H.P. Olney
Sigourney B. Olney
Mother of Mr. Irving Paeff
Dr. and Mrs. R. Townley Paton
Elizabeth Pieper
Stephen James Prentis
Richard Taylor

Two sculptures by Michael Malpass, "Nuts and Bolts" (right) and "Midnight Fair," have been donated to the Laboratory by Richardson Pratt, Jr., president of the Pratt Institute in Brooklyn. The public was invited to view these pieces and 22 other works on display throughout the Laboratory grounds from June 1987 through March 1988. The exhibition, "Nothing But Steel," comprised the work of 19 artists, including Lloyd Harbor resident Christopher Solbert, who conceived the exhibition, selected the artists, and supervised installation of the works.

Contributions totalling $5400 were made to the "Nothing But Steel" fund by:

Mrs. Donald Arthur
Mr. and Mrs. William Braden
Mr. and Mrs. G. Morgan Browne, Jr.
Mr. George W. Cutting, Jr.
Mrs. John P. Campbell
Mr. and Mrs. Miner D. Crary, Jr.
Mr. and Mrs. Norris Darrell
Mr. and Mrs. Charles Dolan
Mr. and Mrs. James A. Eisenman
Mr. and Mrs. Duncan Elder
Mr. and Mrs. Joseph G. Fogg
Mr. and Mrs. Oliver R. Grace
Mr. and Mrs. Henry U. Harris, Jr.
Mrs. Sinclair Hatch
Mr. and Mrs. Robert D. Jay
Mr. and Mrs. Gordon Lamb
Mr. Edward M. Lamont
Mr. and Mrs. George N. Lindsay
Mrs. Charles P. Noyes
Mr. and Mrs. Randall P. McIntyre
Mr. and Mrs. Robert J. Osterhus
Mr. and Mrs. Donald R. Peirce
Mrs. Richardson Pratt
Mr. and Mrs. John R. Reese
Mr. and Mrs. Peter O.A. Solbert
Dr. and Mrs. James D. Watson
Mr. and Mrs. Taggart Whipple



LONG ISLAND BIOLOGICAL ASSOCIATION

The LONG ISLAND BIOLOGICAL ASSOCIATION (LIBA) and Its Relation to the Cold Spring Harbor Laboratory

Biological research at Cold Spring Harbor began in 1890 when the Brooklyn Institute of Arts and Sciences established a summer laboratory here as the field station for its Department of Zoology. Representatives of the Institute were invited by Eugene G. Blackford, at that time the Fish Commissioner of the State of New York, to inspect a site on Bungtown Road across Northern Boulevard 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, whaling, and textile manufacture at the head of Cold Spring Harbor. Bungtown Road, which runs through the Laboratory 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 to locate a Station for Experimental Evolution at Cold Spring Harbor. Charles B. Davenport, who had been directing the Laboratory since 1896, assumed additional duties as director of the Carnegie Institution's facility. It is interesting to note that Mr. Davenport lived in the Victorian house on Route 25A which was built on the site of the Jones' family homestead before the turn of the century. Formerly known as "Carnegie Dormitory," it is now repainted in its original colors and called "Davenport House."

During the early years, scientists at the Laboratory worked out the genetics of eye and hair color in humans. In 1908, they developed the first hybrid corn. Many consider this discovery one of the most significant achievements of this century. Another discovery with far-reaching importance to molecular biology was made by Alfred Hershey in 1952, when he provided key evidence that DNA is the molecule of heredity—work for which he shared the Nobel Prize.

The Long Island Biological Association (LIBA) was founded by a small group of community leaders in 1924 when the Brooklyn Institute decided to discontinue its research at Cold Spring Harbor. For the next 38 years, this association operated the laboratory in conjunction with the Carnegie Institution.

In 1962, the Carnegie and LIBA facilities were merged to form the Cold Spring Harbor Laboratory of Quantitative Biology, and the property owned by LIBA was conveyed to the Laboratory, with LIBA retaining reversionary rights. In addition to its representation on the Board of Trustees of the Laboratory, LIBA became an important source of capital for the funding of specific projects at Cold Spring Harbor, including the building of the James Laboratory Annex, the renovation of Blackford Hall, the rebuilding of Williams House, and the acquisition of the land formerly owned by the Carnegie Institution, culminating with a $600,000 donation toward the cost of the Oliver and Lorraine Grace Auditorium.

LIBA's membership has grown from the small band of residents who saw the importance of maintaining an independent laboratory at Cold Spring Harbor in

Membership in LIBA requires a minimum annual contribution (tax deductible) of $25 per family. Further information can be obtained from the Long Island Biological Association, Post Office Box 100, Cold Spring Harbor, N.Y. 11724 or by telephoning the Laboratory at 516/367-8319.

1924 to an association of more than 500 interested supporters today. Its affairs are handled by a board of 29 directors who are elected to office by the membership at an annual meeting. LIBA members and their friends are encouraged to attend lectures and other events designed to increase their understanding of molecular biology.

In the fall of 1986, the Trustees of the Laboratory recognized the need to establish an annual giving program at Cold Spring Harbor to give the Laboratory the ability to continue to attract the best new Ph.D. scientists. The LIBA Directors responded and established an annual giving program to initially fund four incoming postdoctoral scientists and to augment laboratory support for young staff investigators. Additionally, the Cold Spring Harbor Laboratory Associate Program was conceived to give special recognition to those who contribute $1000 or more to annual giving. This program offers special educational opportunities through "Associates only" lectures, briefings, and workshops.

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CHAIRMAN'S REPORT

1987 was an especially exciting year for the Long Island Biological Association. As the pace of discovery in molecular biology quickened, LIBA's initial efforts to raise annual funds for the Cold Spring Harbor Laboratory met with considerable success.

Membership swelled to 519, and total contributions reached $182,550. A principal reason for this success was support from 94 Cold Spring Harbor Laboratory Associates during our fiscal year ending September 30, 1987.

Dr. David Botstein, Laboratory Trustee from MIT, addressed the Annual Meeting with an interesting speech on "Genetic Fingerprints," and Dr. Ernst Mayr from the Museum of Comparative Zoology in Cambridge gave the Dorcas Cummings Lecture on "What Is Evolution All About?" These programs were augmented by a series of open houses to familiarize members and guests with the exciting developments at the Laboratory. In addition, the Associates were invited to attend "hands-on" laboratory workshops. At one workshop, they cut DNA with several restriction enzymes, each of which cleaved at specific nucleotide sequences within the DNA molecule. The resulting DNA restriction fragments were then separated according to size using gel electrophoresis. At a second workshop, Associates added a plasmid DNA to bacterial cells and demonstrated that the transformed cells thrived in a medium containing the antibiotic ampicillin, which killed the normal bacterial cells.

These two experiments were highly effective in giving the participants a real understanding of laboratory procedures and some of the exciting possibilities brought about through genetic engineering. To further their understanding of molecular biology, Associates were invited to interesting lectures and scientific briefings during the year.

DNA Literacy Program Director David Micklos (left) explains use of micropipet to Mrs. Nicholas Parks and her son Nicholas at a special Associate's workshop on DNA fingerprinting.

E. Mayr, G.W. Cutting, Jr., and B.D. Clarkson at the Dorcus Cummings lecture.

As part of the allocation of the contributions received, fellowships were awarded to Toshiki Tsurimoto, Paul Walton, Roymarie Ballester, and Gokul Das. Moreover, staff scientists Nouria Hernandez and David Spector were awarded grants from the New Investigator Start-up Fund, which was established through LIBA contributions. Funds were also applied to the Science Meetings Program, including an important meeting in September, "Modern Approaches to Vaccines," at which the problem of AIDS was a major topic.

LIBA is taking an increasing responsibility for expanding the community's knowledge and understanding of basic molecular biology. Through the continued interest and strong support of its membership, LIBA is sharing in the excitement and progress of this fast-growing science.

George W. Cutting, Jr., Chairman

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Banbury Center

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Fevzi Daldal

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Takashi Toda

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Esa Kuismanen
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Mary Chapman