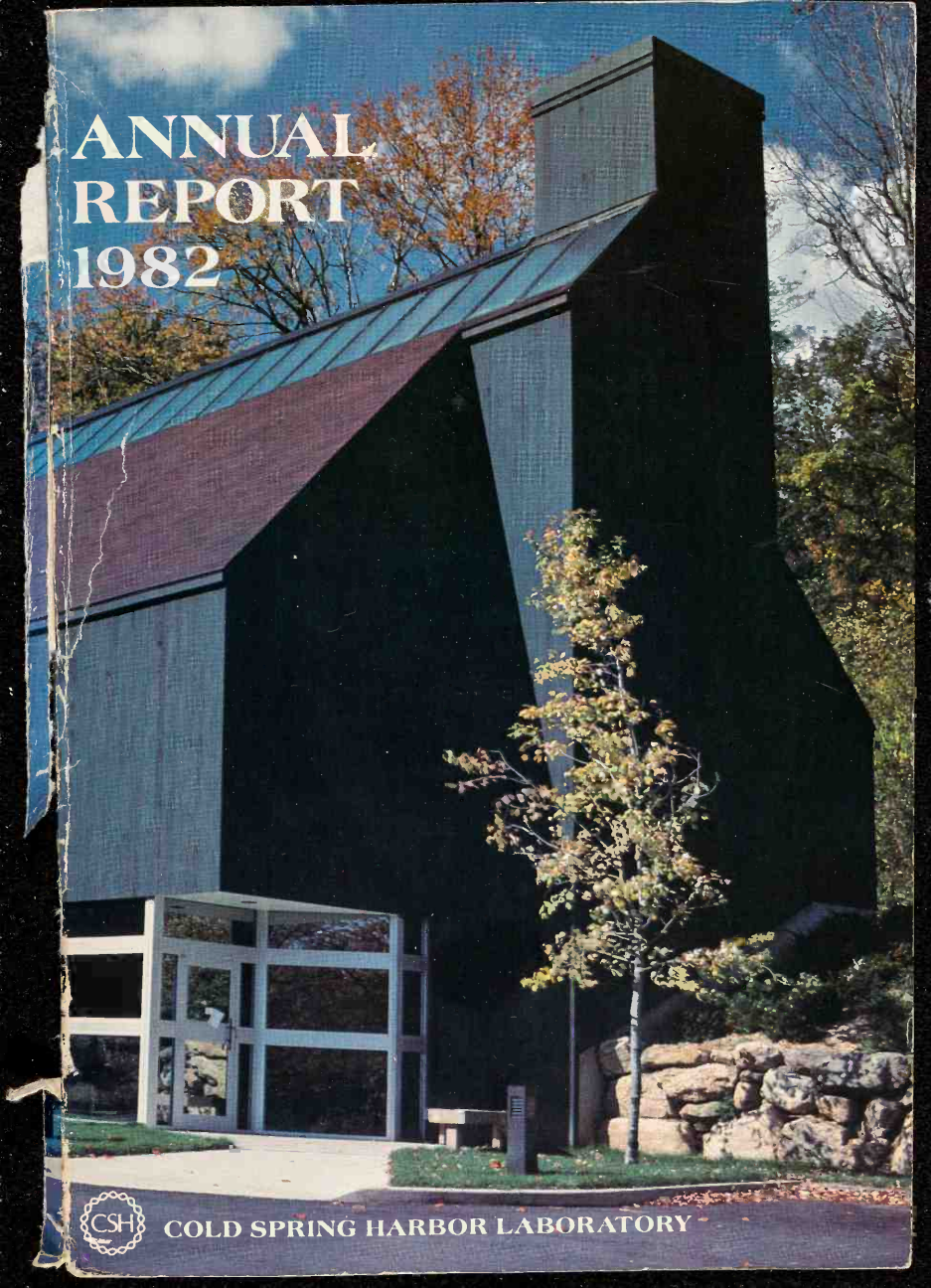
ANNUAL REPORT 1982



COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1982

COLD SPRING HARBOR LABORATORY

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

1982 Annual Report
Editors: Annette Kirk, Elizabeth Ritcey
Photographers: Herb Parsons, Joan James

Cover: Reginald G. Harris Building, dedicated May 27, 1982. Photo by Ross Meurer.

COLD SPRING HARBOR LABORATORY

COLD SPRING HARBOR, LONG ISLAND, NEW YORK

OFFICERS OF THE CORPORATION

Walter H. Page, *Chairperson*
Dr. Bayard Clarkson, *Vice-Chairperson*
Dr. Norton D. Zinder, *Secretary*
Robert L. Cummings, *Treasurer*
Roderick H. Cushman, *Assistant Treasurer*
Dr. James D. Watson, *Director*
William R. Udry, *Administrative Director*

BOARD OF TRUSTEES

Institutional Trustees

Albert Einstein College of Medicine
Dr. Matthew Scharff

Columbia University
Dr. Charles Cantor

Duke University
Dr. Robert Webster

Long Island Biological Association
Mr. Edward Pulling

Massachusetts Institute of Technology
Dr. Boris Magasanik

Memorial Sloan Kettering Cancer Center
Dr. Bayard Clarkson

New York University Medical Center
Dr. Claudio Basilico

The Rockefeller University
Dr. Norton D. Zinder

State University of New York, Stony Brook
Dr. Thomas E. Shenk

University of Wisconsin
Dr. Masayasu Nomura

Wawepex Society
Mr. Townsend J. Knight

Yale University
Dr. Charles F. Stevens

Individual Trustees

John F. Carr
Robert L. Cummings
Roderick H. Cushman
Walter Frank, Jr.
Mrs. Mary Jeanne Harris
Amb. John P. Humes
Dr. Ralph Landau
Mrs. Mary Lindsay
Walter H. Page
William S. Robertson
Alexander C. Tomlinson
Dr. James D. Watson

Honorary Trustees

Dr. Harry Eagle
Dr. H. Bentley Glass
Dr. Alexander Hollaender

Officers and trustees listed are as of December 31, 1982

DIRECTOR'S REPORT 1982

Now 30 years have passed since Francis Crick and I discovered the double helix. We knew from the moment we first put together our two-stranded model for the precise arrangement of the atoms in DNA that we had found a uniquely wonderful molecule, the knowledge of which would transform the nature of biological research, if not of man's perception of his own meaning within our universe. Our spring of 1953 thus was filled with the exhilaration that came from the winning of a very big race as well as the thrill that came from the realization that we were the first to perceive the key secret of the gene that gave it the power of accurate self-replication.

Soon we moved on to worrying about how the key features of the double helix could be definitively proved correct and then how we might determine the way in which the genetic information within it was used to order the amino acids within polypeptides. Solving these problems was clearly not going to be easy, and never did we see the need to fantasize as to where DNA research might take us over the long haul. The genetic engineering tricks of today were beyond our wildest imagination, with neither Francis nor I having the slightest addiction to science fiction when our science itself could be so immensely rewarding.

Now I can only feel very fortunate to have lived during the era that already by its 20th birthday (1973) had given us an elegant, practical way to make recombinant DNA and through it a virtually unlimited power to probe the remaining key mystery of life. What has been learned about DNA in just 30 years perhaps only has a parallel in the intellectual achievements that marked the revolution in atomic physics which began with Rutherford and Bohr in 1912 and culminated with the building by Fermi and Szilard of the first atomic pile in 1942. And like with that grand era of physics, the early days of DNA involved the participation of a small group of highly perceptive scientists. For the most part, they were close friends and took real pleasure in the accomplishments of their peers even when at times it meant that another had first achieved the objective they had also keenly wanted.

Fortunately, there were so many good discoveries to be made that virtually all the pioneers with DNA did experiments of great importance, and I do not remember the first decade of the double helix as a period of happy winners and unlucky losers. Of course, here I am referring only to those scientists who, after the double helix was found, stopped thinking about whether DNA was important, knowing that from 1953 on the only sensible way to attack genetics was to start with the base pairs of DNA. There was, of course, a small coterie of malcontents who disliked the double helix from the start. Given that bias, they had little chance for future success, and to judge by their later bitter objections, first to the general validity of the central dogma (DNA→RNA→protein) and then much later to any use of recombinant DNA, they must have known that real science was passing them by.

And while the fundamental significance of many newly reported experiments was often debatable, the experimental facts themselves were seldom that controversial. To be sure, certain results were misinterpreted long enough to reach the print of a respectable journal, but such errors were few and far between and through them the course of DNA research has probably not suffered more than a month or two delay.

Of even less consequence has been actual fabrication of false data. For the most part, these forgeries were reported as semi-anticipated extensions of the conventional scientific wisdom of the moment. For example, the recent claim that a protein kinase cascade gives rise to the cancerous phenotype built upon the prior discovery that the oncogenic protein coded by the Rous sarcoma virus was a protein kinase. But almost from the moment the cascade was proposed, an undercurrent of rumors arose that no graduate student, much less mature scientist, had ever accomplished that much science in such a short time. And when the troubling fact became known that key experiments could not be repeated in other labs, the burden of proof quickly passed back to the lab making the sensational claims. To be sure, the protein kinase cascade dominated the conversations of retrovirologists for several months, but the only real scientist seriously harmed was the supervisor of the now revealed to be long-term compulsive faker.

Massive fakeries of the protein kinase cascade variety have been very rare occurrences in the

world of molecular biology. The most sensational of these soon generated skepticism from labs doing similar research which desired independent confirmation before accepting the claims at face value. In each such case the putative facts were too important to be ignored, and of necessity other labs began to repeat the experiments. On the other hand, when only marginally interesting claims are made, no one is likely to repeat them. Scientifically irrelevant fakes, however, could be more common occurrences, since advancement in many academic institutions depends upon the number of published papers. Unfortunately, libraries, like our own, all too often subscribe to many effectively unread periodicals which serve as sitting ducks for inventors of irrelevant data. By so subscribing, we keep in existence journals whose only honest justification is their availability for the occasional important article that has been mistakenly rejected by the major journals of the moment.

It has thus made good sense to accept as correct the facts that we read in journals like *Nature, Cell,* or the *Journal of Molecular Biology*. To be sure, we may sometimes reject the way some of these facts are interpreted, and it is often by such questioning that we move science forward. But we should not make too much of a point about where we have occasionally gone wrong. The past 30 years of DNA research have not been dominated by frequent reversals of scientific direction. Instead, it has been an era of decisive experiments that rightfully have generated virtual immediate acceptance.

During the early days of DNA research there was no way to guarantee that practical benefits for mankind would emerge, and there were indeed well-known scientists, like the celebrated Australian immunologist Macfarlane Burnet, who wrote with almost passionate conviction as late as 1962 that the worlds of molecular genetics and medicine would never meaningfully come together. In his view, we had no more to offer society than a group of high-powered philosophers worried about the meaning of life or the nature of free will. Then I might also have predicted that our one day knowing the complete sequence of the DNA of a human chromosome was an even less likely event than my witnessing a genie arise out of a bottle in the Arabian Desert. Fortunately, I had the good sense never to put in print what I thought the future would bring.

Nowadays we hear much more often the opposite complaint that because of the recombinant DNA procedures that promise to bring about great advances in medicine, agriculture, and possibly the chemical industry itself, we shall be forced to surrender our highly valued academic freedom for the nasty bustle of the competitive market place. To those like me brought up in a depression era when a scientist choosing a career in biology could look forward to a life of almost monkish austerity, it is still a shock to see friends driving cars that in the past were totally off limits to any academic, save those few either born rich or fortunate enough to be a highly paid consultant to the chemical or securities industries.

We must take care, however, not to be dominated by petty jealousy of our new breed of molecular biologist-entrepreneur. I am not among those who believe that poverty enriches the soul and remember well how my American fellowship in a still impoverished, just post-war Europe let me virtually commute between the best labs in England and on the continent. My scientific career was thus greatly advanced. At worst I anticipate that my now much more affluent peers will at least share the costs of a joint dinner and at best dream that they will soon sense the virtues of enlightened philanthropy and see the Cold Spring Harbor Laboratory which helped shape their scientific careers as the obvious institution for buildings that bear their names.

I also do not believe we should be that much concerned about the fact that a large number of senior academic molecular biologists will be spending a noticeable fraction of their time consulting with industry. Such arrangements have for decades been the norm with chemists, and I have never heard the argument convincingly made that chemistry would be a more exciting subject if it could be divorced from industry. That chemistry today lacks much of the excitement that it had 30 years ago more likely reflects the fact that important new discoveries in chemistry are much harder now to make. As a consequence, many of the better graduate students who in the past would have gone into chemistry now opt for careers in molecular biology-biochemistry.

We should, moreover, take note of the fact that much, if not the majority, of high-quality science teaching is done by younger scientists not yet known to the outside world. It is this younger group who, furthermore, does much of the incisive research that outsiders normally credit to the senior faculty. And those individuals who do annoy us by their excessive outside advising are seldom those still capable of either inspiring or teaching well our better youth. It is not that academics are absent for a day or even a week of talks with industry that really bothers their peers. Instead, it is usually the fact that this absence comes on top of their inability (disinclination?) to teach well, a trait long noticeable before they began to consult and which shunted them away from maintrack teaching assignments.

Of course, there are other scientists who both teach and consult well, and we are better off for their existence. There may, in fact, be little correlation between being a consultant and the quality of teaching. So, I would be surprised if the quality of academic life were to rise perceptibly if we were to stop all consulting arrangements with industry. Thus, given the increasingly eroding scientific salaries, even within our major universities, it may be a blessing to all concerned that consulting arrangements make possible family incomes for selected senior faculty that actually relate to the financial requirements of full-time intellectuals

colon and lung cancer cell lines. Why such large genes are used to code for such relatively small proteins is most unclear. Whether still other RNA genes exist is not yet known, nor do we have any idea as to the differences in function between the H.RAS, N.RAS, and K.RAS genes. It is tempting to follow Jim Feramisco's hunch that they may be involved in the modulation of signals initiated when specific receptor proteins recognize their respective agonists. Until, however, real biochemistry is done with the RAS proteins, we shall have no way to test such speculations.

The Cassette Model Moves to the Level of Enzymology

Over the past several years our Yeast Group has provided crucial molecular evidence for the concept that the silent mating-type cassettes at the storage sites *HML* and *HMR* donate copies of themselves that move to and replace functional cassettes at the *MAT* site. But how this happens at the molecular level was until very recently a total mystery. Then, Jeff Strathern, working with other members of the Yeast Group, observed a unique double-stranded cut within the *MAT* DNA in strains frequently switching mating type (homothallic strains) but no such breaks in DNA from non-switching strains. This result suggested the presence within switching cells of a specific endonuclease that recognizes a sequence found at the *MAT* locus. Such an enzyme was looked for and found by Richard Kostriken working with Fred Heffron in Demerec Lab. They call their enzyme YZ endo. The finding that double-stranded cuts initiate cassette movements closely followed upon new data from John Szostak's lab at the Sidney Farber Center which indicated ordinary genetic recombination in yeast also largely proceeds with double-stranded cuts. When we better understand both processes at the molecular level, we may well find that they share many common steps.

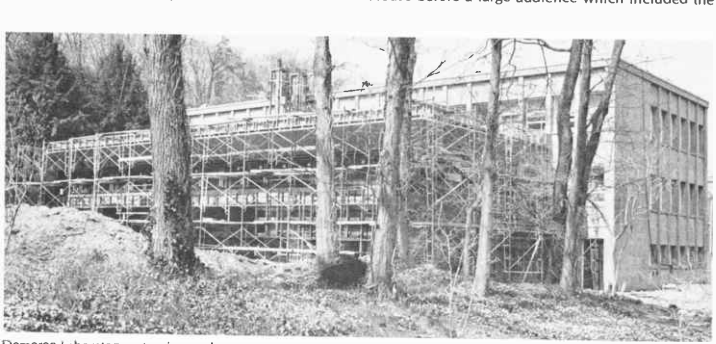
The First of Our Exxon Scientists Arrives

The agreement we concluded in May of 1982 with Exxon specified that up to six Exxon scientists will work here at Cold Spring Harbor over the next several years doing research of joint interest to ourselves and Exxon. Early in the fall, Susan Bonitz, the first of those appointments, joined the Yeast Group. Prior to her arrival here, Susan was for a year with Genentech, after finishing her Ph.D. at Columbia University on the structure of the yeast mitchondrial genome. Within Delbrück Lab Susan will initially work on RNA splicing mechanisms in yeast mitochondria.

Key to our being able to provide adequate research facilities for the Exxon scientists is the construction of a badly needed new addition to Demerec Lab. Work on this addition began in the fall of 1982, with a target finishing date of July 1, 1983. This 6000 sq. ft. addition will let us initiate major new efforts in protein chemistry, in vitro mutagenesis, and genetic recombination at the DNA level. The equipment for these labs, as well as the funds needed for the addition, are being funded largely through our agreement with Exxon. Soon our scientists will have the opportunity to work with equipment commensurate with the doing of the most sophisticated forms of recombinant DNA research.

Dedication of the Reginald G. Harris Research Building

On May 27, 1982 we dedicated the Reginald G. Harris Building, a most attractive new facility that at long last gives us adequate holding space for the mice, rats, rabbits, and guinea pigs that we utilize in our cancer research as well as facilities for the breeding of those unique mouse strains used by Lee Silver in his studies on the T locus. The opening ceremony was held on the lawn below Davenport House before a large audience which included the



Demerec Laboratory extension under construction

unable to moonlight on weekends say as taxi drivers or security guards.

So, instead of focusing on the imputed evil tradeoffs of the salary supplements given to individual scientists by industry, our academic institutions might best see how they themselves might be recipients of equally invaluable industrial largesse. Just as with consulting, we should not expect a one-way flow of benefits; it only makes sense for industry to ask how it can direct help to those who might in return benefit them. Here molecular biology occupies a privileged position since this is still a discipline almost totally lacking at the high levels, if not the middle levels, of management. While there now exist many chemists in the top ranks of industry, there are virtually no molecular biologists so sited. High-level advice as to how to properly utilize their techniques has to come from the outside. And instituional help per se, as opposed to assistance from individuals, may often be required to let the benefit of the recombinant DNA-hybridoma revolutions be rapidly assimilated by our industries so that they can remain competitive with the outside world.

The arrangement we concluded a year ago with Exxon may, in fact, serve as a model for such programs of mutual help. We saw the imperative to radically expand our facilities for protein chemistry and nucleic acid chemistry at the micro level, both to let us probe even deeper the nature of the genes that cause cancer, as well as to begin to explore the genes that control the development of nervous systems. But we saw no immediate way to obtain the funds to add the new space to Demerec, our laboratory best suited for such work. At the same time, Exxon, seeing the long-term need to have a first-rate research group in biotechnology, needed help in moving into a field with which they had no previous practical experience. We thus agreed on a joint program of research in which we help Exxon recruit a number of promising young molecular biologists who will first come to CSH and use our new facilities for recombinant DNA research and then move on to the modern, new research labs that soon will be completed for Exxon in Clinton, New Jersey.

While here, the Exxon scientists will also be members of our scientific staff and will work on problems of joint interest to both Exxon and this Laboratory. No product-oriented research will be carried on here, the main emphasis being the development of recombinant DNA procedures that may someday hasten the development of commercially useful genetically engineered enzymes. We in no way see our academic freedom or potential for excellence threatened; just the opposite, we now have the funds not only to add a major new addition to Demerec Lab, but also to obtain the equipment and additional staff and postdocs that will let recombinant DNA research proceed here at the rapid pace that necessarily characterizes the best of the recombinant DNA world.

At Cold Spring Harbor we can thus anticipate with great pleasure the next decade of DNA research. It would be most surprising, indeed, if it does not also dazzle us with the unexpected and produce much well-deserved satisfaction for those who combine optimism with the capacity for sustained intelligent hard work. I am, of course, assuming that the federal government will continue to provide the main support for our research as well as that of other leading institutions. Here I believe that there is no way that our nation can continue to help lead the world without the exploiting of science to the fullest extent. And given that of all the fields of science, molecular biology now shows the greatest promise of development for the public good, I believe we would be virtually silly to worry excessively as to whether our scientific endeavors might suddenly be downgraded to the back burner. We are thus in the position of a front runner who has the capacity of staying ahead. This is an opportunity we must not squander.

Highlights of the Year

Cancer Gene Research Becomes Even More Exciting

Last year I reported successes in the cloning of a DNA segment, isolated from a human bladder cancer cell line, which has the capacity to convert the mouse cell line NIH3T3 into its transformed cancerous equivalent. This research done in Demerec Laboratory by Michael Wigler and his associates was soon followed by similar feats at MIT and NIH. Each of these labs isolated the same 5.6-kb gene called Hu-H.RAS. Hu-H.RAS is closely homologous to the oncogene of the Harvey sarcoma virus that Ed Scolnick and his group at NCI have shown to be a GTP binding protein located at the cytoplasmic surface of the plasma membrane. Subsequent DNA sequence analysis revealed that Hu-H.RAS codes for a 189-amino acid protein that differs from its homolog found in normal cells by only a single amino acid replacement, glycine to valine at position 12. Somehow this single change converts a normal protein into one that makes its respective cell cancerous. How this happens is still a total mystery since no one has any idea of the function of Hu-H.RAS.

Recently, two additional members of the human RAS gene family have been cloned by the Wigler group. Each codes for a protein very similar to Hu-H.RAS. One, called Hu-N.RAS, isolated from a human neuroblastoma cell line, is 13.5-kb in length and contains much longer introns than found in the Hu-H.RAS. Even larger is the 45-kb DNA segment that codes for the third RAS gene, Hu-K.RAS, whose cancerous equivalents are found in several human

attendees of our annual RNA Tumor Virus Meeting. Speaking first was Harry Eagle of the Albert Einstein College of Medicine, who in the past has served us so well as Chairman of our Board of Trustees. Giving the main speech was Vincent DeVita, Director of the National Cancer Institute, who related the problems he faced in attempting to fund increasing numbers of first-class research proposals while facing a static level of federal support for the National Cancer Institute.

After solving several teething problems with the air-conditioning and heating systems, the Harris Building works very well indeed. Over the past month we have been modifying still unoccupied first-floor rooms into a laboratory for mouse embryology that shall first be used for a course on the Molecular Embryology of the Mouse to be held in July of 1983. Afterward, we shall use these new lab facilities for introducing new forms of recombinant DNA vectors into early mouse embryos, with the expectation that many of their respective genes will be functionally integrated into the chromosomes of the host embryos. Thus, through possession of the Harris facility, we cannot only extend our efforts toward understanding cancer, but perhaps also play a leading role in the development of procedures for the genetic engineering of the mouse.

A First-class Symposium on "The Structures of DNA"

The realization in 1979 that certain segments of DNA might twist to the left as well as to the right brought increased attention to the exact molecular conformations of DNA and to the physical and chemical techniques used in their study. The time had thus arrived for a fruitful interaction between those physicists and chemists concerned with the precise arrangements of atoms within DNA and those scientists concerned with its replication and transcription. So we decided to hold our 47th Symposium on "The Structures of DNA." The opening address was given by Alex Rich, the discoverer of left-handed DNA, and Aaron Klug provided a most perceptive summary of the major advances reported during the seven information-packed days of the Symposium. By the end of the meeting we realized that our expectations for much intellectual enlightenment were more than exceeded, and many participants told us that the 1982 Symposium was the best meeting they had ever attended. Few conferences now extend as long as the Symposium, and the fact that so many participants attended all the sessions reaffirms our belief that the Symposium continues to be a major asset toward the advancement of modern biology.

The Past Summer Brings Almost 3000 Scientists to Cold Spring Harbor

In no past year have so many persons come to Cold Spring Harbor for our various meetings and courses. Our spring set of meetings was dominated by recombinant DNA, starting with the first meeting ever devoted to the increasingly important subject of Heat Shock. Then successive weeks witnessed meetings on In Vitro Mutagenesis, RNA Processing, and RNA Tumor Viruses. Our nine-week period of summer courses commenced as soon as our Symposium ended. Then our late-summer set of meetings started up. These were dominated by various sets of viruses—SV40, Polyoma and Adenoviruses, Phage, Herpes, Papilloma, and Poxvirus—into which was interposed our Cell Proliferation meeting on Teratocarcinoma Stem Cells.

Our six courses on Molecular Genetics and Cells, held in James and Delbrück Labs, all represented continuation of courses given in previous years. Most of our courses in Neurobiology also continued successfully. One brand-new offering, a combined workshop/course on Molecular Neurobiology, was

Harris Building, view from parking lot; interior view

initiated through funds provided by the Sloan Foundation. It was organized by Ron McKay of our staff and Jeremy Brockes, then still at Caltech. The excitement that had emerged by its conclusion led to the obvious suggestion that we focus the 1983 Symposium on this subject.

Financing such a large number of summer courses over the future now seems problematical. We find that even when we obtain firm federal study section support, administrative decisions frequently greatly reduce the monies we finally receive. Now it appears that substantial help from private foundations will be essential if we are to help provide a continuing flow of highly trained individuals into science. Already I must express our great indebtedness to the Klingenstein Fund for their positive response to my frantic last-minute cries for help when the National Institute of Mental Health did not provide the support we had expected.

LIBA Initiates a Fund Drive for a New Auditorium

In the fall, LIBA committed itself to a fund drive to raise $500,000 toward the estimated $2,000,000 cost of a 360-seat auditorium building to be sited across Bungtown Road from our Bush Lecture Hall. Toward this end, Edward Pulling assembled and distributed to all LIBA members a most appealing brochure outlining the plans for the building as well as the ways in which it will be used. Not only has the brochure been very effective in mobilizing LIBA members who already have pledged over 60% of their goal, but it also led one of our neighbors who wishes now to remain anonymous to pledge a further million-dollar gift toward the construction costs. We have thus commenced working drawings for the auditorium, with the goal of starting construction by the late fall of 1983 so that it will be ready for dedication at the start of the 50th Cold Spring Harbor Symposium in 1985.

In looking forward to what promises to be a marvelous new addition to our facilities, we can again take comfort in the fact that our LIBA neighbors remain such effective and devoted champions of our efforts. Here we should note that Edward Pulling, the Chairman of LIBA, has now provided extraordinarily effective leadership for 15 straight years.

Continued Upgrading of Our Physical Plants

This past year witnessed replacement of totally inadequate heating and air-conditioning equipment within Demerec Lab by a series of heat pumps that now perform both heating and cooling functions. Not only was the original equipment out of date, but it represented planning for labs containing many fewer scientists and very few heat-producing machines. Equally important was the complete rebuilding of the Demerec labs now occupied by Richard Roberts and Dave Kurtz, as well as partial reworking of Michael Wigler's lab. In McClintock lab, the rooms renovated 12 years ago for neurophysiological experiments have been greatly upgraded to permit hybridoma and recombinant DNA research. In addition, a dark room and a new microscopy room have been created on the second floor. The former sheep shed, a storage space for Symposium volumes when I became director 15 years ago and more recently our Mouse House, has been completely renovated again into most attractive office and mini-lab space for neurobiology. It provides Ron McKay and Susan Hockfield a semi-civilized retreat when the summer courses force them to vacate their labs in McClintock and Jones Labs.

Mouse House lab

Urey Cottage, formerly a tiny summer house constructed by unemployed carpenters during the depression and since added onto several times in the process of becoming a year-round residence, was effectively gutted in the fall as the first step in its transformation into the new home for our publications department now housed in Nichols. During the winter a wing was built facing the hill on its west side, a shed extension added to the south, and a porch constructed on the east side that overlooks the harbor. The final renovations and refurnishings should be completed by late summer, thereby giving our crowded Nichols administration the additional space they so badly need.

A dignified new look was also given to the main reading room of the Library building through the placement of greatly expanded shelves for our periodicals and serials collections.

Firm Decisions Made to Construct a North Addition to James Lab and a Squash Court-Exercise Facility near the Tennis Court

Our tentative plans last year for a major new addition to James Lab have now solidified into firm working drawings approved by the National Cancer Institute which shall provide partial funding. When

Urey Cottage under renovation

its new north wing is completed, James Lab will at last have a year-round hybridoma facility as well as sufficient lab space so that each year's commencement of summer courses does not automatically set back the research of the year-round James staff. Happily, our architects correctly estimated what the addition would cost, and the low bid of $1, 297,000 was within our committed resources. A contract has already been signed which shall allow construction to start in late June of this year (1983).

Our trustees also approved the plans for a squash court that will be partially built into the hill leading up to the tennis court. Originally conceived solely as a squash court, it is now planned to contain also a separate room for an exercise machine as well as appropriate shower and rest room facilities. Working drawings are now being made, and we anticipate using an outside contractor so as not to further overburden our buildings and grounds personnel. Construction is planned to begin by early fall, so that, with luck, the much-needed addition will be available for use by lab personnel in late winter.

Planning Commences for Expanded Facilities for Plant Molecular Biology

Already two scientists (Russell Malmberg and Stephen Dellaporta) in Delbrück Lab are working full-time with plants, and both Jim Hicks and Jeff Strathern are devoting smaller parts of their efforts to research with plants. Concurrently, we have initiated a very successful summer course on Plant Molecular Biology that will be given for the third time in 1983. Unfortunately, we see no way to expand Delbrück into a building large enough to permit the Yeast Group to continue flourishing as well as to establish an equally effective research team focused on plants and their potential for genetic engineering.

For the moment, we have a small, satellite, plant molecular biology lab located at Uplands Farm, a former dairy farm that belonged to Mrs. George Nichols and which was willed to the Nature Conservancy upon her death in late 1981. We are using for plant research her former greenhouses, as well as a field where Barbara McClintock and Stephen Dellaporta can grow corn during the coming summer (1983). We have also applied for funds from the National Science Foundation to convert the Uplands Farm garage, in which we now store and ship our books, into a lab for six to eight scientists. Hopefully, we shall start by early January the renovations needed to transform this solidly built brick building into a functional lab-office facility.

We realize that these plans are very ambitious, if not audacious, considering the currently low funding level for plant molecular biology within the United States. There are signs, however, that our governmental agencies are seriously considering rapidly expanding their support for this area. In addition, the possibility exists that we might be able to

fund much of this research through arrangements with large corporations that see recombinant DNA procedures as major future tools for agricultural research.

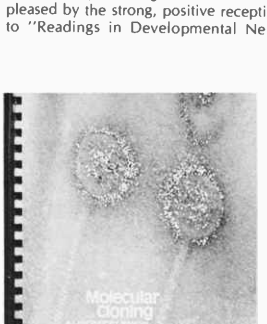
Our Molecular Cloning Manual Has Already Sold 18,000 Copies

Publication of "Molecular Cloning," a manual for genetic engineering by Tom Maniatis, Edward Fritsch, and Joe Sambrook occurred in June 1982. Prior to its appearance we had orders for more than 5000 copies and were assured of a best seller. But we did not in any way anticipate the broadly based appreciation it would quickly receive and its rapid adoption by many, many university courses. Already (late June 1983) over 18,000 copies have been sold, almost twice the number of any previous book published by us, and it seems likely that at least 25,000 copies of the first edition will be purchased. We have plans for a revised edition to appear in 1984, and hopefully we can maintain much of the student market that we have captured.

This year also marked the publication of many other fine books, including the massive 1400-page "RNA Tumor Viruses" edited by Robin Weiss, Natalie Teich, Harold Varmus, and John Coffin. Because of its size it could not be priced cheaply, but nonetheless it has already sold nearly 1500 copies. We should also note the rapid publication, in a most-handsome form, of "Heat Shock," a volume arising out of a meeting on this topic and which appeared in print only 5½ months after the meeting. Such speedy publication owes much to a major editing effort by Alfred Tissières who remained in Cold Spring Harbor for the month following the May meeting. We have also been most pleased by the strong, positive reception accorded to "Readings in Developmental Neurobiology" edited with critical comments by Paul Patterson and Dale Purves. And we note with great satisfaction the appearance of our 1981 Symposium volume "The Organization of the Cytoplasm" and the ninth volume in our Cell Proliferation series "Growth of Cells in Hormonally Defined Media." Both sets of books represent major editing efforts by our publications staff.

Nancy Ford

The number of volumes sold in 1983 was 37,337 compared to 16,056 in 1981. Although much of this increase reflects the spectacular sales of the "Molecular Cloning" manual, subtraction of its 1982 sales of 13,103 still leaves 24,234 sales, a most substantial advance over 1981. We should note that 1982 represents the tenth year that our Publications Office has been headed by Nancy Ford, who deserves much credit for the loyal, intelligent way she has efficiently guided our publishing effort to its current state as a major supplier of advanced books in molecular biology.



Molecular Cloning Manual

More Major Changes in Our Scientific Staff

Departing in August of 1982 after 12 years at Cold Spring Harbor was David Zipser. David came here from a tenured position at Columbia University to help reestablish genetics as a major focus in our intellectual life. Since then his lab has welcomed many younger scientists whose careers first began to flourish here at Cold Spring Harbor. Over the past several years David's main interests have switched to the brain and how computer modeling may help us understand how information is processed by integrated sets of nerve cells. Through moving as a Research Professor to a Cognitive Science Group at the University of California, La Jolla, David will be able to interact with other scientists of similar interests.

Leaving for an Assistant Professorship at the University of Alabama Medical School was Jeffrey

David Zipser

Engler who first as a Postdoc and then as a Staff Investigator worked so effectively on adenovirus DNA. Also moving to a medical school environment was Manuel Perucho who has joined the Microbiology Department at the Stony Brook Medical School. Manuel played a key role in the isolation of the human bladder cancer cell gene. We shall also miss Jim Stringer who has moved on from a Staff Investigator position in James Lab to the University of Cincinnati Medical School where he will be an Assistant Professor of Microbiology. Departing at the end of the year to help found California Biotechnology, Inc., was John Fiddes who as a Senior Staff Investigator in James Lab greatly advanced our capacity to do high-level recombinant DNA research.

In the spring of 1983 Maggie So and Fred Heffron left their positions as Senior Staff Investigators to join the scientific staff of the Scripps Research Institute in La Jolla. Likewise moving to Scripps Research Institute was Rasika Harshey who as a Staff Investigator associated with Ahmad Bukhari's Lab did many imaginative experiments on phage μ.

Leaving their Postdoctoral slots here were Bruce Anderson to run a lab at Fairfield Hospital in Australia, Marilyn Anderson to join a Plant Cell Biology Group at the University of Melbourne, Hans Engeser to return to Germany, Maria Goradia to do research at the College of Physicians and Surgeons of Columbia University, Margaret Hightower to move to the Population Council Center for Biomedical Research in New York City, Deborah and Theodore Kwoh to join the teaching staff at the Veterinary Microbiology Department of the University of Saskatchewan, Sandra LeMaster to move with her husband Jim Stringer to Cincinnati, Sam Silberstein to return to his position at the National Bureau of Standards in Washington, Joseph Sorge to join the staff at the Scripps Research Institute in La Jolla, and Nikos Vamvakopoulos to return to Greece.

Promoted to Staff Investigator from their Postdoctoral positions here were Russell Malmberg, Fumio Matsumura, Earl Ruley, and Paul Thomas. Likewise joining our staff as Staff Investigators were Fevzi Daldal, a Turkish citizen who after receiving his Ph.D. in France was a postdoc at Harvard Medical School with Dan Frankel, and Ed Harlow who did his Ph.D. at Imperial Cancer Research Fund with Lionel Crawford. And moving up to Senior Staff Investigator positions were Bruce Stillman, Jim Lin, and Ron McKay. Also newly appointed as Senior Staff Investigator was Mary-Jane Gething, who also comes to us from the Imperial Cancer Research Fund where she was on the scientific staff after completing her Ph.D. in her native Australia.

Three new Additions to Our Senior Scientist Ranks

At their spring meeting our Trustees approved the promotion of Yakov Gluzman, Amar Klar, and Jeffrey Strathern to the rank of Senior Scientist beginning July 1, 1982. Each position carries a rolling commitment of five years of salary support, providing the closest approximation to tenure that we can honestly offer considering our limited endowment funds. So promoting them signifies our belief that they have the capacity for continued highly creative research.

Our Ability to Grow Intellectually Continues to Depend Upon Our Robertson Research Funds

This year marks the tenth anniversary of Charles S. Robertson's major gift to us that established in 1973 the Robertson Research Fund. Now it is virtually impossible to conceive the healthy functioning of our Lab without the funds that it provides to renovate and equip our major laboratories as well as to provide much-needed fellowship support for younger scientists. The Robertson Research Fund Fellows during this year were Kenji Shimizu and Masao Yamada, both from Japan, and David Beach from England. Robertson money was also essential in permitting Robert Franza to join Jim Garrels' group to use its sophisticated technology for 2-D protein gels to examine the unique features of cancer cells.

A Very Successful Year for Our Banbury Center

When Charles S. Robertson donated his Banbury Lane estate to us, he did so with the knowledge that we would use it as a center for advanced teaching in neurobiology as well as for the holding of high-level conferences in biology. Last year, the fifth year the Banbury Center has been in full operation, was indeed most successful, witnessing a wide variety of meetings that collectively brought some 400 leading scientists to discuss problems ranging from the feasibility for the genetic engineering of man

and the risks we face from exposure to DNA damaging agents to the nature of senile dementias like Alzheimer's disease. In virtually every case we were able to fund these meetings adequately, and so the Banbury Center had nearly a balanced budget. Our early fears that we might not be able to put on outstanding small conferences without a virtually hemorrhagic drain on the Laboratory's general funds have thus happily receded, at least temporarily, from our thoughts.

Succeeding Victor McElheny as Director of the Banbury Center is Michael Shodell. After obtaining his Ph.D. from Berkeley, Mike was for five years on the staff at the Imperial Cancer Research Fund in London. Upon returning to the States he combined teaching at nearby C.W. Post College with a freelance career in science journalism. His strong background in cancer research fits in nicely with our aim to maintain the Banbury Center as a site for small meetings on Cancer Risk Assessment. Equally valuable is his interest in journalism, given our desire to also use our Banbury facilities to promote the better understanding and use of science by the general public.

We Decide to Explain Ourselves Better to the Outside World

As we grow larger, to a size that soon will encompass more than 100 scientists and over 300 employees in total, it no longer makes sense to act as if the outside world is not interested in us and that no harm comes from many Long Island residents believing that we are a small, marine biology-oriented annex to the Cold Spring Harbor Fish Hatchery located across 25A. Not only do we do science that the general public would take pleasure in knowing, we are still not very known to the major executives of the many large companies whose corporate headquarters are in the New York region. We thus initiated a search for a new staff member to function as an Information Officer as well as to commence a more organized effort to raise monies for specialized projects. The person we eventually recruited, David Micklos, fills neatly all the qualifications we were seeking. He was trained as a biologist, spent two years teaching science in Botswana as a member of the Peace Corps, has professional teaching experience as a photojournalist, and has held a position with the major Public Relations firm, Hill and Knowlton.

For his first task David has prepared a most attractive brochure on our history and purposes that is not burdened down with the necessarily dense technical vocabulary found in the scientific sections of this Annual Report. Now he is working closely with Mike Shodell of our Banbury Center to initiate both a Corporate Sponsor Program aimed at bringing us into closer contact with companies actually engaged in recombinant DNA research and a Corporate Affiliate Program that should help major corporations not directly involved in recombinant DNA research appreciate the changes that biotechnology is likely to bring to both our individual lives and the ways our industries must function to maintain competitive positions on the world-wide scene.

Our Administrative Staff Continues to Function Well Under Trying Physical Conditions

Nichols Building, currently the center of our administrative activities as well as our publishing efforts, will get a major internal renovation when our Publishing Department moves up to their new, expanded Urey Cottage headquarters. The inherently dingy entrance hallway of Nichols will be opened up and many of the offices rearranged to handle much more efficiently our Accounting, Grants Management, and Personnel sections. Already we have fixed up new space for a business-oriented computer (Basic 4) which soon will record all of our accounting and grant management actions.

The fact that despite there being so little space now devoted to administration we still are in control of an operating budget that will grow to 17 million dollars in 1983 reflects the loyal, intelligent devotion to our purposes by our Comptroller Bill Keen and Administrative Director Bill Udry. Now newly promoted to the key role of Assistant Administrative Director is John Maroney, who formerly served us so well as Purchasing Director. Appointed as our Purchasing Agent was Peter Rice. We now miss Pat Hickey who recently resigned as Personnel Director, and we are now engaged in a search for his replacement. Grants Management continues to be very well handled by Steve Kron, ably assisted by Susan Schultz.

We Must Soon Acquire a Much Larger Computer to Aid Our DNA Research

The ability now to sequence very long DNA segments has created the need for both a means to store this sequence information as well as procedures for scanning such sequences for biologically important signals and for comparing given DNA segments to other such sequences. Now we use a modestly small DEC PDP 11/44 microcomputer for these tasks. Increasingly, however, we find this computer inadequate for our needs which by now would best be handled by the much higher-capacity VAX computer, also a DEC product, for which many newly developed DNA sequence analysis programs are available. We are already actively seeking the $500,000 sum needed to purchase our own VAX. When available, it will be located in a specially designed space for computers on the ground floor of the new auditorium building that we plan to have ready by June 1985.

We should note that our current PDP 11/44 machine also now handles 34 data processing terminals located in many offices in all our major scientific buildings. The availability of these data processing terminals has greatly expanded our ability to speedily turn out manuscripts and grant applications, and we have already passed the point where we would ever consider existing without the availability of centralized computer facilities. Here I should mention the key role played by Rich Roberts in bringing a workable computer to the aid of our staff and of the great skills that our resident computer expert Mike Balamuth brings to those who need his help.

Our Buildings and Grounds Department Performs Beyond the Call of Duty

Unlike most institutions where the Buildings and Grounds Department largely handles routine maintenance and grounds upkeep, we run in a very different way. Before coming here, Jack Richards, our Superintendent of Buildings and Grounds, was a successful contractor and so has the skills needed to let the Lab itself often supervise major lab renovations as well as the construction of new buildings. We thus have not used an outside contractor for the south addition to Demerec, with Jack directly dealing with sub-contractors of known reputation for careful workmanship. Such procedures can save us much needed monies as well as insuring more rapid completion of badly needed facilities.

Likewise, most impressive has been the professional way Hans Trede and his grounds staff have landscaped our newly completed labs as well as the new parking lot next to the Harris Building. I note also with great satisfaction the clearing and planting of grass in the great field to the north of Nichols. In this process a small, spring-fed pond has been created which already has attracted our resident mallards and barn swallows.

Continued Leadership from Our Board of Trustees

All major decisions such as the go-ahead signal for a new laboratory reflect lengthy deliberations by our Board of Trustees. We are indeed fortunate that we have a Board on which both distinguished scientists and lay persons serve. This mixed composition can let us move rapidly, for around one table the key scientific, esthetic, and financial inputs can be obtained. Until now, most such decisions were easy to reach since new research and teaching labs could be generated by the renovation of older, effectively unused buildings. Now we can no longer move this way, and to increase further our research and teaching capabilities we must construct new buildings. The new and almost finished south extension of Demerec and the about to be started north extension of James Lab represent the new way we must act. But in so expanding our physical plant we risk changing our quiet whaling-era appearance to that of the more ordinary research university. So we have been more than careful to instruct our architects to see that their new designs will still let us take delight in walking down Bungtown Road.

At the same time, we have to realize that the generation of new lab benches automatically must lead to new parking slots as well as to new dwelling places for the scientists who use them. An *Ad Hoc Infrastructure Committee* chaired by Bayard Clarkson has just been set up by our Board to examine these problems. They have a target date of October 1983 to come up with practical solutions for parking and housing that will improve the way of life for our staff as well as reassure our local community that we have no intention, in any way, of losing the bucolic feeling that since our founding has enriched the scientific life at Cold Spring Harbor.

Retiring from our Board at the conclusion of their statutory six-year terms were Bache Bleecker, Emilo Collado, and Mrs. Franz Schneider. All functioned most effectively in our behalf, and we trust that we can count on their continued support. Returning to our Board to again represent the Wawepex Society is Townsend Knight, a lawyer for the New York law firm of Curtis, Mallet-Prevost, Colt and Mosle. Newly elected for the first time as Individual Trustees are Oliver R. Grace of Cove Neck, a distinguished investor who has long served the world of medical research so well by his leadership of the Cancer Research Institute of New York, and Ralph Landau, one of our nation's most noted chemical engineers, vice president of the National Academy of Engineering, founder of the Halcon Corporation, and who for 30 years has maintained a home nearby on Long Island Sound at Asharoken.

This Is My 15th Consecutive Annual Report

In January of 1968 the trustees designated me as the new director, with my duties actively to commence in June of that year upon the completion of the spring term at Harvard. It was a momentous period of my life—I was about to turn 40, my book the *Double Helix* was to be published in the USA in February, coming out in April in London, and in late March I entered married life with Elizabeth Lewis, then a student at Radcliffe.

The Cold Spring Harbor Laboratory I took my young bride to that summer of 1968 was very different from today. It was physically run down, underfunded, and badly in need of staff. Even then, however, I never felt the Lab's future in doubt. Our site was beautiful, our traditions so great, and our friends and admirerers numerous.

Since those first days I have worked steadily to promote our causes. That we are now proclaimed a very visible success story owes much to many, many special people and institutions. I here mention only those closest to me. First I must acknowledge Harvard University for letting me start

out as director while retaining the security of a Harvard professorship. For eight years I tried to serve well both worlds, and my leaving of Harvard in no way reflected any lack of respect or affection for that great institution. I must also credit the perceptive support and friendship I have received from Bentley Glass, the late Robert Olney, Harry Eagle, and Walter Page, the four chairmen of our Board of Trustees that I have had the privilege to serve under.

Equally important has been the assistance of Edward Pulling and his late wife Lucy in introducing me and Liz into the warm, civilized world of the Long Island North Shore. I have also profited greatly from my 14-year association with our now Assistant Director Joe Sambrook. Together we have worked hard to make the Lab a world-class participant in cancer research. We would not have succeeded nearly so well without the astute financial know-how of our Administrative Director Bill Udry. He has helped generate the steady positive cash flow that has given us the ability to provide the modern laboratories and equipment needed to do important science. And with pleasure I acknowledge the steady leadership and good taste of Jack Richards, whose feeling for buildings has been a key ingredient in giving us a physical environment that delights not only our eyes but our intellect.

But most of all I am indebted to my wife Liz for the supportive and stylish way she has played the necessarily difficult role of the Director's wife while simultaneously giving me the family life I deeply cherish.

June 24, 1983 *J.D. Watson*

YEAR-ROUND RESEARCH

TUMOR VIRUSES

Molecular Biology of Tumor Viruses
Nucleic Acid Chemistry
Electron Microscopy
Protein Synthesis
DNA Synthesis

MOVABLE GENETIC ELEMENTS

Insertion Elements and Plasmids
Yeast and Plant Genetics
Molecular Genetics of
Pathogenic and Virulence Factors
Bacterial Transposition

VERTEBRATE GENE STRUCTURE AND EXPRESSION

Mammalian Cell Genetics
Hormonal Control of Gene Expression
Genes for the Major Structural Proteins
Structure and Expression of
Eukaryotic Genes
Molecular Genetics of the Mouse
Mammalian Cell Genetics

CELL BIOLOGY

Mammalian Stress Response
Biology of the Cytoskeleton
Quest 2-D Gel Laboratory

NEUROBIOLOGY

Leech Neurobiology
Anatomical Studies with
Monoclonal Antibodies
Molecular Studies of the Nervous System

First row: M. Mathews; M. Yamada, S. Hughes, D. Heltman, T. Kost
Second row: W. Osheroff; J.-Z Li; C. Bray, E. Waldvogel, J. Johansen; D. O'Loane
Third row: W. Boorstein, D. Pascucci; Yeast Group
Fourth row: B. Stillman, P. Lalik, M. Walsh; M.A. Ruley, E. Taparowsky; G. Binns, W. Welch

First row: *S. Hinton, G. Penzi, A. Bukhari, T. Patterson, B. Vogel; M. Manos, K. Van Doren*
Second row: *S. Hocktield; M. Costa; Y. Katoh; L. Edlund; K. O'Neill*
Third row: *C. Schley; B. Vogel; B. Zipser; Z. Manzoor*
Fourth row: *M. Rossini; M. Goldtarb, D. Birnbaum; G. Blose, S. Blose, N. Hattner, B. Daniels*
Fifth row: *W. Huse, M. Deluca; P. Meyer; C. Deal, E. Billyard; J. Emanuele, L. Cascio*

TUMOR VIRUSES

For many years, adenoviruses and SV40 have been the central obsession of the Tumor Virus Group. From time to time we discuss the possibility of switching to other topics but always we are drawn back to the DNA tumor viruses. They remain objects of extraordinary intellectual interest and they continue to serve as the major source of ideas that enliven studies of the molecular biology of eukaryotic organisms in general.

MOLECULAR BIOLOGY OF TUMOR VIRUSES

J. Sambrook, J. Fiddes, M.-J. Gething, Y. Gluzman, T. Grodzicker, E. Harlow, E. Ruley, J. Stringer, W. Topp, *B. Anderson, M. Anderson, J. Brandsma, M. Hightower, M. Roth, M. Yamada, B. Ahrens, P. Barkley, M. Cahn, R. Chisum, M. DeLuca, M. Dermody-Weisbrod, A. Devine, C. Doyle, L. Garbarini, M. Goodwin, R. Greene, D. Hanahan, D. Keller, R. McGuirk, M. Manos, M. Merle, D. Moll, M. Ramundo, L. Rodgers, J. Scal, D. Smith, C. Stephens, L. van der Wal, K. Van Doren, J. Wiggins*

Transformation and Viral Tumor Induction

W.C. Topp, M.J. Hightower, M.B. Ramundo, D.M. Smith, M. Dermody-Weisbrod, M. Cahn

This past year the research in the group again centered about two projects, the induction of breast fibroadenomas in female rats by group-D adenoviruses and the alteration in nutritional requirements for cellular proliferation that accompany oncogenic transformation by DNA viruses.

In collaboration with John Shanley and Claire Shellabarger at Brookhaven, we have determined that the inducibility of breast fibroadenomas by human adenovirus type 9 is hormone dependent, the virus being nononcogenic in ovariectomized female animals. Reimplantation of diethylstilbestrol (DES) does not restore oncogenicity and castration of males does not render them susceptible. The exact nature of this hormone dependence is under active investigation, and preliminary results suggest that the tumors themselves lack cytoplasmic estrogen receptors. Specific experiments are designed to determine the status of progesterone receptors and the progesterone dependence of tumor induction.

From a genetic standpoint, two interesting facts have been determined. First, although unusually similar one to another by the criteria of DNA/DNA heteroduplex analysis or restriction mapping, very few of the group-D viruses are active for fibroadenoma induction. Both Ad9 and Ad10 induce fibroadenomas in virtually 100% of female animals injected at birth but at least 10 other group-D viruses we have tested fail to do so. So far there is nothing about either the structure or the pathology of these various serotypes that could provide a clue to explain this variability, and experiments are underway to isolate in vitro recombinants (in collaboration with Tom Shenk) between oncogenic and nononcogenic group-D viruses. Mapping of the relevant sequences in this way will help us to identify the gene products and allow us to begin to study mechanisms.

The second interesting phenomenon is the variability in susceptibility to viral fibroadenoma induction amongst inbred strains of rats. To date Wistar/Furth, Long-Evans, Buffalo, and Brown Norway rats have been found to respond to viral injection whereas Fisher, Lewis, and ACI rats are resistant. There is an apparent correlation between susceptibility and MHC haplotype with the *a* and *l* haplotypes being resistant and the *u*, *b*, and *n* haplotypes sensitive. The F_1 generation of a cross between ACI (*a*) and W/Fu (*u*) is 100% resistant and an N_2 backcross to the W/Fu parent is now in progress. The hypothesis that susceptibility to viral fibroadenoma is linked to MHC will be tested in three ways. First, the N_2 generation will be MHC typed using monoclonal reagents provided by Abe Fuks and a correlation sought between MHC type and fibroadenoma appearance. Second, two more strains of a supposed sensitive haplotype and two of a supposed resistant will be tested. Finally, Lewis inbreds which are congenic to either ACI or W/Fu at MHC will be challenged. Other possible explanations for the apparent genetic dominance of resistance include interferon inducibility, and experiments along these lines will be conducted in parallel.

Significant advances in our understanding of the altered growth requirements of transformed cells have been achieved in collaboration with Don McClure in the Cancer Center at the University of California, San Diego. Our experiments are designed around viral transformation of an established rat cell line, REF52. In addition to nutritional factors such as insulin, transferrin, hydrocortisone, and fatty acid, this line requires two mitogens, epidermal growth factor/vasopressin and the apoproteins of the high-density lipoprotein complex. SV40 transformation is accompanied by the obviation of the requirement of one of the two mitogenic factors for serum-free cellular proliferation. The phenotype of the transformants is only partially transformed, with most lines growing only poorly in soft agar. Selection of soft agar subclones produces lines that have obviated the requirement for both mitogens and these lines are tumorigenic. Nutritional requirements do not further change upon selection of tumor-derived sublines. The soft agar sublines are not highly tumorigenic, tumors appearing only after 3 months, so it is of interest that the complete obviation of requirement for mitogenic stimulation precedes the acquisition of oncogenic growth potential and is not coincidental with it. In the past, cloning efficiency in serum-containing soft agar has been found to be the most reliable correlate of cellular tumorigenicity, and the acquisition of this property also precedes oncogenicity in our system. However, only the highly oncogenic tumor-derived lines give good growth in serum-free soft agar in the absence of added mitogens, suggesting that it is this property that may be closely linked.

In a parallel set of experiments, we examined the loss of the actin cable bundles that has often been said to accompany the acquisition of anchorage-independent growth potential. Here again, we

found that cable loss preceded the appearance of oncogenic growth potential; however, in nononcogenic lines, the cables returned when the cells were cultured serum free. Only oncogenic lines were found to lack cables in serum-free monolayer cultures. It would appear that, on the basis of these results, SV40 transformation results in the acquired sensitivity to serum factors that produce anchorage-independent growth and loss of actin cables. However, this is not sufficient alteration to allow tumor growth and it is only those cells that exhibit these properties in the absence of added serum factors.

Infectivity of a Plasmid Clone of Adenovirus 5

D. Hanahan, Y. Gluzman

A full-length copy of an *Eco*RI-resistant variant of Ad5 has been cloned in *Escherichia coli* by linkage to the plasmid pXf3. The remnants of the Ad terminal protein were removed with NaOH, and the duplex molecule reformed by annealing. RI linkers were added to the flush ends. Following ligation to RI-cleaved pXf3, the recombinant was used to transform *E. coli* DH1. The clone that arose (pxAd5) contained full-length Ad5, which was releasable from the plasmid with *Eco*RI. No sign of rearrangements was detected.

The clone was examined for its ability to infect human cell lines (HeLa and 293). The cloned circular plasmid was not infectious. The RI-cleaved recombinant produced plaques when transfected into 293 cells, which carry and express adenovirus early proteins from regions 1A and 1B. Viral DNA was prepared and was shown to be RI resistant, which is not a characteristic of either the control viral DNAs used in the transfections or of other Ad5 virus used in the laboratory. The 3′ ends of Ad5 and the XAd viral DNA were labeled using T4 polymerase, cleaved with *Hha*I, and analyzed on a 20% polyacrylamide gel, using a sequencing ladder to provide markers differing by 1 bp. The ends of the XAd virus were precisely the size of wild-type virus. Thus, in the course of establishing an infection, the cloned virus DNA was relieved of the extra nucleotides on its ends and restored to its original configuration.

The specific infectivity of RI-cloned pXAd is about 5% of normal Pronased viral DNA. In vitro, RI ends are ~50% as efficient as ends carrying the terminal protein (Tamanoi and Stillman, *Proc. Natl. Acad. Sci. 79*: 2221 [1982]). Since the RI ends can function as origins of replication, the low infectivity may result from a competition between replication and degradation. When a bona fide Ad end (the 700-bp *Cla*B fragment) is ligated onto linearized pXAd, and the ligated DNA transfected into cells, the virus that appear carry the extra *Cla*B fragment on the right end, along with the original RI end embedded about 700 bp into the new end. This is consistent with other data that indicate that the Ad origin of replication is in part a free end, and if embedded, it is not active. The observation that initiation of RI-linked ends produces wild-type ends in virus bears on possible mechanisms of initiation.

Cloning of cDNA of SV40 Large T Antigen and Its Propagation as SV40 Virus

Y. Gluzman, J. Fiddes, B. Ahrens

The early region of SV40 codes for two early proteins (large T and small t antigens). The single transcript produced from the early region serves as a splicing template to originate two different messages. These messages, which have the same acceptor but two different donor sites, are translated into large T and small t antigens. The role of large T antigen in the initiation of viral DNA replication and initiation of transformation is well established. Using mutants with a deletion in the small t region, it was found that the complete integrity of small t antigen is not absolutely required in either lytic growth of SV40 or for transformation of some cells in vitro. However, most of these deletion mutants produced a truncated small t antigen. The influence of the altered proteins remains unclear. To bypass this problem, we cloned a complete cDNA copy of the SV40 large T antigen mRNA and placed it under the control of the bona fide SV40 regulatory region. The cloning of the cDNA was synthesized from SV40 early mRNA produced during infection of 293 cells with a helper-free Ad5 vector carrying the SV40 early region under control of the late Ad2 promotor. The cDNA was reconstructed to be colinear with the early SV40 region and was reconstructed with the SV40 late region. This construction resulted in production of the complete SV40 genome, with the exception that the early region has the cDNA for large T antigen. This DNA was used to transfect COS-1 cells to propagate a virus population containing the altered early region. Very slow growth was also observed after direct transfection on CV-1 cells, although plaques appeared after 20 days. The virus population grown on COS-1 cells was infectious and could replicate SV40 DNA on CV-1 cells, although with greatly reduced efficiency. The preliminary marker-rescue experiments located deficiency in the cDNA construct to *Hin*dIII B fragment which spans the splicing region. At present, we are investigating the role of small t antigen in complementing the growth of SV40 cDNA virus, the transforming potential of cDNA, and transcriptional controls of messages, produced from cDNA template.

Helper-free Adenovirus-5 Vectors

Y. Gluzman, K. VanDoren, D. Hanahan

In the last annual report, we reported the construction of helper-independent adenovirus-5 (Ad5) vectors. Since then, we have cloned and propagated several different genes of interest as recombinant

helper-independent viruses. These genes include: (1) SV40 T antigen-coding region under control of late Ad2 promoter; (2) wild-type SV40 early region with its own promoter and origin of DNA replication; (3) SV40 early region with its promoter but mutated origin of DNA replication; and (4) neomycin resistance gene from TN 5 under control of the SV40 promoter.

The biological functions of these genes were evaluated in a variety of assays. The vector containing the SV40 T antigen-coding region under control of the major Ad2 late promoter was examined for the production of T antigen. This construct produced large amounts of T antigen during lytic infection of 293 cells as judged by polyacrylamide gel electrophoresis with or without immunoprecipitation. The wild-type early region with its own promoter produced T antigen during nonlytic infections in a variety of cells as judged by immunofluorescence. Vectors containing the early region of SV40 with and without a functional origin of replication were assayed for their ability to transform Rat 2 or HS74BM (human fibroblast) cells. SV40 inserts lacking a functional origin of replication transformed cells as efficiently as the wild-type early region. These cells formed foci on monolayers of cells and efficiently expressed T antigen as judged by immunofluorescence. Several lines of HS74BM-transformed cells have been established and analysis of the DNA integrated into the genome is underway. A similar vector containing the neomycin resistance gene of TN 5 under control of the SV40 early promoter was assayed on Rat 2 and CV-1 cells. Cells were efficiently transformed to resistance to the drug G418 (neomycin analog). Preliminary results on the integration of this DNA into the genome of Rat 2 cells indicate that there are multiple insertions of the viral DNA as well as amplification of the integrated sequences. Further analysis of these DNAs as well as the DNAs isolated from neomycin-resistant CV-1 colonies is currently in progress.

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

Y. Gluzman, M. Manos, B. Ahrens

Mutant SV40 DNA insertions have been rescued from a variety of permissive cells transformed by UV-irradiated SV40. Viral DNA was rescued by fusion of these lines with COS-1 cells. Fusion with COS-1 cells provides a functional T antigen in *trans* that allows SV40 insertions in transformed cells to excise and replicate in the heterokaryons formed. The rescued SV40 inserts were cloned into the plasmid vector pK1. This cloning procedure restores a complete SV40 genome containing the early region (*Bgl*I–*Bam*HI) from the mutant inserts. These DNAs were analyzed by marker-rescue analysis, and some of them by DNA sequencing. A total of seven mutants were isolated (C6-1, C6-2, C2, C8, C11, T22, and BSC-SV1). The mutants contain lesions in different parts of T antigen that render the protein incompetent in viral DNA replication but functional in transforming cells in vitro. The mutations in C6-1 and C6-2 were determined by sequence analysis and found to be: nucleotide numbers 5074 and 5011 in C6-1 and nucleotide number 4360 in C6-2, which is 277 nucleotides away from the mRNA splice joint. Mutations in C6-1 affect both large T and small t antigens, while the mutation in C6-2 lies in the unique part of large T antigen. The mutations in C2 and C11 were mapped to the C-terminal part of T antigen encoded between nucleotides 3373 and 2848. The T22 mutation resides in the aminoterminal third of the protein. C2 and C6 (containing both the C6-1 and C6-2 mutations) appear to have an enhanced tumorigenicity in hamsters.

Illegitimate Recombination between Adenovirus and SV40 Genomes

L. Ling, M. Manos, Y. Gluzman

Different mechanisms of DNA recombination might lead to different final products. Analysis of the final products of recombination has been useful in studying the mechanisms of transposition and insertions of different movable elements. We have chosen to analyze junctions of adenovirus-SV40 recombinants because these junctions are the final results of illegitimate recombination occurring between two nonhomologous viruses: adenovirus 2 and SV40. The nucleotide sequences of six Ad2-SV40 junctions from three Ad2-SV40 hybrid viruses (Ad2**HEY, Ad2**LEY, and Ad2*D1) were determined. Comparison of parental adenovirus 2 and SV40 DNA sequences with the sequence at the Ad2-SV40 junctions revealed that five out of six junctions are abrupt transitions from Ad2 to SV40 DNA, and in one case (Ad2**LEY, right junction) there is an additional nucleotide at the junction, which cannot be ascribed to either DNA. Ad2**HEY and Ad2*D1 right junctions are identical and Ad2**LEY and Ad2*ND4 left junctions are identical, a result that strongly suggests these Ad2-SV40 hybrids arose by recombination between the linear Ad2 DNA and circular SV40 DNA, followed by recombination between Ad2 DNA and SV40 DNA present in the Ad2-SV40 hybrid DNA. The unambiguous transition of Ad2 DNA into SV40 DNA at the junction sites is an example of recombination events that have apparently occurred without any homology at the recombination site.

Transformation of *Escherichia coli*

D. Hanahan

During the last year, a study on plasmid transformation of *Escherichia coli* was continued both in James Laboratory and at Harvard University. The influence of the conditions under which DNA and cells are combined on the efficiency with which plasmid molecules produce transformed cells was examined

in considerable detail. Multivalent cations are necessary for transformation, and an extensive comparison of different combinations of cations has produced one set that is very effective in facilitating transformation by plasmids. About one in 500 plasmid molecules effects a transformed cell. This represents about a 100× improvement over the classical method of Mandel and Higa, which employs Ca^{++} alone.

Plasmid transformation can be loosely divided into two stages—uptake across the cell envelope and establishment as an autonomously replicating genetic element. DNA competition and plasmid cotransformation experiments suggest that uptake is efficient under these conditions and that multiple plasmids can be taken up with independent probability. Establishment may now be the rate-limiting step. Higher eukaryotic DNA inhibits transformation in *cis* but not in *trans*, and this inhibition is seen both in transformation by naked DNA or following in vitro packaging of recombinant cosmids and this subsequent injection into cells. Since two unrelated ways of putting plasmids into cells produce similar responses, it seems likely that this restrictionlike effect is manifested during establishment. Genetic studies in progress suggest that it may be possible to release this inhibition. The improvements in transformation efficiency are already enjoying wide application in the cloning of cDNAs (see reports by Hughes and Fiddes, for example), where efficient transformations are essential to maximize the yields of precious cDNA preparations.

Expresssion of SV40 Tumor Antigens from Adenovirus Promoters and Controlling Elements

T. Grodzicker, M. Yamada, M. Merle, C. Stephens

In an effort to study regulation of adenoviral transcription, processing, and translation, we have constructed a series of adenovirus-SV40 hybrid viruses that contain the SV40 *A* gene downstream from different adenovirus promoters. The SV40 DNA used contains the SV40 T antigen-coding region but is missing the SV40 early promoter and T antigen-binding sites. Thus, expression of T antigen is under the control of adenovirus regulatory elements. The SV40 *A* gene is a useful marker because reagents exist that allow the accurate quantitation of SV40 T antigen mRNA and protein in infected cells. Furthermore, the entire SV40 sequence and the adenoviral sequences in the region of interest are known (see Tooze, *DNA tumor viruses* [1980]; Gingeras et al., *J. Biol. Chem. 257:* 13475 [1982]). We also wanted to construct viruses that would produce large quantities of SV40 T antigen that could be easily purified and used in biochemical studies. These studies were conducted in collaboration with Dr. R. Tjian and C. Thummel at the University of California at Berkeley.

Precise Positioning of SV40 T Antigen-coding Sequences in Preselected Regions of the Adenoviral Genome

To place the SV40 *A* gene at several positions around the adenoviral major late promoter and late leaders, a combination of in vitro and in vivo recombination was used (see Fig. 1). The starting material was a series of plasmids containing adenoviral DNA linked to the SV40 *A* gene. These plasmids were constructed by S.-L. Hu at Cold Spring Harbor Laboratory. The joint between the Ad2 and SV40 DNA was located at a position on the adenoviral genome where we wanted the SV40 *A* gene to be located in the hybrid virus to be constructed. This Ad2-SV40 insert was ligated to a *Bam*-digested adenoviral DNA-protein complex. The product created would be a virus with a duplication of adenoviral DNA (in the insert and the left arm of the vector). Homologous recombination (see Fig. 1B) would excise the intervening DNA and produce a virus with the SV40 insert positioned at the desired location. We have found that the internal adenoviral fragment of the vector can be present or absent depending on the size of the insert. A favorable packing size for the final virus is probably selected. By using an adenoviral vector with Ad5 sequences in the left arm and Ad2 sequences in the plasmid insert, we can determine where recombination took place in the virus constructs to yield the final hybrid virus. We have found that recombination can occur very close to (within 200 bp) the SV40 insert.

The construct DNAs were cotransfected with human 293 cells with helper wild-type DNA and the resulting virus grown through two cycles of infection in simian CV-1 cells to select viruses that express SV40 T antigen. The SV40 helper function that enables human adenoviruses to grow in monkey cells is encoded by the carboxyterminal end of SV40 T antigen. The final viruses produced are shown in Figure 2. Viruses exist that have the SV40 *A* gene in the third segment of the tripartite leader (Ad-SVR284), and in the i leader (Ad-SVR274). The i leader is an auxiliary leader found frequently on mRNAs initiated at the major late promoter at early and intermediate times after infection, in the intron between the first and second segments of the tripartite leader (Ad-SVR26), in the intron between the second and third segments of the late leader (Ad-SVR280), and in the intron distal to the third segment of the late leader (R289).

Transcription and Processing of SV40 mRNAs in Cells Infected with the Hybrid Viruses

All of the hybrid viruses that contain the SV40 *A* gene in the region of the adenoviral major late promoter make very large amounts of T mRNA. They are made in the same high amounts as adenoviral late mRNAs. All of the virus make SV40 T mRNAs that are spliced and terminated at the position as

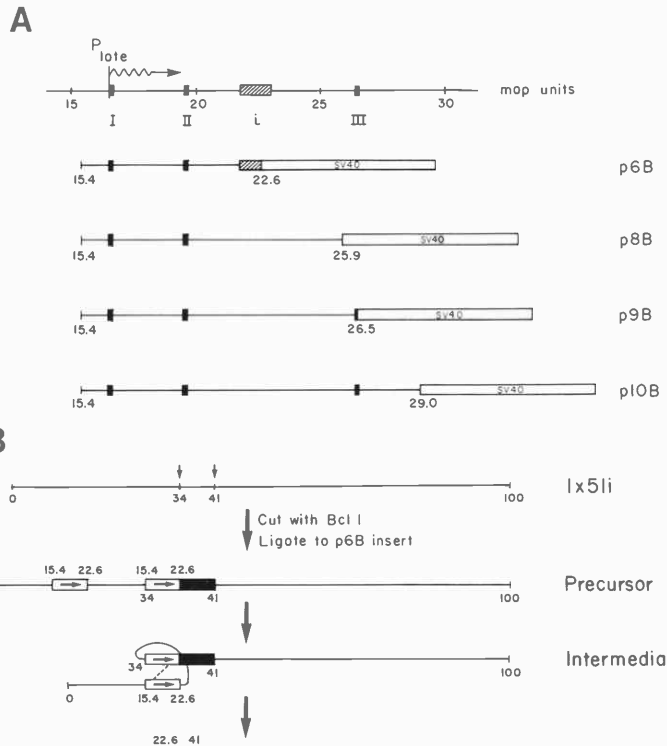


Figure 1

Construction of adenovirus-SV40 hybrid viruses. (*A*) Inserts of viral DNA in plasmids p6B, p8B, p9B, and p10B. On top is depicted a segment of the adenoviral genome, including the late promoter (P_{late}), three late leader regions (small dark boxes), and early i leader segment (hatched box). Below are the four *Bam* inserts that contain different lengths of adenoviral DNA fused to the SV40 *A* gene (open box). (*B*) An example of in vitro/in vivo recombination: the construction of Ad-SVR274. Above is depicted the 1 × 5li adenovirus vector with the *Bcl*I sites marked by arrows. Replacement of the internal 1 × 5li *Bcl*I fragment with the p6B *Bam* insert results in the proposed precursor to Ad-SVR274. The duplication of adenoviral DNA from map positions 15.4 to 22.6 is emphasized by the open boxes with arrows, and the SV40 *A* gene is represented by the dark box. Intramolecular recombination between the duplicated regions of adenoviral DNA, shown in the intermediate, would result in the formation of Ad-SVR274, shown at the bottom. Alternatively, recombination could proceed via an intramolecular intermediate.

authentic SV40 T mRNA. Analyses of SV40 mRNA by S1 nuclease treatment, Northern hybridization, and sandwich hybridization lead to the following conclusions: (1) All T mRNAs are initiated at the adenoviral major late promoter. (2) If the SV40 is inserted distal to any segments of the late leader, they are spliced together normally and found at the 5′ end of SV40 T mRNA (see, for example, Fig. 3).

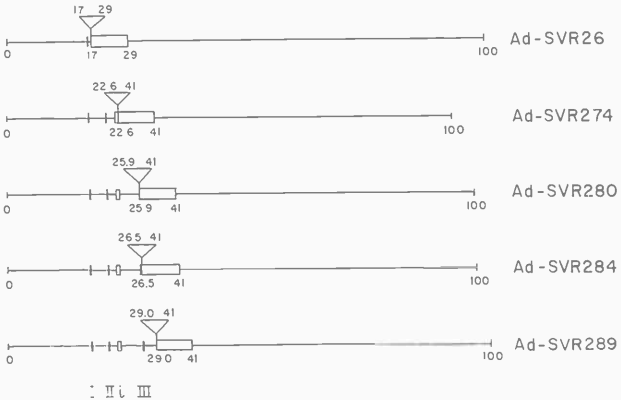


Figure 2
Genome structures of the adenovirus-SV40 hybrid viruses. The genome structure of Ad-SVR26, one of our previous constructions (Thummel et al., *J. Mol. Appl. Genet. 1:* 435 [1982]), is depicted above. The genome structures of five recombinant viruses: Ad-SVR26, Ad-SVR274, Ad-SVR280, AdSVR284, and Ad-SVR289 are shown. The late leader regions (small black boxes), early i leader (small open box), deleted adenovirus DNA (triangles), and SV40 sequences (large open box) are shown. The numbers are adenoviral map units (1 map unit = 360 bp).

When SV40 is in the intron between the first and second leaders (Ad-SVR26), splicing does not occur at the first leader donor splice site about 50% of the time. Instead the transcript is continuous past the first leader into the intron and the SV40 sequences. (3) When SV40 is in the i leader segment, large

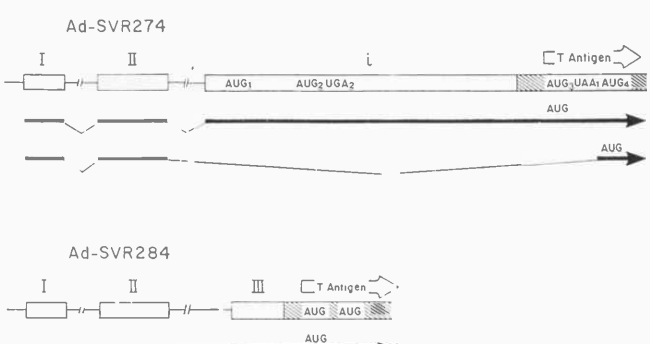


Figure 3
A schematic representation of splicing and translation at the 5′ ends of the Ad-SVR274- and Ad-SVR284-encoded hybrid mRNAs. Portions of the Ad-SVR274 and Ad-SVR284 genomes are depicted. The open boxes represent leader regions, the hatched boxes represent SV40 DNA, and the normal SV40 T antigen-coding region is marked with an open arrow. All AUG triplets in the DNA are marked; they are numbered in the Ad-SVR274 genome so that they can be matched to their appropriate termination codons. The SV40 AUG codons (3 and 4) are both in frame with the T antigen-coding region (Tooze *DNA tumor viruses [1981]).* The two adenoviral AUG triplets (1 and 2) are both out of frame with the T antigen-coding region and are also out of frame with each other (Gingeras et al., *J. Biol. Chem. 257:* 13475 [1982]). The dark lines below the genomes represent the predominant hybrid T mRNAs encoded by each virus. The AUG codons used for T antigen translation are shown above each of the messages.

amounts of T mRNA contain i sequences even though they are normally present at low levels at late times after infection in adenoviral late mRNAs. (4) Several cryptic splice acceptor sites in SV40 sequences are used with reasonable efficiency. For example, there is a very strong splice acceptor at the 5′ end of the SV40 sequences near the AUG initiation codon for T antigen. Presumably this is not used in authentic SV40 mRNAs because there is no donor splice site preceding it. Two other strong SV40 cryptic splice acceptor sites in the 3′ region of the gene are used to produce hybrid mRNAs. These sites are seen in all hybrid SV40 mRNA populations and not in SV40-infected cells. Thus, there is some feature of an adenovirus-infected monkey cell that differs from SV40-infected cells and influences the splicing pattern. (5) Very low amounts of small t mRNA are seen in hybrid-infected cells. Since large T and small t mRNAs differ only in the splice donor sites used to make the mRNA, it is likely that the adenovirus-infected cell is influencing the splicing pattern of mRNAs. However we have not ruled out that t mRNA is particularly unstable.

Translation Control of T mRNAs in Hybrid-infected Cells

In all hybrid-infected cells, the same large amounts of T mRNAs are produced. However different amounts of T protein are made. In Ad-SVR284-infected cells, for example, protein is overproduced to the same level as the mRNA. At least 5% of newly made protein is T antigen and one can isolate at least 2 mg of T antigen from 1 liter of infected cells. T antigens are detected by [^{35}S]methionine labeling of infected cell extracts. Proteins are analyzed on polyacrylamide gels before and after immunoprecipitation with various anti-T antibodies. Proteins are detected by autoradiography or Coomassie blue staining. Cells infected with the other hybrid viruses (see Fig. 2) make about 20-fold less T antigen although the protein is equally stable. However, when T mRNAs produced by different viruses are translated in vitro in reticulocyte lysates, the same amounts of T antigen are synthesized. Thus, there is some translational control in the infected cell that is not present in vitro.

Novel AUG Codons Can Be Used To Produce T Antigen

In some cases, T antigen synthesis does not begin at the normal AUG initiation codon. In Ad-SVR26 (see Fig. 2), a large proportion of T mRNAs carry sequences from the first leader and the intron between the first and second leader before the SV40 sequences. Two AUG codons are located in the intron RNA and are in the same frame as T antigen-coding sequences. Use of one of these AUG codons would produce a T protein about 5K larger than SV40 T antigen, and this is, in fact, found. The first AUG codon has a G at position −3 (GGCATGA) and an A at position +4 and thus falls into the category of efficient starts as proposed by Kozak (*Nucleic Acids Res. 9:* 5233 [1981]). Why translation of this mRNA does not produce as much protein as mRNA in which translation is initiated at the normal T AUG is not clear. The intron AUG may be intrinsically a weaker start sequence, the adenoviral leaders may play an important role, or more sequences than those immediately surrounding the AUG may be important for efficient translation initiation in vivo. In addition, mRNAs that have been produced by splicing at the SV40 cryptic splice acceptor site at the beginning of the SV40 sequences (see Fig. 3, for example) have had the normal T AUG removed by splicing. There is another internal AUG located 13 codons downstream that is in frame with the T AUG. Thus, T antigen initiated at this AUG codon would be close in size to wild-type SV40 T antigen but would differ at the N terminus. In cells infected with hybrid virus that contain T mRNAs with this new splice point, one can find, by immunoprecipitation, T proteins that comigrate with SV40 T antigen. They are efficiently immunoprecipitated with polyclonal T antisera or monoclonal antibodies that recognize determinants in the middle or carboxyl terminus of the molecule. However, they are not immunoprecipitated by monoclonal antibodies that recognize determinants at the amino terminus of the molecule. (Monoclonal antibodies were obtained from D. Lane and E. Harlow.) Thus, these T antigens have the properties predicted for T antigen initiated at the second AUG found in the T antigen-coding sequence.

Efficiency of Translation of T Antigen When the SV40 A Gene Is Transcribed as the Second Gene in a Dicistronic mRNA

There are two hybrid viruses in which the SV40 *A* gene is preceded by coding sequences for part of an adenoviral gene that contains an AUG start codon, coding sequences, and a translation termination codon. In Ad-SVR274 (see Fig. 1), the *A* gene is inserted into the middle of the i leader in the protein-coding sequences (C. Anderson et al., unpubl.). The reading frames of the i protein and T antigen are different so that a termination codon is introduced into the i coding sequence at the beginning of the SV40 insert. In Ad-SVR6 (Thummel et al., *Cell 23:* 825 [1981]; T. Grodzicker et al., unpubl.), the *A* gene is located in the adenoviral early 1B transcription unit about 200 bp downstream from the E1b 15K AUG codon. The 5′ end of the SV40 insert introduces a translation termination codon into the 15K reading frame (see Tooze, *DNA tumor viruses* [1981] for adenoviral sequence). Analyses of SV40 T mRNA by S1 nuclease analysis, R-loop mapping (in collaboration with L. Chow), and Northern blotting have shown that the SV40 sequences are preceded by the truncated adenoviral gene coding sequences in both Ad-SVR6- and Ad-SVR274-infected cells.

Analysis of T antigen protein by labeling with [^{35}S]methionine and immunoprecipitation with a variety of anti-T sera and monoclonal antibodies has shown that Ad-SVR274 produces low levels of a wild-type T antigen. Thus, when the SV40 *A* gene is the second gene in the mRNA, it is translated, albeit inefficiently. In Ad-SVR6-infected cells, T antigen is produced in large amounts and at levels quite commensurate with the levels of T mRNA. Thus, it is translated efficiently when it is the second gene on this mRNA. This situation is unusual for eukaryotic mRNAs where distal coding sequences on a message are not usually translated. The E1b transcription unit is unusual in itself because it codes for two proteins whose AUG starts are 300 bp distant from each other, although the mRNAs for these proteins have common 5′ ends (see Tooze, *DNA tumor viruses [1980];* Bos et al., *Cell 27:* 121 [1981]). Clearly positioning a foreign gene as the second coding sequence in this transcription unit allows for its efficient translation.

Integration of Other Foreign Genes into Adenovirus

T. Grodzicker

Plasmids had been constructed containing the human β-globin gene (either with or without its promoter) coupled to T antigen-coding sequences. The inserts were excised with *Bam* and ligated to *Bam*-digested Ad2/Ad5 1×51 DNA. This Ad2/Ad5 recombinant virus contains only two *Bam* sites and is the most common vector that we use. After transfection into 293 cells along with wild-type helper virus, progeny were grown in CV monkey cells to select viruses that express T antigen. Among the progeny were viruses that contained the β-globin gene and expressed β-globin mRNA. We are presently trying to quantitate the levels of mRNA and determine their 5′ ends.

Based on our previous work with T antigen, we can now position foreign DNA precisely in adenovirus and have found that insertion of the SV40 *A* gene into the third leader gives the highest levels of T mRNA translation. We are now constructing plasmids to be used for inserting foreign genes into adenovirus based on these results. First, we have modified p9B (see Fig. 1) so that the SV40 insert carries its own promoter. Second, there are sites between the adenoviral and SV40 DNA into which foreign DNA can be inserted. Several genes are being inserted into this plasmid. We have also inserted the human α-chorionic gonadotropin gene with and without its poly(A) addition site into the plasmid pALE. This plasmid is similar to that shown in Figure 1, except that adenovirus and SV40 sequences are joined at position 17 on the adenoviral genome. All of these inserts are being put into adenovirus by a combination of in vivo and in vitro recombination and will be analyzed as to what sequences they contain.

Foreign Proteins Expressed on Eukaryotic Cell Surfaces

J. Sambrook, M.-J. Gething

During the past year, we have continued studies on the molecular genetics of cell-surface proteins. Most of our work has involved the gene of influenza virus that codes for the hemagglutinin (HA). This glycoprotein is the major antigen of the influenza viral particle and it is inserted into the lipid membrane that envelops the virion. Apart from its importance to man as a mutable antigen, HA is involved in the penetration of the host cell by the virus, being responsible not only for the initial attachment of the virus to cellular receptors, but also for the fusion of viral and cellular membranes that marks the onset of infection.

The natural form of the HA gene is an RNA molecule of negative polarity. However, in recent years, the gene has been converted into a double-stranded DNA form, cloned and propagated in prokaryotic vectors, and sequenced. In this way, the complete amino acid sequences of the HAs of a large number of strains of influenza virus have been established. Furthermore, a large number of monoclonal antibodies have been raised against HA and these have allowed a detailed analysis of the antigenic structure of HA. Finally, about 5 years ago, the glycoprotein spike of HA was crystallized and its three-dimensional structure was recently determined at the 3-Å level using X-ray crystallography. The culmination of all this work has been a detailed description of the physical domains of the molecule, the location of its major antigenic sites, the points at which it is glycosylated, its organization into trimeric structures, and its orientation with respect to the membrane. At the present time, therefore, HA is the best characterized of all eukaryotic membrane proteins.

The crucial interactions between HA and membranes are mediated by the three separate hydrophobic regions of the molecule: (1) the aminoterminal, hydrophobic signal draws the nascent polypeptide chain into contact with the intracellular membrane system; (2) the carboxyterminal region anchors the completed molecule in the external membranes of both virus and infected cell; and (3) the amino terminus of HA2 subunit of HA which appears to be involved in penetration of the cell membrane during infection.

Because the biosynthesis of HA involves host enzymes and processes during translation, membrane transport, glycosylation, and maturation, it appears to be a profitable model for the study of the integral membrane proteins of eukaryotic cells. One approach to the further analysis of HA, and of integral membrane proteins in general, is to introduce alterations in their amino acid sequences and to analyze the subsequent effects on the biosynthesis, structure, and function of the molecules. It would be of particular interest to determine the effects of alterations in the hydrophobic sequences on the interactions of the protein with membranes, par-

ticularly those involving translocation of the nascent polypeptide strand. The first stage in this process is to synthesize HA from a cloned gene; and much of our work this year has been concerned with the construction and use of recombinant viral genomes consisting of both SV40 and influenza virus sequences that direct the expression of large amounts of HA at the surface of eukaryotic cells.

The clones of the HA gene used in our work were derived from RNA extracted from influenza viral particles using methods that are standardly employed to clone cellular RNAs. The only extra step was the addition of a tract of polyadenylic acid to the 3′ end of the virion RNA to provide a binding site for the oligo(dT) used to prime reverse transcriptase (see Gething et al., *Nature 287:* 301 [1980]). Double-stranded cDNA was inserted into the *Pst*I site of the plasmid pAT153 by dG:dC homopolymeric tailing. Following transfection of *E. coli*, plasmids containing copies of the Japan HA gene were identified by in situ hybridization to colonies and to virion RNAs immobilized on DBM paper. The DNA sequence of the cloned inserts was carried out using the procedures of Maxam and Gilbert. Using these methods, a cDNA copy of the complete Japan HA was obtained (except for 11 nucleotides of the 3′ untranslated region). The exonuclease BAL31 was used to remove both of the sets of homopolymeric C:G and A:T tails used in the construction and also the 5′ untranslated region of the gene. *Hin*dIII linkers were added to the ends of the resected molecules, which were then recloned in pAT153. The resulting population of clones was scanned to identify an individual in which the nucleotide immediately following the *Hin*dIII linker was the A of the initiating AUG codon of the HA-coding sequence. Cleavage of such a clone with *Hin*dIII and *Bam*HI yielded a fragment containing the entire coding sequence of HA in an orientation suitable for insertion into the various vectors described below.

Vector Systems

Because the HA gene occurs naturally in the form of negatively stranded RNA, it contains none of the controlling elements, such as promoters and enhancers, that are required for efficient transcription of conventional DNA genes. Thus, the vectors all were designed so that the HA-coding region was placed under the control of either the SV40 early promoter/enhancer or the SV40 late promoters.

Vectors in Which the HA-coding Sequence Replaces the SV40 Early Genes

As mentioned above, the cloned HA had been reconstructed so that the A residue immediately following the *Hin*dIII linker would correspond to the A of the initiating AUG of the protein. This allowed the HA gene to be inserted into the early region of the SV40 genome (between the *Hin*dIII site at nucleotide 5171 and the *Bam*HI site at nucleotide 2533 so that its initiation codon was almost exactly in the position normally occupied by the initiation codon of large and small T antigens. In addition, at the time that these experiments were undertaken, there were indications from other workers that foreign genes inserted into SV40 recombinant vectors were expressed more efficiently when splice donor and acceptor sites were provided. We therefore built into the original a sequence of DNA containing the intron of the SV40 gene coding for small t antigen. Finally, to ensure that transcripts of the HA gene would be terminated efficiently, the HA sequences were always inserted so that they lay upstream of a poly(A) additional signal (also derived from SV40). It should be noted that the untranslated 3′ region of the HA gene contains the sequence (AAUUAA), closely related to the consensus poly(A) addition signal (AAUAAA), that is believed to function during natural infections with influenza virus. However, it was not clear whether this signal would work efficiently in the form of double-stranded DNA, and a conventional poly(A) addition signal was therefore included in the construct.

The HA gene should therefore be transcribed under the control of the upstream SV40 promoter/enhancer to yield an mRNA that (1) initiates at the usual site, (2) contains a 5′ untranslated region that apart from the last 5 nucleotides is identical to that of conventional SV40 early mRNAs, (3) carries a coding region specifying an HA molecule whose sequence is identical to that of the natural form of the molecule, (4) has the opportunity to be spliced at the donor/acceptor sequences provided by the downstream intron, and (5) terminates at either the poly(A) addition site within the HA gene or at the SV40 site further downstream.

This recombinant viral genome contains the origin of SV40 replication and an intact set of late genes. However, the SV40 early genes have been replaced by the influenza virus HA gene and the recombinant therefore cannot replicate in simian cells unless functional T antigen is supplied. This was done by using as a permissive host the COS-1 line of SV40-transformed simian cells that carry an endogenously expressed copy of the SV40 T antigen gene.

Vectors in Which the HA-coding Sequence Replaces the SV40 Late Genes

To place the HA-coding sequences under the control of the SV40 late promoter(s), the HA gene was inserted into the late region of the SV40 genome between the *Hpa*II site at nucleotide 346 and the *Bam*HI site at nucleotide 2533. The resulting recombinant viral genome contains the origin of SV40 replication and an intact set of SV40 early genes. The HA-coding sequences are joined to the noncoding sequences of SV40 that would normally be transcribed into the untranslated 5′ region of

SV40 late mRNAs. At late times during lytic infection of permissive simian cells, this recombinant genome should express an unspliced, hybrid mRNA consisting of untranslated SV40 sequences at its 5′ end and an intact set of HA-coding sequences. Polyadenylation could occur either at the site within the 3′ untranslated sequences of the HA gene or at the normal site for late SV40 mRNAs at nucleotide 2674. Translation of this RNA should begin at the first AUG, which is located at the beginning of the HA-coding sequences. Because the late-replacement vector contains a functional origin of DNA replication and an intact set of SV40 early genes, it will replicate its DNA efficiently in permissive simian cells. However, the late genes of SV40 have been deleted and production of infectious virions containing the recombinant genome therefore requires that SV40 capsid proteins be supplied by a complementing helper virus such as *dl*1055, an early deletion mutant of SV40.

The late-replacement vector is similar to those used previously by many other workers to express cloned genes derived from a variety of different organisms; and more recently, to express cloned HA genes from other strains of influenza virus.

Production of Viral Particles Carrying SV40-influenza Recombinant Genomes

All the intermediate constructions were carried out in bacterial plasmids of various sorts: The final recombinant viral genomes also were propagated in bacterial plasmids until their structures had been verified. They were then excised from the plasmids (by cleavage with *Bam*HI), purified by gel electrophoresis, and ligated at low concentrations of DNA to generate closed circular molecules, which were then introduced into the appropriate host cells using DEAE-dextran as a facilitator. The early-replacement vectors were introduced into COS-1 cells in the absence of a helper viral DNA: The late-replacement vector was introduced into CV-1 cells together with an equal amount of the DNA of the SV40 deletion mutant *dl*1055. Only a small proportion of cultured mammalian cells are competent at any one time to take up DNA, so that very few cells of the population are infected initially. However, during the next several days, each of these cells undergoes a lytic infection and produces over a million viral particles that are able to spread into neighboring cells and infect them with high efficiency. Usually, the lysate obtained from the first set of infected cells needs to be passed serially once or twice to obtain a high-titer virus stock. In the case of the early-replacement vector, this virus stock consists exclusively of recombinant genomes encapsidated in SV40 coat proteins; in the case of the late-replacement vector, it consists of approximately equal numbers of helper virus particles (*dl*1055) and recombinants. Both virus stocks can be used to infect permissive cells with high efficiency and to induce in them a lytic cycle of virus growth. During such productive infections, the viral genomes are transported to the nucleus where they are liberated from their capsids. The early promoter soon becomes active and genes under its control are expressed. By 12 hours after infection, viral DNA replication is under way and genes begin to be expressed from the late promoter with high efficiency. By 36–48 hours after infection, the newly synthesized viral genomes begin to be assembled into progeny viral particles—a process that continues for another 24 hours, when the cells detach from their substrate and die.

HA Is Expressed Efficiently from Both the Early and the Late SV40 Promoters

The simplest way to measure the quantity of HA synthesized in cells infected with the early- or the late-replacement vectors is by solid-phase radioimmune assay. In this test, extracts of the infected cells are allowed to react with HA-specific polyclonal antibody that has been adsorbed onto a polyvinyl chloride surface. HA is known to have several different epitopes, so it would be expected that a given molecule of HA might bind to the adsorbed antibody through one antigenic site, leaving the others available for reaction. The number of these available sites (and hence the number of HA molecules) can then be titrated with radiolabeled antibody.

When this test was applied to extracts made from cells infected with either the early- or the late-replacement vector, it was found that the amount of HA detected increased as the course of infection proceeded. By 62 hours, when the lytic infection was in its terminal phase, cells infected with the late-replacement vector contained approximately 6×10^8 molecules of HA per cell, while those infected with the early-replacement vector contained approximately 2.5×10^6 molecules of HA per cell. For comparison, a simian cell at a late stage during infection with influenza virus will contain about 5×10^7 molecules of HA . These results show that the influenza virus HA gene is expressed with high efficiency when it is coupled to either the early or the late SV40 promoters.

Originally, the fact that COS-1 cells infected with the early-replacement vector synthesized 10-fold less HA than CV-1 cells infected with the late-replacement vector was thought to be simply a reflection of the relative strengths of the early and late SV40 promoters. However, we now know that other factors are involved that play a much more important role in determining the amount of HA that is synthesized by these recombinant viruses. Chief amongst these is the presence or absence from the early-replacement vector of the small DNA fragment that carries the intron of small t antigen.

The Small t Intron Leads to Genetic Instability of the Early-replacement Vector

It was clear from the studies described above that splicing of mRNA is not required for expression of

the HA gene from the late-replacement vector. To test whether the intron sequence built into the early-replacement vector was necessary for expression of HA, we constructed a derivative that is identical to the original early-replacement vector in every respect except that it lacks the intron. A virus stock containing this new recombinant was raised and propagated in COS-1 cells in the absence of helper virus. When its ability to express functional HA was compared with that of the original, intron$^+$ recombinant virus, we found that the intron$^-$ virus stock was of a much higher titer, so that it could infect more cells of the population. Furthermore, each of these infected cells produced more HA than cells infected with the intron$^+$ virus.

Analysis of the recombinant DNA molecules isolated from cells infected with sequential passages of the intron$^+$ virus showed a rapid increase in the proportion of rearranged genomes. By the fourth and fifth passage, the stock was essentially noninfectious and contained a preponderance of molecules shorter than that of the original construct. A number of the rearranged DNA molecules from the fourth-passage intron$^+$ stock were cloned into the bacterial plasmid Xf3, analyzed by restriction enzyme digestion, and sequenced by the Maxam-Gilbert procedure. The results showed that many forms of rearrangement had occurred, including (1) simple deletions, 50 bp to 1 kb in length, both within the HA gene and across both of the SV40-HA junctions, (2) deletion of a segment of the HA gene, together with an insertion of a similar-sized piece of SV40 DNA, derived from a distant region of the viral genome, (3) deletion of the SV40-HA junction downstream of the HA-coding region, together with an inversion of the sequence of SV40 DNA lying immediately downstream from the junction. These changes are reminiscent of the sorts of rearrangement that were shown several years ago to occur in the SV40 genome when the virus was serially passaged many times in succession at high multiplicity. The genome of the intron$^+$ recombinant however, seems to be particularly vulnerable to such rearrangements. By contrast, the recombinant genome lacking an intron is completely stable. No rearrangement of its genome could be detected by restriction analysis and Southern hybridization after six sequential passages at high multiplicity; the intron$^-$ virus stock showed no reduction in infectivity with increasing passage number, and it continued to direct the synthesis of large quantities of HA.

We therefore conclude that the provision of splice junctions is not required for efficient expression of influenza virus HA cloned within either the early- or the late-replacement vectors. The functional, unspliced mRNA for HA is apparently synthesized with great efficiency no matter whether the HA gene is presented to the cell in the form of double-stranded DNA or as negatively stranded RNA. It might be tempting to argue that the presence of the intron in the early-replacement vector is actually detrimental to the expression of the HA gene. However, the rapid accumulation of rearrangements in the recombinant genome suggests that the deleterious effects of the intron are mediated more at the level of DNA than RNA. Why genomic instability should be correlated with the presence of the intron remains to be elucidated.

The HA Synthesized by SV40-HA Recombinants Is Biologically Active

To establish that the HA expressed by the SV40-HA recombinants was authentic both in structure and biological activity, a series of assays were performed on cells infected with either the early- or the late-expression vectors. These included immunoprecipitation, cytoplasmic and cell-surface immunofluorescence, and erythrocyte binding. By all these and other tests, the protein was indistinguishable from HA produced naturally in influenza virus-infected cells. These results have been described in detail previously and will be discussed only briefly here.

Immunoprecipitation. Extracts of cells infected with the recombinant SV40 HA viruses contained a protein that was specifically precipitated by an HA antiserum and was indistinguishable in size from authentic, glycosylated HA precipitated from extracts of cells infected with influenza virus. So much of this protein did the recombinant cells contain that it was not necessary to use immunoprecipitation to detect it; HA could be seen either as a band stained with Coomassie blue after the extracts of infected cells had been analyzed by SDS-polyacrylamide gel electrophoresis, or as a prominent radioactive species when extracts were prepared from infected cells that had been labeled with [^{35}S]methionine.

Cell-free Translation of mRNA. HA-specific mRNAs isolated from authentic, influenza virus-infected cells and from cells infected with SV40-HA recombinants can be translated in lysates of rabbit reticulocytes into a protein of molecular weight 63,000 that is the nonglycosylated form of HA. When the translation was carried out in the presence of dog pancreas membranes, the apparent molecular weight of both HA products increased to that of the in vitro, glycosylated form of HA (75,000). Thus, the two forms of HA synthesized in vitro from recombinant mRNA are identical to the corresponding forms synthesized from authentic HA mRNA.

Immunofluorescence. Cells infected with the SV40-HA vectors displayed bright, cytoplasmic fluorescence with the Golgi apparatus staining with particular intensity. The surface of the cells also stained specifically with a uniform, dimmer fluorescence. The distribution of fluorescence in cells infected with influenza virus was similar to that displayed by recombinant-infected cells, but of lower intensity.

Binding of Erythrocytes. Cells infected with the early- or the late-replacement vector adsorbed a dense carpet of erythrocytes onto their surfaces.

It is clear from these studies that both types of recombinant viruses express large quantities of HA,

which appears normal in all respects; its molecular weight is indistinguishable from that of authentic HA and it is displayed on the infected cell's surface in a glycosylated form that is both antigenically and biologically active. Thus, a protein that is normally encoded by a negative-strand RNA genome can be expressed in copious amounts when double-stranded DNA copies of its coding sequences are harnessed to strong SV40 promoters. To our knowledge, influenza virus HA is the first integral membrane protein to be expressed in eukaryotic cells from recombinant DNA vectors. It is therefore possible to begin to think of using site-directed mutagenesis to dissect those parts of the molecule that are important in its structure, function, and biosynthesis and have been inaccessible to genetic analysis until now.

Removing the Carboxyterminal Hydrophobic Sequence Converts HA from an Integral Membrane Protein into a Secreted Protein

As discussed above, HA is normally anchored in the plasma membrane of the infected cell or in the envelope of influenza virus by a tract of nonpolar amino acids that are coded by the distal sequences of the HA gene. These sequences were removed from the standard late-replacement vector to create a new vector that differs from its parent in only one significant way: It lacks the DNA sequences coding for the 38 amino acids that normally are found at the C-terminus of HA. Substituting for them is a stretch of 11 amino acids, largely polar in nature, that is encoded by the dG:dC homopolymer tail, a synthetic *Bam*HI linker, and a short sequence of SV40 DNA.

This "anchor-minus" mutant synthesizes as much HA as the standard late-replacement vector. However, instead of being entirely cell-associated (in the Golgi or on the cell surface), the mutant HA is found largely in the medium. The synthesis and secretion of the anchor-minus HA is so efficient that it is the only labeled protein that can be detected in the medium after labeling the infected cells for 1 hour with [^{35}S]methionine. The most straightforward explanation of the mutant 's properties is that the removal of the C-terminal hydrophobic sequences results in a loss of anchoring function, so that the nascent polypeptide, instead of remaining attached to the luminal face of the rough endoplasmic reticulum, passes completely through the membrane. Once free in the lumen, the mutant form of HA is treated by the cell as if it were an authentic secretory protein and is discharged into the medium.

The secreted form of HA differs from the membrane-bound form in only one major respect—its rate of glycosylation. The complex oligosaccharides that are added to membrane and secretory proteins are composed of "core" and "terminal" regions that are added in the rough endoplasmic reticulum and the Golgi apparatus, respectively. The inner core region, linked to asparagine, contains a minimum of two *N*-acetylglucosamine and three mannose residues. While fewer in number than those originally added, all of these core sugars are donated as a part of a preformed group from an activated donor molecule—dolichol. As far as is known, all secretory and integral membrane proteins share the same asparagine-linked oligosaccharide core. However, the final pattern of glycosylation differs greatly from protein to protein. Most of these differences are generated during the passage of the protein through the Golgi apparatus, as a consequence of the activity of a series of specific mannosidase and glycosyl transferase enzymes. Since, in general, all copies of a particular protein show the same pattern of glycosylation, there must be information contained in either the sequence or the folding of the polypeptide chain that determines what alterations are to be made to the original precursor oligosaccharide. It is therefore interesting that the anchor-minus and the wild-type, which differ so little in primary amino acid sequence, should show differences in the rates at which they become glycosylated. The core-glycosylated form of wild-type HA (M_r = 73,000), which can be detected after a short period of labeling (~10 min), is chased completely into its final glycosylated form within a matter of minutes. By contrast, the population of anchor-minus molecules takes up to 2 hours to become completely glycosylated. Even though the rate of glycosylation of anchor-minus HA is comparatively slow, its pattern of labeling with radioactive sugars is very similar to that of wild-type HA and it therefore appears that final composition of the oligosaccharides attached to the two proteins is not significantly different.

These results lead to the following conclusions: (1) The hydrophobic amino acid sequence at the C-terminus of HA is required to anchor the protein in the outer membrane of the cell. Removal of this sequence converts HA from an integral membrane protein into a secretory protein. It therefore follows that signal sequences apart, there is no unique sequence of amino acids common to all secretory proteins that automatically confers on them the ability to be secreted. (2) The slow rate of glycosylation of the anchor-minus HA indicates that secretory proteins in general may be processed more slowly than integral membrane proteins. This difference may lie at the level of transport to the Golgi apparatus or passage through it. (3) The signals governing the pattern of glycosylation of HA cannot lie in the 38 amino acids that normally comprise the carboxyl terminus of the molecule.

Future Work

During the next year, we intend to extend this work in several directions. First, we would like to develop cell lines that constitutively synthesize HA in large quantities, and we are exploring a variety of ways (1) to establish stable cell lines that contain multiple copies of the HA gene and (2) to place the HA-

coding sequences under the control of inducible promoters. Second, we would like to refine our analysis of the functional domains of the HA molecule by synthesizing forms of the protein that contain single amino acid substitutions in the crucial hydrophobic regions. Finally, we would like to analyze the properties of chimeric molecules in which the hydrophobic regions of HA have been replaced by those of another integral membrane protein.

Recognition Signals for Sorting Surface Proteins

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

A fundamental principle of cell architecture is the segregation of cell functions into distinct subcellular regions. This partitioning requires a directed transport of structural and enzymatic components, since molecules are not synthesized everywhere in the cell. The best-understood system of directional transport is the pathway by which secretory and cell-surface proteins reach the plasma membrane (Sabatini et al., *J. Cell Biol. 92:* 1 [1983]). In cells that form selective barriers, such as kidney distal tubule epithilia, the plasma membrane is itself polarized into at least two surface domains. Each domain contains a subset of surface proteins, indicating that some mechanism for sorting surface proteins is active in these cells (Louvard, *Proc. Natl. Acad. Sci.72:* 4132 [1980]). We have been investigating this sorting process, taking advantage of the observation that certain viral glycoproteins are directionally transported to either the apical or basolateral surface domains in polarized epithelial cells (Rodriguez-Boulan and Sabatini, *Proc. Natl. Acad. Sci. 75:* 5071 [1978]). Glycosylation does not play a role in this process (Roth et al., *Proc. Natl. Acad. Sci. 76:* 6430 [1979]; Green et al., *J. Cell Biol. 89:* 230 [1981]), and recently we have determined that influenza virus hemagglutinins (HAs) are sorted to the apical surface of polarized AGMK cells infected with recombinant SV40 viruses. Thus, some feature of the HA polypeptide serves as a recognition signal for cell-sorting mechanisms.

To determine the location of this "sorting signal" on HA, we have begun construction of both deleted and chimeric HA molecules using the cloned HA gene inserted into SV40 vectors (Gething and Sambrook, *Nature 293:* 620 [1981]). As a first approach, we wish to determine on which side of the membrane-spanning region of HA the sorting signal resides. HA molecules with the extreme carboxyterminal "tail" either truncated or extended have been constructed. Construction is underway of a chimeric HA with sequences coding for the external HA spike spliced in frame to sequences coding for the hydrophobic membrane spanning region and cytoplasmic "tail" of the HSV-I gC glycoprotein. In addition, the HSV gC glycoprotein gene has been inserted into an SV40 expression vector. The biological properties of each of these recombinant molecules will be studied in CV-1 cells and in polarized AGMK primary epithelial cell cultures. In collaboration with E. Ruley, we are preparing a polyoma expression vector that may be of great use for studies of the sorting of chimeric glycoproteins in the polarized continuous mouse epithelial cell line, MME (Roth et al., *J. Virol. 45:* 1065 [1983]).

Publications

Gething, M.-J. and J. Sambrook. 1981. Cell-surface expression of influenza hemagglutinin from a cloned DNA copy of the RNA gene. *Nature 293:* 620.

——. 1982. The expression of influenza virus hemagglutinin from SV40-HA recombinant vectors. In *Eukaryotic viral vectors* (ed. Y. Gluzman), p. 20. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

——. 1982. Secreted and cytoplasmic forms of influenza virus hemagglutinin. *Nature 300:* 598.

Gething, M.-J., I. Heiland, and J. Sambrook. 1982. Expression of a cloned copy of the influenza virus hemagglutinin gene in prokaryotic and eukaryotic cells. Proc. Munich Symposium of Microbiology, *Prospective biological products for viral diseases* (ed P.A. Bachmann), p. 81. Taylor and Francis.

Gluzman, Y. and B. Ahrens. 1982. SV40 early mutants that are defective for viral DNA synthesis but competent for transformation of cultured rat and simian cells. *Virology* (in press).

Gluzman, Y. and K. VanDoren. 1983. Palidromic adenovirus type 5-simian virus 40 hybrid. *J. Virol.* (in press).

Klessig, D.F., T. Grodzicker, and V. Gleghon. 1983. Construction of human cell lines which contain and express the adenovirus DNA binding protein gene by cotransformation with the HSV-1 tk gene. (Submitted.)

Klessig, D.F., M.P. Quinlan, and T. Grodzicker. 1982. Proteins containing only half of the coding information of early region 1b of adenovirus are functional in human cells transformed with the herpes simplex type 1 thymidine kinase gene and adenovirus type 2 DNA. *J. Virol. 41:* 423.

Ling, L.E., M.M. Manos, and Y. Gluzman. 1982. Sequence of the junction in adenovirus 2-SV40 hybrids: Examples of illegitimate recombination. *Nucleic Acids Res.* (in press).

Maniatis, T., E. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Mathews, M.B. and T. Grodzicker. 1981. Virus-associated RNAs of naturally occurring strains and variants of group C adenoviruses. *J. Virol. 38:* 849.

Sambrook, J. and M.-J. Gething. 1983. In *Experimental manipulation of gene expression* (ed. M. Inouye). Academic Press, New York. (In Press.)

Thummel, C., R. Tjian, and T. Grodzicker. 1981. Expression of SV40 T antigen under control of adenovirus promoters. *Cell 23:* 825.

——. 1982. Construction of adenovirus expression vector by site-directed *in vivo* recombination. *J. Mol. Appl. Genet. 1:* 435.

Thummel, C., R. Tjian, S.-L. Hu, and T. Grodzicker. Regulatory sequences required for efficient expression of SV40 T antigen from the adenovirus late promoter. *Cell* (in press).

NUCLEIC ACID CHEMISTRY

R.J. Roberts, T.R. Gingeras, *J.E. Brooks, N. D'Anna, J. Dellaporta, G. Freyer, Y. Katoh, C. Keller, P.M. Lin, P.A. Myers, D. O'Loane, K. O'Neil, B. Parsons, M. Wallace*

During the past year, the Nucleic Acid Chemistry group has continued to accumulate data toward the elucidation of the Ad2 DNA sequence. In addition, we have begun characterizing mRNA splice points in early region-IV mRNAs. The development of computer programs to analyze these sequences has continued and is now focusing on computer-graphic routines. Our long-standing interest in restriction endonucleases and methylases has been maintained, and a major effort is being directed towards the characterization of the *Pae*R7 retriction-modification system, which we have cloned into *Escherichia coli*.

Adenovirus-2 Genomic Sequence

R.J. Roberts, K. O'Neill, B. Parsons

At the present time, two main blocks of sequence have been collected. The first segment consists of 16,000 nucleotides located between 0% and 43% of the genome, of which 0–36% is in final form, and confirmatory data is being collected between coordinates 36% and 43%. The second main block of sequence is located at the right end of the genome and consists of 4000 nucleotides of completed sequence between genome coordinates 89% and 100%. In addition, all but a few nucleotides of the region between genome coordinates 66% and 70% have been obtained. As a result of the information available from this laboratory and from others, most notably the groups of F. Galibert (Hôpital Saint-Louis, Paris), U. Pettersson (University of Uppsala, Sweden), and J. Sussenbach (University of Utrecht, Holland), more than 95% of the complete sequence is now available. As soon as an uninterrupted sequence for the Ad2 genome is obtained by combining our data with the data from these other laboratories, the remaining task will be to identify and confirm those differences that occur between our strain and the strains used in these other laboratories. Because we already have a great deal of data corresponding to regions sequenced in other laboratories, we know that these strain differences are rather limited and so far number less than 10.

From the analysis of the sequences that are presently available in final form, it is apparent that the information content of the Ad2 genome is extraordinarily high. There are numerous examples of overlapping genes. This can occur through the use of different reading frames on the same strand and also by the use of overlapping reading frames on complementary strands. Nowhere is this more pronounced than within the region between genome coordinates 14% and 30%, which at one time was considered the silent region of the Ad2 genome! It is now almost certain that one long open reading frame, located on the *l* strand between coordinates 14.2% and coordinate 23%, encodes the main body of the Ad2 DNA polymerase. At both the C terminus and the N terminus of this gene, there are overlapping reading frames on the same strand. One of these, which overlaps the C terminus, is known to encode the N terminus of the IVa_2 polypeptide. Within the main body of the gene, there are two other reading frames that are open on the *r* strand. Recent evidence from C. Anderson (Brookhaven) and J. Lewis (Seattle) indicates that polyadenylated mRNAs, from which these reading frames can be translated in vitro, are present in Ad2-infected cells. In one case, the corresponding mRNA is entirely contained within the intron that is removed during the splicing of the first and second leaders of late mRNA, whereas the second is contained within those mRNAs that carry the i leader, which was first identified by T. Broker et al. (Electron Microscopy Section). Other significant stretches of open reading frame also exist within this region, however so far there is no information to suggest that they are used. The sequences in this region also contain other biological information. Both the late promoter and the promoter for the IVa_2 mRNA lie within it, as do the signals that direct the splicing of the first, second, and i leaders of late mRNAs.

Computer Program Development

T.R. Gingeras, C. Keller, R.J. Roberts

For several years now, we have been developing computer software designed to aid both in the assembly of DNA sequences and in the subsequent analysis of the data. These programs are written in FORTRAN, a language that is relatively transportable to other computers, and copies of the programs have been widely distributed.

During the course of the last year, we have made considerable improvements in many of our programs that are concerned with the assembly of DNA sequences. In particular, we have streamlined the two main programs that handle the data base of information about the various M13 clones that we obtain together with the sequence reactions that have been carried out on these clones. We have also streamlined the procedure by which primary pieces of sequence data that overlap are joined together. Finally, a new program, SCROLL, has been developed in collaboration with M. Corcoran and P. Rice. This program takes a main segment of completed sequence and extracts from the master data base of primary sequence all of those segments that were used to deduce the complete sequence. These segments are then aligned with respect to the main

sequence in such a way that the accuracy of that sequence, as judged by the number of times that an individual base has been determined independently, can be readily visualized. The output from this program is extremely useful during the final stages of the sequencing project when it is desired to recheck those regions that may be most susceptible to error.

We have also made considerable progress in writing software that gives a graphic representation of the information content of a sequence using a high-resolution graphics terminal. Thus, we have developed programs that will accurately display restriction enzyme maps and open translational reading frames. In addition, we have programs that will allow graphic display of searches, either for specific nucleotide sequences or for information about specific peptides. It is already clear to us that the use of computer graphics in an interactive mode provides a powerful tool with which to access the information content of a long sequence.

Restriction Endonucleases

J.E. Brooks, P.M. Lin, P.A. Myers

The list of restriction endonucleases continues to grow, and at the present time 398 different enzymes have been found with at least 91 different specificities represented. For the last 2 years, we have been involved in a joint project with D. Comb and I. Schildkraut (New England Biolabs) during which we have examined more than 170 strains for restriction endonucleases. So far a total of 47 type-II enzymes have been found and 11 new specificities identified. The 15 enzymes discovered during the last year are listed in Table 1. In addition, we have also determined recognition sequences for the previously described enzymes *Bst*XI and *Bsp*1286. *Bst*XI recognizes the sequence CCANNNNNTNTGG and is only the second example of an enzyme that recognizes this rather unusual sequence pattern. *Bsp*1286 recognizes the sequence

```
  G   C
GAGCAC
  T   T
```

and is identical in specificity with two other enzymes (*Sdu*I and *Nsp*[7524] II) that have recently been described by other laboratories.

Early Region IV of Adenovirus 2

G. Freyer, Y. Katoh

Early region IV of Ad2 maps between coordinates 91.3% and 100% on the viral genome and is expressed early in infection. Transcription of this region has been studied extensively by electron microscopy and is known to be complex. Some of the polypeptides derived from this region have been partially characterized; however, this region's role in the Ad2 life cycle is not well understood. We have been studying the splicing patterns of the mRNAs produced from this region at the sequence level by cloning cDNAs corresponding to early region-IV mRNAs.

Initially, a cDNA library was constructed in the conventional way using mRNA isolated early during Ad2 infection of HeLa cells. Eighty early region-IV clones were isolated, and sequence analysis of a representative selection enabled us to identify donor and acceptor splice sites for several of the known mRNAs. In addition, two of the cDNAs that we have sequenced correspond to mRNAs that had not previously been reported. One surprising finding was that the large intron lying between coordinates 92% and 95%, which is common to several early region-IV mRNAs, did not contain unique donor and acceptor splice sites. Unfortunately, most of the clones that we isolated were not full length and, in particular, were lacking the 5′ leader sequence. Since the mRNAs that arise from early region IV fall into families based upon the particular acceptor splice site to which this 5′ leader sequence is joined, we were not able to discover whether the heterogeneity in the large intron correlated with specific families of mRNA, or whether all families would show this heterogeneity.

To answer this question, we have devised a new strategy for the construction of a cDNA library that leads to an increase in the frequency of full-length cDNA clones. To this end, we synthesized a short oligonucleotide of 15 bases that exactly matches a

Table 1

Strain	*Enzyme*	*Recognition sequence*
Actinomadura madurae	*Amal* (*Nru*I)	TCGCGA
Halococcus agglomeratus	*Hag*I	unknown
Nocardia brasiliensis	*Nbr*I (*Nae*I)	GCCGGC
Nocardia brasiliensis	*Nba*I (*Nae*I)	GCCGGC
Nocardia dassonvillei	*Nda*I (*Nar*I)	GGCGCC
Nocardia minima	*Nmi*I (*Kpn*I)	GGTACC
Nocardia otitidis caviarum	*Noc*I (*Pst*I)	CTGCAG
Rhodococcus rhodochrous	*Rrh*I (*Sal*I)	GTCGAC
	*Rrh*II	unknown
Rhodococcus species	*Rhs*I (*Bam*HI)	GGTACC
Rhodococcus species	*Rhp*I (*Sal*I)	GTCGAC
	*Rhp*II	unknown
Rhodococcus species	*Rhe*I (*Sal*I)	GTCGAC
Streptomyces dysgalactiae	*Sdy*I (*Sau*96I)	GGNCC
Vibrio harveyi	*Vha*I (*Hae*III)	GGCC

portion of the early region-IV leader sequence. The four 5′ nucleotides were complementary to the 5′ extension that is left when DNA is cut with *Sal*I (TCGA). This single-stranded oligonucleotide was then ligated onto the *Sal*I end of a pBR322 vector that had been prepared by digestion with *Hin*dIII and *Sal*I. Poly(A) plus mRNA isolated from Ad2-infected HeLa cells was then used to make cDNA using oligo(dT) priming. This mixed population of cDNAs was then annealed to the vector and second-strand synthesis carried out, such that the oligonucleotide that had been ligated to the vector could serve as a primer for its synthesis. This was followed by ligation and transformation. A large number of early region-IV cDNA clones have been isolated from this library. Plasmid DNA from these clones was sequenced directly using the original oligonucleotide as a primer, and more than 80% of the clones examined were found to contain the 5′ leader sequence. Since the first leader is extremely short, these sequence reactions also reveal the position of the first splice in each cDNA. A second synthetic oligonucleotide that maps close to the junction sequences of the large intron will be used to finish the characterization of these clones.

The method that we have used for the production of these early region-IV cDNAs containing an intact 5′ leader sequence was remarkably successful. To test its more general applicability, we plan to employ a similar strategy for the cloning of early region-IIB cDNAs where the mRNAs are present in much lower amounts.

A Substrate for In Vitro Splicing
G. Freyer

Despite a great deal of work, there has so far been little success in the characterization of a good in vitro system able to accomplish mRNA splicing. One problem in this area is the difficulty of isolating large quantities of a suitable substrate with which to assay splicing activity because the nuclear precursors of these mRNAs are present in exceedingly low quantities. In an attempt to overcome this difficulty, we have constructed a recombinant plasmid that is designed to produce an accurate Ad2 late transcript under the control of an *E. coli* promoter. The scheme for the construction of the clone was as follows. A restriction fragment containing the Ad2 major late start site was cloned into pBR322. A strong terminator from phage fd was then inserted downstream at the *Bam*HI site of pBR322. The leftwards lambda promoter (P_L) was then inserted next to the Ad2 sequences such that the Pribnow box lay approximately the same number of nucleotides away from the Ad2 late start as it does for the lambda leftwards start site. The Ad2 *Hin*dIIIB fragment was then inserted so as to reconstruct a continuous section of Ad2 sequence beginning at the major late start site at 16.6% on the Ad2 genome and continuing out to 31.5%. Finally, a poly(dA)-poly(dT) stretch was added so that transcripts ending in poly(A) could be produced by in vitro transcription with *E. coli* RNA polymerase.

Initial studies have shown that in vitro the *E. coli* RNA polymerase can make a specific transcript containing Ad2 sequences which can be easily purified by virtue of its poly(A) tail. Direct RNA sequence analysis of the transcript produced in vitro shows that it initiates to the left of the normal Ad2 late transcript start site. Furthermore, the level of transcripts produced in vitro are lower than those usually observed with lambda P_L. We are currently attempting to remove two bases from the region between the Pribnow box and the transcription start site so as to ensure that the in vitro transcript exactly matches, at its 5′ end, the in vivo Ad2 transcript. Once that is completed, we will then attempt to use this RNA as a substrate with which to assay in vitro mRNA splicing.

Cloned Restriction-modification Genes
J.E. Brooks, T.R. Gingeras

We have continued our work on the cloning and characterization of type-II restriction-modification systems. The proteins encoded by these systems are of interest, not only as invaluable tools to the molecular biologist, but also as a means of studying specific DNA-protein interactions. During the past year, we have concentrated on two plasmid-borne systems, *Pae*R7 and *Eco*RII. The *Pae*R7 system was isolated from a plasmid of *Pseudomonas aeruginosa*. We have succeeded in transferring these genes into a pBR322 vector and propagating them in *E. coli* (Gingeras and Brooks, *Proc. Natl. Acad. Sci. 80*: 402 [1983]). By a series of BAL31 nuclease digestions, we have been able to clone separately the restriction endonuclease and modification methylase genes. Clones containing only the endonuclease gene are of particular interest because extracts of these clones display restriction endonuclease activity, although there is no apparent restriction of the growth of infecting phages. Furthermore, chromosomal and phage DNAs isolated from these host cells are not protected against cleavage by *Pae*R7 in vitro.

The subclones obtained by BAL31 deletion have been examined for their levels of expression of the endonuclease and/or methylase both in vivo and in vitro (Table 2). In addition, restriction maps of these subclones have been obtained and in several cases deletion end points have been sequenced. From this analysis, it appears that both genes are under the control of a common promoter with the methylase gene lying proximal to the promoter and the restriction endonuclease gene lying distal to it. In collaboration with G. Theriault, this controlling region has been sequenced and contains a sequence identical to that of the *E. coli* ara^c promoter. Clones that are endonuclease$^+$, methylase$^-$ (restriction$^-$ in vivo) have deleted either part or all of this region and characteristically show low endonuclease levels (Table 2). The determination of the complete nucleotide sequence of the *Pae*R7 system, as well as efforts to isolate and identify the protein products made by this system, are now in progress.

Table 2
Properties of Clones Containing the PaeR7 Restriction-modification System

Clones[a]	Phenotype[b] in vitro R	in vitro M	in vivo R	in vivo M	Endonuclease levels[c]	Stability upon retransformation[d]
1. RM 3.8	+	+	10^{-5}	1.0	1	+
2. Δ 1.4	+	+	10^{-5}	1.0	10^{-2}–10^{-1}	–
3. Δ 1.3	+	+	1.0	0.1	10^{-3}–10^{-2}	–
4. R 2.3	+	–	1.0	0	10^{-3}–10^{-2}	–
5. R 1.9	+	–	1.0	0	$<10^{-3}$	–
6. M 2.7	–	+	0	1.0	NA[e]	+

[a]Δ clones are deletion mutants formed after treatment of the RM3.8 clone with BAL31 nuclease.

[b]In vitro phenotype refers to restriction endonuclease and modification methylase activity, respectively, on Ad2 DNA substrates by crude extracts; in vivo phenotype refers to restriction and modification, respectively, of φ80 phage used to infect the various host clones.

[c]A pBR322 derivative plasmid containing one *Pae*R7 site is linearized at a distal site and used as a substrate on which to measure endonuclease activity. The endonuclease activity of RM3.8 is standardized to 1.0.

[d]Stability of these constructs is measured as their ability to maintain initially measured level of phage restriction after two or three rounds of retransformation.

[e]NA, not applicable.

E. coli also carries plasmid-borne restriction-modification systems. We have been characterizing the *Eco*RII restriction-modification system. This system differs from the *Pae* system in that modification is based on cytosine rather than adenine methylation and its recognition site is CC↑GG (the internal cytosine is modified). This is also the recognition sequence for the *Eco dcm* methylase found in *E. coli* strains, and such sites are reported to be "hot spots" of recombination in *E. coli* (Coulondre et al., *Nature 274*: 775 [1978]). We have now isolated the *Eco*RII restriction–modification genes on a 5-kb fragment and have begun BAL31 deletion experiments to characterize the order and position of the genes. Comparative studies involving *Pae*R7, *Eco*RII, and *Eco*RI systems (Newman et al., *J. Biol. Chem. 256*: 2131 [1981]; Greene et al., *J. Biol. Chem. 256*: 2143 [1981]) have been initiated to see if plasmid-borne systems share common features of organization and control of expression, as well as to explore their possible sequence homology, which might indicate that they have a common evolutionary origin. Additionally, we are in the process of exploring the role that the *Eco dcm* methylase gene plays in *E.coli*. This will be done by introducing clones expressing *Eco*RII methylase into *dcm*⁻ cells. In addition to being identical in its enzymatic specificity, preliminary evidence shows *Eco*RII and *dcm* to be homologous in DNA sequence, as assayed by Southern blot analysis.

As we noted in last year's report, the availability of cloned DNA methylase genes recognizing a variety of site-specific nucleotide sequences presents the possibility of investigating the effect of DNA modification on the control of gene expression in eukaryotic cells. Recently, there has been accumulating evidence that DNA methylation is important in eukaryotic gene regulation. Because it has been technically impossible to isolate eukaryotic DNA methylase genes and difficult to study their protein products, we have constructed eukaryotic expression vectors containing a variety of DNA methylation genes from prokaryotic cells. Development of these heterologous systems will make it possible to study the role of site-specific DNA modification during eukaryotic gene expression. We have begun by transferring the M · *Eco dam* gene into *Saccharomyces cerevisiae* using the yeast-*E. coli* shuttle vector YEP228. This was accomplished after we had isolated and sequenced this gene (Brooks et al., *Nucleic Acids Res.* [1983, in press]) which methylates the sequence G^{m}ATC. Knowing the complete sequence of this gene enabled us, in collaboration with R. Kostriken and F. Heffron (Mechanism of Transposition Section), to obtain expression in yeast cells. We have found that all of the G^{m}ATC sequences are modified in yeast cells containing this recombinant plasmid.

Although adenine or cytosine methylation does not occur normally in the yeast genome (D. Swinton and S. Hattman, pers. comm.), the appearance of such significant levels of DNA modification does not appear to affect any of 12 different metabolic pathways tested nor does it affect the rate of growth of these cells. *dam*⁺ yeast clones are now being used to study nucleosome structure of the genome by digesting chromatin with restriction endonucleases that recognize GATC and either require (*Dpn*I) or are blocked (*Mbo*I) by *dam* expression.

The *dam* gene has also been transferred into another eukaryotic expression vector pXKH (D. Hanahan) and, in collaboration with D. Kurtz (Hormonal Control of Gene Expression Section), has been transformed into cultured mouse L cells. The results from these experiments appear positive for expression of *dam* and are currently being analyzed.

More in line with the type of DNA modification that is normally found in eukaryotic cells (5-methylcytosine), we have begun work on transferring two cytosine methylase genes (from type-II restriction-modification systems) into eukaryotic host cells.

These methylase genes are the *Eco*RII methylase gene from *E. coli* ($^{m}CC^{A}_{T}GG$) and the *Bcn*I methylase gene from *Bacillus centrosporus* ($C^{m}C^{G}_{C}GG$). In general, we are fusing these prokaryotic methylase genes to an inducible eukaryotic promoter so that it will be possible to control expression of these modification processes. In collaboration with D. Kurtz, we wish to determine if modification at $Cp^{m}CpG$ or mCpCpG will affect the levels of gene expression of specific gene products made within the host cells.

Publications

Brooks, J.E., R.M. Blumenthal, and T.R. Gingeras. 1982. Isolation and characterization of *Escherichia coli dam* methylase gene. In *Genetic exchange: A celebration, and a new generation* (ed. U.N. Streips et al.), vol. I, p. 221. Marcel Dekker, New York.

Brooks, J.E., R.M. Blumenthal, and T.R. Gingeras. 1983. Isolation and characterization of the *E. coli dam* methylase gene. *Nucleic Acids Res.* (in press).

Fitzgerald, G.F., C. Daly, L.R. Brown, and T.R. Gingeras. 1982. ScrFI: A new sequence-specific endonuclease from *Streptococcus cremoris*. *Nucleic Acids Res. 10*: 8171.

Gingeras, T.R. 1982. Computers and DNA sequences: A natural combination. In *Statistical analysis of DNA sequence data* (ed. B.S. Weir). Marcel Dekker, New York. (In press.)

Gingeras, T.R. and J.E. Brooks. 1983. Cloned restriction/modification from *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. 80*: 402.

Gingeras, T.R., R.M. Blumenthal, R.J. Roberts, and J.E. Brooks. 1982. The isolation and characterization of the *E. coli dam* methylase gene. In *Metabolism and enzymology of nucleic acids* (ed. J. Zelinka and J. Balan), p. 329. Publishing House of the Slovak Academy of Sciences.

Gingeras, T.R., D. Sciaky, R.E. Gelinas, J. Bing-Dong, C. Yen, M.M. Kelly, P.A. Bullock, B.L. Parsons, K.E. O'Neill, and R.J. Roberts. 1982. Nucleotide sequences from the adenovirus-2 genome. *J. Biol. Chem. 257*: 13475.

Modrich, P. and R.J. Roberts. 1982. Type II restriction and modification enzymes. In *Nucleases* (ed. S.A. Linn and R.J. Roberts), p. 109. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Roberts, R.J. 1982. Restriction endonucleases. In *Nucleases* (ed. S.A. Linn and R.J. Roberts), p. 311. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Roberts, R.J. 1982. Restriction and modification enzymes and their recognition sequences. *Nucleic Acids Res. 10*: r117.

———. 1983. Restriction and modification enzymes and their recognition sequences. *Nucleic Acids Res. 10*: r135.

Roberts, R.J., T.R. Gingeras, D. Sciaky, R.E. Gelinas, J. Bing-Dong, C. Yen, M.M. Kelly, P.A. Bullock, B.L. Parsons, K.E. O'Neill. 1983. Nucleotide sequences from the adenovirus-2 genome. *Cold Spring Harbor Symp. Quant. Biol. 47*: 1025.

ELECTRON MICROSCOPY

T.R. Broker, L.T. Chow, J.A Engler, M. Rossini, *R.A. Guilfoyle, M.S. Hoppe, R. Galli, W. Osheroff, A.J. Pelletier, D. Campanelli, M. Hallaran*

We have concentrated on four major research projects during the past year: (1) transcription of and cellular transformation by human papilloma virus DNA contained in a variety of shuttle-expression vectors; (2) completion of the DNA sequence of group-B human adenovirus type 7 (Ad7) between chromosomal map coordinates 8.8 and 31.5, interpretation of the RNA regulatory and processing signals and coding regions, and comparison with the analogous DNA sequences of the group-C adenovirus types 2 and 5 (Ad2, Ad5); (3) analysis of regulatory interactions of adenovirus early regions 1A and 2A using a combination of site-directed mutagenesis of cloned DNAs, DNA sequencing and microinjection, and indirect immunofluorescent detection of protein products of the cloned genes; and (4) characterization of the structures and sequence relations of four chicken β-tubulin genes. Each of these systems and the results obtained are discussed below in detail.

Dr. Peter Howley of the National Cancer Institute and T.R.B. co-organized a Papilloma Virus Meeting, which was held at Cold Spring Harbor Laboratory from September 14–18, 1982. The conference attracted 120 molecular geneticists, cell biologists, and clinicians, as described in detail elsewhere in this Annual Report.

Human Papilloma Virus RNA Transcription

L. Chow, T. Broker, R. Galli, D. Campanelli, A. Pelletier

We initiated the study of HPV-1 transcription in the fall of 1981 before the viral DNA was sequenced. We chose pBR322 derivative vectors pSV08 and pSV010 (from R. Myers and T. Tjian, *Proc. Natl. Acad. Sci. 77:* 6491 [1980]). These vectors are deleted for the pBR322 poison sequence which inhibits DNA replication in eukaryotic cells. They contain the SV40 origin of replication but no major cap sites of SV40 early or late mRNAs. Neither vector has a complete copy of the SV40 72-bp enhancer sequence necessary for SV40 early RNA transcription (Banerji et al., *Cell 27:* 299 [1981]; Benoist and Chambon, *Nature 290:* 304 [1981]). At the time, they were felt to be SV40 promoter-minus, with little likelihood of interference with the expression of the inserted genes. The recombinant plasmids are able to replicate in COS cells (SV40-transformed monkey CV-1 cells; Gluzman, *Cell 23:* 182 [1981]). For lack of information on the genomic organization of HPV-1, we cloned full-length chromosomes (extracted from viruses isolated from deep plantar warts) opened in a number of different circular permutations using the *Bam*HI restriction site, both of the *Eco*RI restriction sites, and three of the four *Bgl*II restriction sites (Fig. 1). We anticipated that transcription units would be uninterrupted in at least one of the constructs. Recombinant plasmids were transfected into COS cells using a calcium phosphate precipitation procedure. Cytoplasmic RNAs were isolated 48 hours posttransfection and examined by electron microscopy after hybridization to linearized HPV-1 recombinant DNA (Figs. 2 and 3; Chow and Broker 1983). The results can be summarized briefly as follows:

(1) The HPV-containing plasmids were replicated to very high copy number in the COS cells.

(2) The activity of the HPV-1 promoter(s) is extremely low. This was quite unexpected because of the high gene dosage. Rare transcripts comprising less than 0.001% of the poly(A)-containing mRNAs originated from minor SV40 early or late cap sites, depending on the orientation of HPV-1 in the clone relative to SV40. The transcripts extended over 40–80% of one and the same strand of HPV-1, with none complementary to the other strand.

(3) Two spliced RNAs were observed (Figs. 2 and 3). The main body of the major species corresponds to the predominant species seen in BPV-1-transformed mouse cells (Heilman et al., *Virology 119:* 22 [1982]). The main body of the minor species corresponds to the BPV-1 mRNA encoding the major capsid protein (L. Engel, C. Heilman, and P. Howley, pers. comm.). Each has a 5′ leader sequence spliced to the main body. Both species correlate well with the open reading frames of the HPV-1 DNA sequence subsequently published (Danos et al., *EMBO J. 1:* 231 [1982]) (Fig. 1). The 3′ ends of the RNA as mapped by electron microscopy (EM) also correspond to the locations of AATAAA signals for cleavage and polyadenylation found in the DNA sequence at the ends of the two gene blocks (Fig. 1).

(4) Splice acceptor sites of the main message body remain the same irrespective of whether the 5′ ends of the RNAs were derived from the early or late SV40 promoter and cap site.

(5) Primary transcripts initiated from the SV40 minor early promoter extend completely through the pBR322 vector sequences and on to one or the other HPV poly(A) site. During their processing, two donor and one acceptor splice sites (and one leader segment) were derived from the antisense sequences of the ampicillin gene in pBR322.

(6) Indirect immunofluorescence assays for the HPV capsid protein using antibodies directed against disrupted HPV-1 virions gave negative results in these transfected cells, consistent with the low amount of viral RNA.

We also transfected the plasmids into HeLa cells

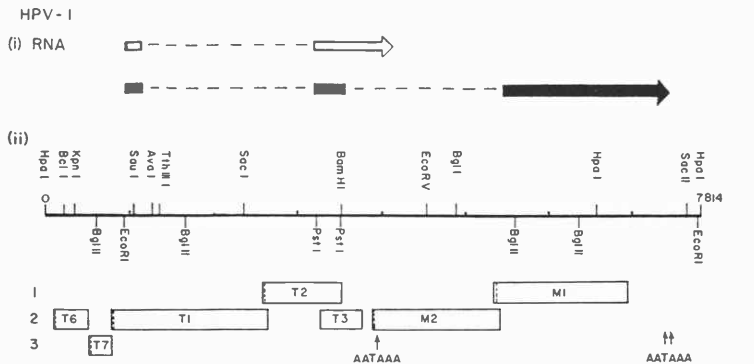


Figure 1
Organization and expression of human papilloma virus type-1a. (*i*) HPV-1 RNA transcripts generated from a surrogate SV40 late RNA promoter in an expression vector transfected into COS cells (Chow and Broker 1983) (see Fig. 2). (*ii*) Human papilloma virus type 1a, sequenced by Danos et al. (*EMBO J. 1:* 231 [1982]; *J. Virol. 46:* 557 [1983]). The circular genome is represented as a linear molecule opened at a site upstream of the transformation genes. Selected restriction endonuclease cleavage sites are indicated. Significant open translation regions in each of the three reading frames of the sense strand are shown. Dashed bars in open frames mark the positions of the first potential AUG initiation codon. Open translation frames are labeled by T (transformation region) or M (morphogenesis region). The locations of promoters and the DNA replication origins are not known. Splicing of primary RNA transcripts (see Fig. 3) potentially would join open translation frames in presently undefined combinations. Vertical arrows indicate the positions of AATAAA sequences, potential signals for 3′ cleavage and polyadenylation of transcripts. Dots above the axis indicate 1000-bp intervals from the index position.

because transcription of some genes has been reported to be better in HeLa than in COS cells, even though they will not replicate and amplify. Again, HPV-1 mRNAs remained extremely rare, as assayed by EM analysis of RNA:DNA heteroduplexes. In another attempt to increase the activity of the HPV-1 promoter, we treated the transfected HeLa or COS cells with a low dose of azacytidine. This base analog inhibits cytidine methylation, which has been shown to be associated with transcriptionally inactive genes. Still we saw no improvement of HPV-1 transcription.

A drawback in trying to study gene expression by transient expression in transfected cells is the low percentage of cells that successfully take up DNA (typically 5–20%). We have tried other protocols that have produced high (>70%) efficiency of transfection in certain cell types. Again, there was hardly any HPV-1 RNA in the cytoplasm. We conclude that transcription of HPV-1 is unusually restricted in otherwise successful expression vectors, as it also is in warts in situ.

Transformation of tk⁻ Cells by Cloned Human Papilloma Viral DNA

One way to ensure that each cell in a culture has many copies of the HPV-1 DNA sequence is to isolate transformed cells containing HPV-1 DNA. We elected to use the herpes simplex virus type-1 thymidine kinase (*tk*) gene as a selectable marker. The *tk* gene (without a promoter) can initiate transcription from within the coding region (apparently from an adventitious "TATA" sequence and 5′ cap site), and the shortened RNA is translated from an internal, in-phase AUG initiation codon into a somewhat defective protein (Zipser et al., *Proc. Natl. Acad. Sci. 78:* 6276 [1981]; Roberts and Axel, *Cell 29:* 109 [1982]). Cells can become successfully transformed and HAT-resistant with such a defective *tk* gene only when it is highly expressed from amplified *tk* genes present at several hundred copies (*ibid*). Long stretches of DNA next to the *tk* gene are also amplified in the process. Therefore, we chose to make HPV-1 recombinants containing a promoterless *tk* gene. The recombinants were transfected into mouse Ltk⁻ cells and human 143 tk⁻ cells using a calcium phosphate precipitation procedure, and the cells were put under HAT selection. We expected that each surviving cell would have several hundred copies of the HPV-1 DNA.

Two major types of tk⁺ HPV-1 recombinants were constructed. Four recombinants were in a pBR322 vector and four in a pBR322 derivative, *pSVΔPH, a vector that we modified from plasmid pSVΔPH (Treisman et al., in *Eukaryotic Viral Vectors*, p. 63, Cold Spring Harbor Laboratory [1982]). Two of the

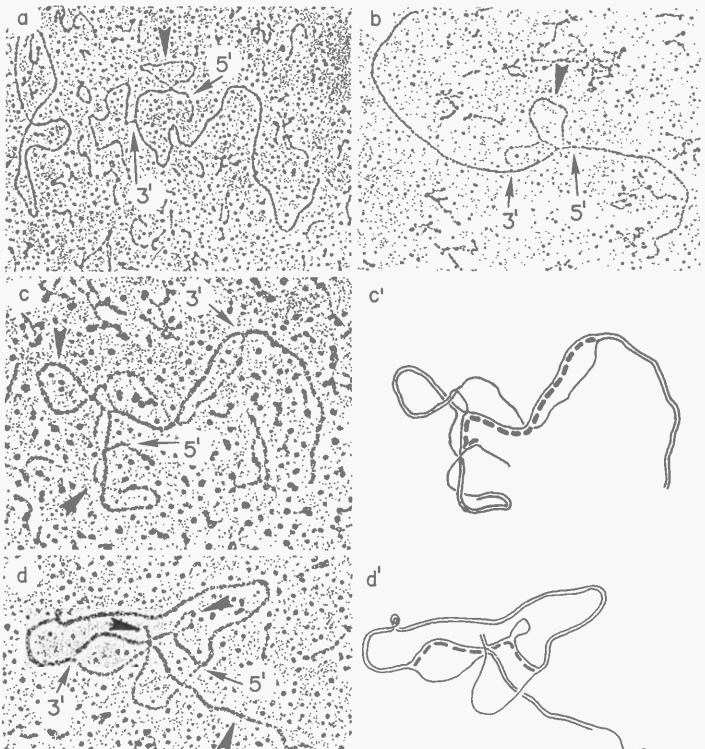


Figure 2

Electron micrographs of RNA transcripts of HPV-1. (a) Total cytoplasmic RNA was isolated 48 hr posttransfection of COS-1 cells with HPV-1:pSV08 plasmid D2 (cf. Fig. 3A). It was annealed to plasmid 6.3 DNA, which consists of HPV-1 DNA opened at the *Bgl*II site at nucleotide 594 and inserted into the pSV010 *Bgl*II site in the polylinker. Plasmid 6.3 was linearized prior to R-loop formation at its *Cla*I site in the polylinker on the side of the vector (near SV40 cf. Fig. 3A). The RNA is annealed to a terminal fork in the partially denatured DNA. (*b*) RNA from the same preparation was annealed to plasmid 55.2 DNA, which consists of HPV-1 DNA opened at the *Bgl*II site at HPV-1 nucleotide 5581 and inserted into the pSV010 *Bgl*II site in the polylinker in the same orientation as plasmid 6.3. Plasmid 55.2 was linearized at the *Cla*I site in the polylinker prior to R-loop formation. The 5′ and 3′ ends of the RNAs are labeled with arrows and the DNA deletion loop corresponding to the single intervening sequence in each RNA is marked with a large arrowhead. (c) and (c′) RNA from the same COS cell extract was annealed to plasmid 6.3 DNA, as in a. The RNA termini and the two DNA deletion loops corresponding to the two intervening sequences removed from the RNA are indicated as in a and *b*. In the interpretive tracing, RNA is depicted with the dashed line. The locations of the 5′ ends of both species of RNA (cf. schematic illustration in Fig. 3A) suggest they were initiated from an SV40 late promoter in the vector. (*d*) and (*d′*) Total cytoplasmic RNA isolated 48 hr posttransfection of COS-1 cells with HPV-1:pSV010 plasmid B38 (cf. Fig. 3B) was annealed to plasmid 6.3 DNA to form R loops. The DNA deletion loop corresponding to the first intervening sequence (IVS) encoded by the pSV010 vector is indicated with an arrowhead. The DNA was linearized at the *Xor*II site in the vector, which is within the second IVS, as reflected by the short and long duplex DNA tails, also marked by large arrowheads. In the interpretive tracing, RNA is depicted with the dashed line. (See Fig. 3B for a graphic representation.)

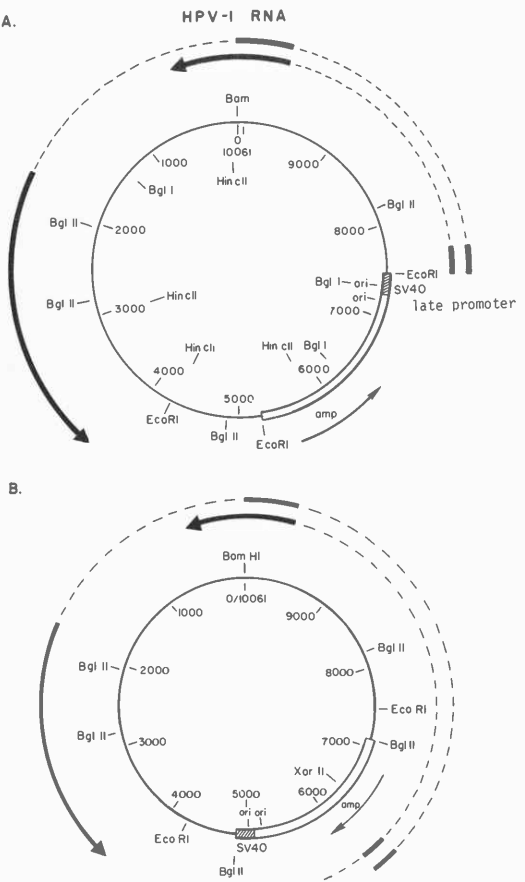


Figure 3
Human papilloma virus RNA transcripts (Chow and Broker 1983). The spliced RNAs generated from *A*, an SV40 late promoter in the vector pSV08, or *B*, an SV40 early promoter in vector pSV010 transfected into COS cells, were mapped by electron microscopical analyses of heteroduplexes or R loops formed between cytoplasmic RNAs and linearized cloned DNAs. In *B*, note the use of cryptic donor and acceptor splice sites derived from pBR322 sequences in the vector, and the RNA "leader" segment equivalent to part of the antisense strand of the *amp* gene. The SV40 and the pBR322 replication origins are marked. The conserved HPV-1-specific sequences of the hybrid transcripts from the two plasmids are identical. The alignments of the RNAs derived from the late SV40 late promoter in *A* with the open translation frames are shown in Fig. 1. The nucleotide numbering system was recently altered so that the *Bam*HI restriction site is at nucleotide 3538.

recombinants are shown in Figure 4, A and B. *pSVΔPH lacks the pBR322 poison sequence; it contains one copy of the SV40 enhancer and the SV40 origin of replication and, due to a deletion in the 21-bp repetition in the SV40 segment, lacks SV40 promoter activity. In these recombinants, the HPV-1 viral DNA was linearized at the *Eco*RI site at nucleotide 7777, which is upstream from both a "TATA"-like sequence in the putative HPV promoter region (Danos et al., *EMBO J. 1:* 231 [1982]) and all open reading frames. The HPV was inserted into the vector in different locations and orientations, relative to the transcription direction of the *tk* gene as well as the SV40 enhancer sequence. Very few tk⁺ colonies were recovered from cells transformed with clones containing pBR322 as a vector (about 30 transformants/μg DNA). When the pBR322 derivative lacking the poison sequence was used as the vector (see Fig. 4B), tk⁺ colonies were readily recovered (about 3000 transformants/μg DNA). A large number of transformants were isolated and grown up under HAT selection. Southern blot analyses of both low-molecular-weight and total cellular DNA of the tk⁺ transformants confirmed the presence of HPV-1 DNA. To our surprise, the copy number in each transformed cell was fewer than 10, indicating the recombinants were not amplified. Therefore, unlike in previous reports, the attenuated *tk* gene was sufficient to confer HAT resistance. It is possible that the SV40 enhancer had an effect on the cryptic *tk* promoter and stimulated its activity sufficiently without requiring high gene dosage. Similarly, in the few tk⁺ colonies that arose in cultures transformed by recombinants of poison sequence-containing pBR322-*tk*-HPV1 that had no SV40 sequences (see Fig. 4A), the input DNA was not amplified. This suggests that an HPV enhancer-like effect on the transcription of the truncated *tk* gene has occurred. An enhancer sequence has been located in the BPV-1 sequence which stimulates *tk* transformation by the intact HSV-1 *tk* gene (Lusky et al., in *Eukaryotic Virol Vectors*, p. 99, Cold Spring Harbor Laboratory [1982]).

HPV-1 recombinants in some of the transformants have integrated. But some apparently exist as plasmids, as demonstrated by Southern blotting experiments. A number of plasmids have been recovered from Hirt lysates of the transformed cells into *E. coli* bacteria using ampicillin selection. Some have small DNA rearrangements and others have gross deletions; they are now being mapped by EM analysis of DNA:DNA heteroduplexes.

We have isolated cytoplasmic RNAs from tk⁺ transformants where the DNA rearrangements did not involve HPV-1 sequences. Unfortunately, no HPV-1-specific RNA was found, even when the transformants were treated with 10^{-7} M 12-*O*-tetradecanoylphorbal-13-acetate (TPA), a phorbol ester known to increase the transcription of BPV-1 viral sequences in BPV-1-infected mouse embryo fibroblast cells (Amtmann and Sauer, *J. Virol. 43:* 59 [1982]). These HPV-1-*tk*-SV *Ori* recombinant plasmids were also transfected into COS and HeLa cells. The level of HPV-1 RNA transcription was not higher than in our original experiments. Therefore, the SV40 enhancer had no detectable effect on the HPV promoter(s).

Transformation of Primary Rat Cells

Recombinant plasmid I-24 is an HPV-1 recombinant we have cloned using *pSVΔPH as a vector without including the *tk* gene (Fig. 4C). It was used to transform primary baby rat kidney cells. Morphological transformants (foci) appeared in 10–14 days. Southern blot experiments showed that HPV-1 recombinant DNA existed in these cells as plasmids with fewer than 10 copies per cell. Plasmids were rescued from Hirt lysates of the transformed cells into *E. coli* HB101 via ampicillin selection. The majority of the recovered plasmids were identical to the input DNA, as judged by three different restriction endonuclease digestions (*Eco*RI, *Bgl*II, and *Hin*dIII).

Cytoplasmic RNA was isolated from TPA (phorbol ester) treated or untreated transformants. Despite the successful expression of the HPV transformation genes, our preliminary results indicate the level of HPV-1-specific mRNA remains miniscule. I-24 plasmid was also used to transfect COS and HeLa cells to no avail. Again, it appeared that the SV40 enhancer has little effect in this system.

In summary, the results from all our experiments indicate that the transcriptional promoter(s) of HPV-1 is very inefficient. Although we can not rule out that part or all of it has been separated from the rest of the transcription unit during DNA cloning, it clearly can not be the only cause of inactivity. We conclude this because, in two of the recombinants we made, HPV-1 was linearized with *Bgl*II cleavage in the morphogenesis region M_1 (see Fig. 1), well upstream from the segment in which the promoter is suspected to be located (between M_1 and T6). Cells transfected with these clones did not produce more HPV-1 RNA than those cloned at the *Eco*RI site at nucleotide 7777.

Adenovirus-7 DNA Sequence and Interpretation

J. Engler, M. Hoppe

In the 1981 Annual Report, many of the features of the Ad7 DNA sequence between map coordinates 8.8 and 31.5 were discussed. Resolution of the few remaining uncertainties allowed the preparation of the complete sequence analysis shown in Figure 5. The protein IX and IVa2 coding regions were described in detail in previous reports. The longest open translation frame (map coordinates 23.0–13.8) encodes a 140-kD protein, which has recently been shown to be a DNA polymerase (Enomoto et al., *Proc. Natl. Acad. Sci. 78:* 6779 [1981]; Stillman and Tamanoi, *Cold Spring Harbor Symp. Quant. Biol.*

Figure 4
Shuttle-expression vectors for HPV-1. HPV-1 virion DNA from a single plantar wart was linearized by partial digestion with *Eco*RI restriction endonuclease and inserted into vectors we constructed from elements available from others, as described below. The clones selected for continued study were those in which the HPV-1 was opened at the *Eco*RI site at nucleotide 7777, presumably upstream from the promoter for the T region. (*A*) A pBR322 clone of a truncated herpes simplex virus type-1 thymidine kinase (*tk*) gene was obtained from Zipser et al. (*Proc. Natl. Acad. Sci. 78:* 6276 [1981]). The truncated *tk* gene apparently is transcribed from an adventitious TATA "promoterlike" sequence within the coding region of the gene, and translation of the mRNA utilizes an in-phase AUG initiation codon, as indicated. We removed the *Eco*RI site in pBR322 by restricting, filling in the cohesive ends, and religating the blunt ends. The HPV-1 was then recombined into the *Eco*RI site downstream of the *tk* gene. (*B*) pSVΔPH was obtained from Dr. Michael Green (see Treisman et al., in *Eukaryotic viral vectors*, p. 63. Cold Spring Harbor Laboratory [1982]). A deletion in the SV40 sequences removes the "21-bp repetitions" and part of one of the 72-bp "enhancer sequences" to eliminate SV40 early promoter activity. The vector retains enhancer activity from the remaining intact 72-bp repetition. The plasmid also has a deletion of one of the three T antigen binding sites constituting the replication origin but retains a diminished capacity for amplification in COS cells. The poison sequence in pBR322 that inhibits replication in eukaryotic cells is deleted. We first excised the globin gene from pSVΔPH. The *Bam*HI site in the pBR322 sequences of this vector was then converted to an *Eco*RI site via linker addition (˚pSVΔPH). The *Eco*RI–*Sal*I fragment containing the HSV-1 *tk* gene described in *A* was used to replace the *Eco*RI–*Sal*I fragment of ˚pSVΔPH. Finally, the HPV-1 genome was inserted into the *Eco*RI site of the composite vector. (*C*) The *Bam*HI site in the pSVΔPH (as in *B*) was converted to an *Eco*RI site, and HPV-1 was inserted into this site.

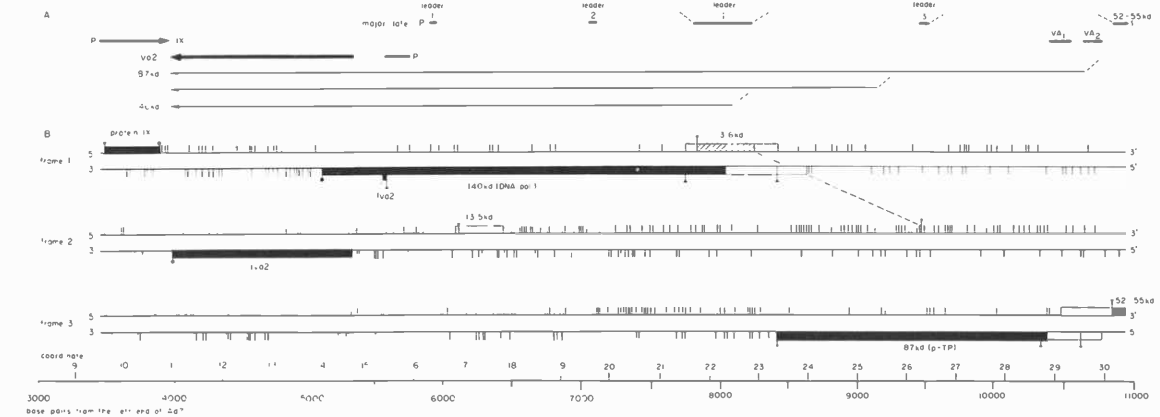


Figure 5

Adenovirus-7 (Ad7) DNA sequence analysis between coordinates 9.5 and 31 (Engler et al. 1983). (*A*) The distance from the left end of Ad7 is given in base pairs and in chromosome coordinates. Thin lines designate early mRNAs from region E2B of Ad2; thick lines show the positions of the promoter and leader segments for the late mRNAs for proteins IX, IVa2, the i leader, the viral-associated (VA) RNA genes, and the 5′ end of the main body for late region 1 (the gene for the 52-kD-55-kD protein). (*B*) Vertical lines show the positions of stop codons in each potential translation frame. The first ATG in each major reading frame is shown with a triangle and the first stop codon with a filled circle. Frames that are thought to encode proteins are shown as open or filled rectangles; those that are highly conserved between Ad7 and Ad2 and Ad5 are shown as filled rectangles. The hatched rectangle shows the position of the 13.6-kD polypeptide encoded by the i and third leaders equivalent to that identified in Ad2 by Virtanen et al. (*Nucleic Acids Res. 10:* 2539 [1982]). The 13.5-kD protein-coding region (Lewis and Mathews, *Cell 21:* 303 [1980]; C.W. Anderson and J.B. Lewis, pers. comm.) is at the same position and of the same length in Ad2 and Ad7, and is generally conserved in sequence except for a significantly higher number of arginine residues in Ad7.

47: 741 [1983]). On the basis of DNA-negative mutations mapping within the gene, the polymerase is essential for viral replication. As reported last year, the coding sequences for the carboxyl terminus of the polymerase overlap those for the amino terminus of the IVa2 protein. Ad2 DNA sequence over this same region was concurrently determined by Gingeras et al. (*J. Biol. Chem. 257:* 13475 [1982]) and by Alestrom et al. (*J. Biol. Chem. 257:* 13492 [1982]). The few sequence differences between Ad7 and Ad5 or Ad2 in this region suggest that the greater selective pressure has been on the polymerase; most differences are in the degenerate positions of the codon triplets of the polymerase, where they make little or no difference in the specified amino acids, but they do alter the IVa2 protein, which is translated in a different phase. The splice junctions of the mRNA for the polymerase have not yet been determined; hence, it is not clear whether translation starts in the upstream leader segment (map coordinates 39.5–39.0) or at the first AUG initiation codon in the main body of the RNA at coordinate 21.4. As shown by the solid black bar in Figure 5, the region of close homology in the Ad7 and the Ad2 genes for the polymerase extends to coordinate 22.3, well upstream from the first AUG codon. This and the large size of the polymerase lend support to the speculation that the amino terminus of the polymerase is encoded in the leader.

The coding region for the 87-kD protein, which is covalently linked to the 5′ termini of the DNA and essential for the initiation of DNA replication (Challberg et al., *Proc. Natl. Acad. Sci. 77:*15105[1980]); Stillman et al., *Cell 23:* 497 [1981]), is also transcribed from the leftward-transcribed strand, between coordinates 28.5 and 23.0. As with the polymerase genes and mRNAs, it is not yet known whether translation of the spliced messages begins in the common leader from coordinate 39.5–39.0 or in the main body of the message. The 5′ end sequences of the open translation frame of Ad7 are not very similar to those of Ad2 between coordinates 29.8 and 28.8. The overall nucleotide sequence homology of Ad7 and Ad2 within the *l*-strand-coding regions is 80%.

A 13.6-kD protein (Lewis and Mathews, *Cell 21:* 303[1980]) specified at early to intermediate times after infection by the i leader of the *r*-strand mRNAs originating from the major late promoter at 16.6 (Chow et al., *J. Mol. Biol. 134:* 265 [1979]). Part or most of its coding sequences overlap the coding region for the polymerase on the opposite strand, a novel situation among eukaryotes. By analogy to the Ad2 13.6-kD gene, the message for which has been sequenced as a cDNA (Virtanen et al., *Nucleic Acids Res. 10:* 2539 [1982]), the termination codon for the Ad7 13.6-kD protein is probably introduced after the splice of the i leader to the third leader segment of the *r*-strand transcripts in which it is included. The only place the three leader segments of the late messages are not strictly colinear is near the beginning of the third leader, where Ad2 has an extra triplet codon (for the 13.6-kD protein) compared with Ad7. The carboxy-terminal halves of the Ad7 and Ad2 13.6-kD proteins differ significantly, unlike the entire remainder of this genetic interval. The inferred sequences of the amino termini of the Ad7 and Ad2 genes for the 52-kD–55-kD genes are very similar, but long in-phase open reading frames just upstream of the known splice site (Akusjärvi and Persson, *Nature 292:* 420 [1981]) are not similar. Nonetheless, these may encode proteins from late messages with splice acceptors for the third leader near coordinate 29 (Chow and Broker, *Cell 15:* 497 [1978]).

Comparison of unassigned open translation frames of Ad7 and Ad2 suggest that only two, both on the *r* strand, are reasonably homologous: One appears to encode a 13.5-kD protein between coordinates 16.9 and 17.9. Direct evidence for such a protein has been found by Drs. J.B. Lewis and C.W. Anderson (pers. comm.) using cell-free translation of selected mRNA, but the RNA itself has not been mapped. The second open frame spans the third leader segment and suggests that the "long third leaders" occasionally seen (Kilpatrick et al., *J. Virol. 30:* 899 [1979]; Chow et al., *Cold Spring Harbor Symp. Quant. Biol. 44:* 401 [1980]) in *r*-strand messages at intermediate times after infection might encode minor proteins.

Following a very productive and interactive five years at Cold Spring Harbor Laboratory, Jeff Engler accepted a position as an Assistant Professor of Biochemistry at the University of Alabama-Birmingham School of Medicine.

Adenoviral Early Gene Regulation

M. Rossini, R. Guilfoyle, J. Engler, W. Osheroff, M. Hoppe

We have continued to study the regulatory interaction between human adenoviral genes expressed at early times after infection by combining site-directed mutagenesis and microinjection techniques. We have determined that the products of early region 1A (E1A) differentially regulate the two primary promoters for the single-stranded DNA binding protein (DBP), one active at early times and the other at late times. By microinjecting different E2A clones containing the DBP gene in association with its early promoter (at map coordinate 75), with its late promoter (at coordinate 72) or with both the early and the late promoters, we have shown that coinjection of a plasmid that expresses early region 1A stimulates the expression of the DBP mRNA transcribed from its early promoter and inhibits the production of the DNA binding protein (DBP) from its late promoter (Table 1) (M. Rossini, in prep.). A modulation of the inhibitory effect on the E2A plasmid with the late promoter has been obtained by varying the E1A DNA concentration, providing more

Table 1
Expression of the 72-kD DNA Binding Protein after Microinjection of E2A Clones into Tk-*ts* 13 Cell Nuclei

Plasmid injected	*Ad DNA insert coordinates*	*E2A promoter present*	*Percent nuclei positive for 72-kD protein*
pAd207 + buffer	59.5–70.7	none	3
pAd207 + HE4[a]			2
pBR730 + buffer	59.5–75.9	early + late	15
pBR730 + HE4[a]			81
pFAG5 + buffer	59.5–75.9[b]	early	20
pFAG5 + HE4[a]			87
pAd15.1 + buffer	50.7–72.8	late	53
pAd15.1 + HE4[a]			16

About 10^{-11} ml of solution containing plasmid DNAs at a final concentration of 250 μg/ml were injected into 100–200 cell nuclei in each experiment. The average percentage of positive nuclei has been calculated from 5 to 10 separate experiments. The 72-kD protein was visualized by indirect immunofluorescence 20–24 hr after microinjection using a hamster anti 72-kD serum (c-783 provided by A. Lewis) as the first antibodies.

[a]*Plasmid HE4 includes adenovirus DNA sequences from coordinates 0–4.5. Similar results were obtained when an E1A + E1B Be5 plasmid: (coordinates 0–9.1) was coinjected.*

[b]*The DNA segment between coordinates 68.4 and 72.8 containing the late promoter has been deleted.*

evidence that the inhibition is a direct function of E1A products. A primary objective of the past year has been the determination of which E1A products are responsible for the interactions with the E2A promoters.

From microinjection tests using E1A plasmids with short deletions introduced by site-directed mutagenesis into different parts of the gene (N. Stow, *J. Virol. 37:* 171 [1981]), we suggested last year that the carboxyl terminus of the E1A proteins includes control functions that lead to the activation or repression of the DBP gene. More detailed understanding has been achieved recently through the use of two additional E1A mutant plasmids, pEK *pm*975 and *phr*1. The pEK *pm*975 (obtained from Dr. A. Berk, University of California, Los Angeles), a single base-pair transversion at the donor splice site of the 12S mRNA, eliminates its production, but does not alter the synthesis of 13S mRNA or its protein product (Montell et al., *Nature 295:* 380 [1982]). Reciprocally, the mutation in adenovirus type 5 (Ad5) *hr*1 introduces a termination codon into the 13S mRNA but leaves the 12S mRNA intact (Esche et al., *J. Mol. Biol. 142:* 399 [1980]; Ricciardi et al., *Proc. Natl. Acad. Sci. 78:* 6121 [1981]). Coinjection of an E1A clone derived from *hr*1 failed to activate the E2A early promoter clone, but this E1A mutant (with the intact 12S mRNA) is still able to inhibit the production of the DBP from its late promoter (Table 2). Conversely, the E1A plasmid pEK *pm*975, which generates only the 13S mRNA, maintains the ability to stimulate the expression of the DBP early promoter clone (pFAG5), but no longer inhibits the expression of the DBP late promoter clone (pAd 15.1) (Table 2). These results indicate that early region 1A encodes two distinct functions controlling the expression of the DBP. The stimulatory factor for the early DBP promoter encoded by the 13S mRNA was previously demonstrated in vivo (Montell et al., *Nature 295:* 380 [1982]). The inactivity of the late promoter for DBP in pBR730 (which also contains the early promoter) in the absence of E1A plasmid suggests there is interaction between the two promoters.

On the assumption that stimulation is a consequence of activation of a transcriptional promoter and that inhibition results from repression of a promoter, experiments were designed to identify the exact location and sequence feature for each of the region-2A promoter sites. Sets of deletions in the E2A early and late promoter regions have been constructed by site-directed mutagenesis using BAL31

Table 2
Expression of the 72-kD DNA Binding Protein Gene from Its Early or Late Promoter after Coinjection of the E2A Plasmids with Mutated E1A Plasmids DNAs

			E2A plasmid: *E2A promoter:*	*pFAG5* *early*	*pAd15.1* *late*
E1A plasmid coinjected	*E1A mRNA produced*			*percent of nuclei positive for the 72-kD protein*	
	12S	*13S*			
None	–	–		20	84
HE4 (wt)	+	+		85	35
*phr*1	+	truncated protein		18	36
pEK*pm*975*	–	+		86	83

Cell nuclei were injected and analyzed as described in Table 1.

[a]*Montell, C., E.F. Fisher, M.H. Caruthers, and A.J. Berk,* Nature 295: *380 (1982).*

exonuclease. These mutants are derivatives of two newly constructed E2A clones containing the DBP gene associated with the early promoter (E252.1) or with the late promoter (L251.1) that have been shown to behave in microinjection experiments like the other E2A clones (described in Table 1) in response to coinjection with region E1A. DNA sequence analyses of these mutants have shown that these deletions extend to different positions between nucleotides −132 and −3, upstream from both the early and late mRNA cap sites.

The mutants deleted in the late promoter region have been tested recently in the microinjection system in the presence or in the absence of the wild-type E1A plasmid. The sequences located between −51 and −87 bp upstream from the late transcriptional RNA cap site appear to contain at least part of the E1A regulatory interaction site (Fig. 6). Inspection of the DNA sequence within this region has revealed an interesting feature that consists of a duplicated GCGG sequence flanking a potential stem-loop structure, which can also be regarded as a partial inverted sequence repetition (Fig. 7). Perhaps the binding of an E1A protein to this region blocks entry of RNA polymerase II or its translocation to the preferred initiation site. Alternatively, binding may induce a change in the DNA or chromatin conformation that renders this region transcriptionally inactive. Additional deletions between −87 and −51 are now being created to map more precisely the primary sequence involved in the repressor function. Similar coinjection experiments are underway using deletions of various portions of the sequences upstream from the E2A early promoter to study the effect on E1A stimulation of DBP expression.

Two approaches are now in progress to analyze the E2A transcription products directly. One involves the use of cell-free extracts, prepared from HeLa cell suspension cultures, which contain RNA polymerase II and cellular factors required for faithful transcription in vitro from the E2A early and late promoters (Mathis et al., *Proc. Natl. Acad. Sci. 78:* 7383 [1981]). The parental plasmid, L251.1 and E252.1, have been tested for their template activities in this system and compared to clone p41 (obtained from N. Fraser), which contains the major late promoter of Ad2 (coordinate 16.45). Our results indicate that transcription from the E2A early and late promoters is extremely inefficient relative to the Ad2 major late promoter (30–50-fold reduced), as also observed by others. To facilitate 5′ end analysis of these transcripts by S1 nuclease mapping procedures, it will be necessary to increase these weak signals. To this end, calf thymus RNA polymerase II will be added to supplement the extracts in the transcription reactions.

The second approach we are undertaking to analyze early region 2A transcription directly is by transient expression of the early and late promoter clones transfected into cell monolayers. Experiments are in progress to optimize conditions for the uptake and expression of the E2A plasmids added to hamster cells as calcium-phosphate precipitates.

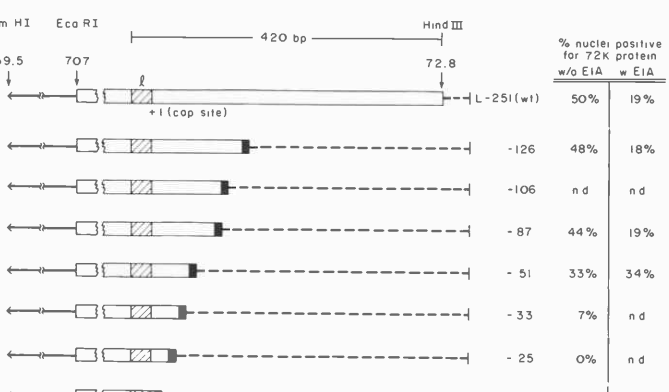


Figure 6

The structures of an Ad5 DBP clone containing just the late DBP promoter region and deletion mutant derivatives affecting the promoter. The sequences upstream of the cap site that are deleted and substituted by pBR322 (broken lines) are illustrated. The nucleotide corresponding to the end point of deletion in each mutant clone is indicated to the right side of each diagram (the 5′ capped nucleotide of the mRNA being at nucleotide position +1). On the far right are the results obtained after microinjection of these clones in the presence or the absence of E1A plasmid reported as percentages of nuclei positive for the DNA-binding protein.

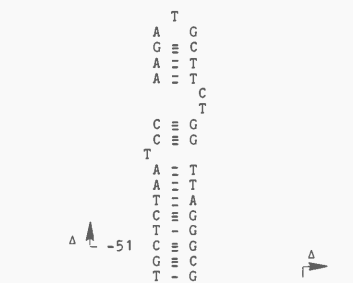


Figure 7

The DNA sequence between nucleotide −88 and −40 located upstream from the E2A late transcription initiation site (Galibert et al., *Gene* 6: 1[1979]). The dyad axis of near symmetry found in this region is shown diagramatically as a hairpin structure. The nucleotides substituted with pBR322 sequences in deletion mutants Ld15 ⁻87 and Ld15 ⁻51 are indicated. The deletion 5′ to nucleotide −51, but not the deletion 5′ to nucleotide −87, reduces the E2 late-promoter repression by the E1A product, as indicated in Fig. 6.

The 72-kD DBP is being analyzed by immunofluorescence and by immunoprecipitation of transfected cell extracts.

Electron Microscopic Heteroduplex Analysis of Chicken β-Tubulin Genes

L.T. Chow

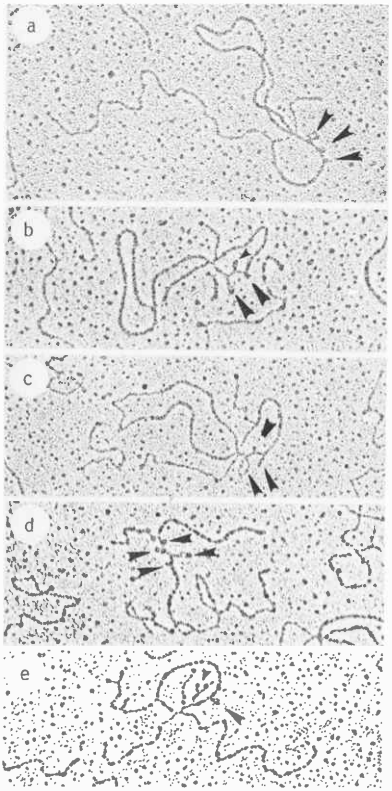
Microtubules are major cytoskeletal structures that participate in the formation of spindles used for chromosomal segregation during cell division. They also are present in the cilia and flagella involved in cellular motion and form some of the cytoplasic fibers that confer cell shape and permit anchorage and locomotion. They are composed of various types of α- and β-tubulin polypeptides, which have been rather highly conserved during evolution. Nonetheless, small but important differences within the α- and β-tubulin gene families dictate subtle structural and functional distinctions; their regulated expression plays a basic role in cellular differentiation.

In a previous collaborative study with Dr. Nicholas Cowan at Princeton, I investigated the structures of some of the human α- and β-tubulin genes (Cowan et al., *Proc. Natl. Acad. Sci. 78:* 4877 [1981]; Wilde et al., *Proc. Natl. Acad. Sci. 79:* 96 [1982]). More recently, in collaboration with Drs. Don Cleveland, Margaret Lopata, and Jane Havercraft of The Johns Hopkins University of Medicine, the structures of the four different β-tubulin genes of chicken were studied. Each of the genes, cloned in pBR322, was characterized by electron microscopic analyses of heteroduplexes formed with a β-tubulin cDNA clone prepared from mature mRNA. Representative heteroduplexes, and interpretative tracings, are presented in Figure 8, a–e. The β-1 and β-2 genes had three intervening sequences (IVS) at similar sites relative to the conserved regions. The IVS ranged from 160 to 500 nucleotides long and were separated from each other by conserved sequences homologous to the pT2 cDNA of about 130 nucleotides each (Fig. 8, a and b). The intervening sequences were located near the 5′ end of each gene, as concluded from the asymmetrical disposition of the pBR322 sequence relative to the cDNA insertion in pT2 DNA in the heteroduplexes (e.g., Fig. 8d). About 250 nucleotides proximal to the 3′ end of the cDNA in pT2 correspond to the untranslated region of the mRNA (Valenzuela et al., *Nature 284:* 650 [1981]). They did not hybridize to the β-1 genomic DNA, indicating sequence divergence in the 3′ untranslated region (Fig. 8a). In contrast, the β-2 clone hybridized to the entire cDNA in pT2 (Fig. 8b). This observation suggests that the cDNA is derived from a message transcribed from the β-2 gene. The β-1 and β-2 genes appear highly homologous in all the other conserved coding segments, since the heteroduplexes with pT2 were formed at fairly stringent conditions.

The β-3 genomic DNA clone hybridized to pT2 DNA at lower frequency than did β-1 or β-2, suggesting some sequence divergence. β-3 appeared to have three or four small intervening sequences of 80–500 nucleotides clustered at the 5′ end of the gene; three of these were located at sites similar to those in the β-1 and β-2 genes (Fig. 8c). Occasionally, an additional homologous sequence was detected at the 5′ end and was possibly followed by a small intervening sequence, as diagramed in Figure 8f. The first two conserved segments hybridized to pT2 only in some of the heteroduplexes at a nonstringent condition. This is most likely due to sequence divergence. About 250 nucleotides in the 3′ untranslated region in the cDNA did not hybridize to β-3 DNA.

The β-4 gene and the pT2 cDNA formed two related heteroduplexes that had three or four small intervening sequences, respectively, near the 5′ end of the gene (Fig. 8, d and e). The first large IVS in the heteroduplexes with three IVS apparently contained sequences redundant to, but separate from, the 3′ half of the first conserved segment. Branch migration led to the formation of a fourth IVS in the heteroduplexes. Consequently, the first conserved region was split into two shorter segments. In fact, most heteroduplexes had four IVS, suggesting a better sequence homology to pT2 in this configuration. Interestingly, of the four chicken β-tubulin genes, only the β-4 gene appears to encode two mRNAs of slightly different lengths, perhaps due to different splicing associated with the apparent duplication of a conserved segment. Again, the 3′ untranslated region in pT2 cDNA did not hybridize to the β-4 DNA.

The arrangement of conserved and intervening


a
b
c
d
e
f
5'
3'
human 5β
chicken β1
chicken β2
chicken β3
chicken β4
chicken β4
(alternate)
0
1
2
3
6
7 Kb

segments in the chicken and human β-tubulin genes is diagramatically summarized in Figure 8f. Interestingly, all three IVS in β-1 and β-2 and the latter three IVS in β-3 and β-4 were located at sites similar to one another and to those detected in a human β-tubulin gene (Cowan et al., *Proc. Natl. Acad. Sci. 78:* 4877 [1981]). Moreover, the pT2 cDNA is more homologous to the human β gene than it is to β-3 and β-4. It is possible that β-3 and β-4 genes may encode polypeptides of substantial sequence divergence from the β-2 polypeptide.

Publications

Broker, T.R. 1982. Human adenovirus serotypes 2, 5, 3, 7, 16, 12 and 31 and adenovirus-SV40 hybrids: Maps of RNA transcripts, protein-coding regions, mutations and restriction endonuclease cleavage sites. In *Genetic maps* (ed. S.J. O'Brien,) vol. II, p. 74. National Institutes of Health, Bethesda, Maryland.

——.1982. Compilation of human and animal papilloma virus DNA restriction maps. In Papilloma virus meeting abstract book, p. 83. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

——. 1983. Animal virus RNA processing. In *Processing of RNA* (ed. D. Apirion). CRC Press, Boca Raton, Florida. (In press.)

Broker, T.R. and L.T. Chow. 1983. Patterns and consequences of adenoviral RNA splicing. *Ex pluribus unum–Ex uno pluribus.* In *DNA makes RNA makes protein* (ed. T.Hunt et al.), p. 171. Elsevier Biomedical Press, Cambridge, England.

Chow, L.T. and T.R. Broker. 1983. Human papilloma virus type 1 RNA transcription and processing in COS-1 cells. In *4th Frederick Cancer Research Center Symposium* (ed. M.L. Pearson and N.L. Sternberg). Raven Press, New York. (In press.)

Chow, L.T., M. Rossini, J.A. Engler, M.P. van Bree, R.A. Guilfoyle, and T.R. Broker. 1982. Organization and expression of the human adenovirus genes encoding the DNA binding protein and the DNA terminal protein. In *Genetic engineering techniques: Recent developments* (ed. P.C. Huang et al.), p. 57. Academic Press, New York.

Engler, J.A. and M.P. van Bree. 1982. The nucleotide sequence of the gene encoding protein IVa_2 in human adenovirus type 7. *Gene 19:* 71.

Engler, J.A., M.S. Hoppe, and M.P. van Bree. 1983. The nucleotide sequence of the genes encoded in early region 2b of human adenovirus type 7. *Gene 21:* 147.

Lopata, M.A., J.C. Havercroft, L.T. Chow, and D.W. Cleveland. 1983. Four unique genes required for beta-tubulin expression in vertebrates. *Cell 32:* 713.

Rossini, M. The role of adenovirus early region 1A in the expression of early regions 2A and 1B. (In preparation.)

Stillman, B., W.C. Topp, and J.A. Engler. 1982. Conserved sequences at the origin of adenovirus DNA replication. *J. Virol. 44:* 53.

Figure 8

The structure of chicken β-tubulin genes. (a–c) Electron micrographs of heteroduplexes between the linearized cDNA clone pT2 and, respectively, the β-1, β-2, and β-3 inserts released from pBR322 with restriction endonucleases. (*d–e*) Micrographs of two related heteroduplexes between pT2 and linearized β-4 DNA showing different structures near the 5′ end. In a–c, pBR322 released from the β tubulin clones has also hybridized to the linearized pT2 DNA causing it to circularize. The 5′ and 3′ ends of each gene were determined by the asymmetrical lengths of the pBR322 sequences in pT2 DNA in the heteroduplexes (as seen in 3D) and are marked with large and small arrowheads in the tracing, respectively. Large and small arrowheads in the electron micrographs point to DNA deletion loops corresponding to the intervening sequences in the four β-tubulin genes. Each micrograph is accompanied by an interpretative tracing: (—) genomic DNA; (. . .) cDNA in pT2; (- - -) vector pBR322 DNA. (*f*) Diagram summarizing the structures of the four chicken clones as well as a human β-tublin gene β-5 (Cowan et al., *Proc. Natl. Acad. Sci. 78:* 4877 [1981]). The first conserved segment and first intervening sequence in β-3 were seen only infrequently. The regions hybridized to pT2 DNA are represented by the solid bars. The redundant sequence in β-4 that did not hybridize to pT2 is represented as an open box. The hatched box in β-2 represents the untranslated region which hybridizes to pT2 but has no homology to the other β-gene clones. The 3′ untranslated regions of β-1, β-3, and β-4 cannot be deduced by this analysis. The corresponding conserved segments in different clones are connected with dashed lines.

PROTEIN SYNTHESIS

M.B. Mathews, B.W. Stillman, F. Tamanoi, *A.M. Francoeur, N.K. Gural, C.H. Herrmann, C. Jackson, P.H. Lalik, S.L. Lemaster, D. Pascucci, P.A. Reichel, D.R. Taylor, G.P. Thomas, E.H. Woodruff*

In 1982 the work of the Protein Synthesis laboratory continued along the lines set out in last year's report. The three main areas of research covered were the adenoviral early proteins, ribonucleoprotein particle (RNP) synthesis, and the stress (heat-shock) response of human cells.

Proteins Required in Adenoviral DNA Synthesis

B.W. Stillman, F. Tamanoi, M.B. Mathews

In annual reports over the last several years, we described analysis of the protein-coding properties of both the late and the early genes of adenovirus 2 (Ad2). Standing in stark contrast with the impressively detailed mapping data, outlined in Fig. 1, is the dearth of information on the roles of these protein species. Of the roughly two dozen early proteins that have been observed, biochemical functions have been ascribed to only three, and it is no coincidence that these all participate in DNA replication, for which we now have a powerful assay system. The three proteins are products of early region E2, which has two major domains. The upstream, promoter-proximal E2A domain codes for the 72,000-dalton DNA binding protein (DBP), a multifunctional protein that is involved in DNA chain elongation and was discussed extensively in last year's report. We now know that the downstream portion, region E2B, encodes at least two proteins—the terminal protein precursor (pTP) and adenoviral DNA polymerase—that are required for DNA chain initiation.

The first step in the initiation reaction is the formation of a covalent complex between the first nucleotide, dCMP, and the terminal protein precursor, pTP. This remains attached to the growing DNA chain and persists on mature viral DNA in the form of a shorter (55,000-dalton) terminal protein that is derived by specific cleavage of the 80,000-dalton precursor. The cleavage is carried out by a virus-encoded protease during maturation of infectious viral particles (see 1980 and 1981 reports). Several laboratories have demonstrated that the initiating complex can be formed in crude extracts of Ad2–infected HeLa cells supplemented with ATP, Mg^{++}, and template DNA. We have fractionated this extract to study the enzymology of the initiation reaction and to determine the functional domains of the pTP protein.

We had previously mapped the gene encoding pTP to the E2B region, a 6.5-kb span that specifies multiple mRNA and protein species. Recently, the DNA sequence of this region was completed by the Nucleic Acid Chemistry Section. In collaboration with Dr. J.E. Smart (Protein Chemistry Section), the location of the sequence coding for pTP was established through partial sequence analysis of the protein. Comparison of this data with the amino acid sequence predicted from the DNA sequence showed that the majority of the coding region for pTP lies between coordinates 23.4 and 28.9 on the viral genome. From this work, we also learned that the mature terminal protein corresponds to the C-terminal portion of pTP, as does an intermediate 62,000-dalton cleavage product. Furthermore, the serine residue that is covalently bound to dCMP or to viral DNA was located at a site near the common C terminus of the three proteins.

On the other hand, the pTP- coding region did *not* overlap the region that, on genetic grounds, was believed to specify a protein involved in DNA replication and also in cell transformation. Mutants of the adenovirus N group, such as Ad5*ts*149, are defective for replication at the restrictive temperature and had been mapped to a stretch of the viral genome between coordinates 18 and 22.5. This span neighbors that encoding pTP and is included in the E2B region. We therefore postulated that the E2B region encodes an additional protein involved in replication, and we set out to test this idea.

Extracts were prepared from Ad5*ts*149-infected cells grown at either the permissive or restrictive temperature and were tested for their ability to support DNA synthesis. The extracts prepared at the permissive temperature were active for DNA replication and could not be inactivated by incubation at the restrictive temperature (39.5°C). However, Ad5*ts*149 extracts prepared from cells grown at the restrictive temperature were inactive for both DNA synthesis and pTP-dCMP complex formation. These results suggested that the mutation in Ad5*ts*149 causes a defect in a protein that functions in the initiation of DNA replication. We purified this protein in functional form from cells infected with wild-type virus using an assay in which protein fractions were tested for their ability to complement defective extracts for DNA replication. The purified protein can restore both pTP-dCMP complex formation and full-length DNA synthesis. It has an apparent molecular weight of 140,000 daltons, is tightly bound to pTP, and—most strikingly—contains DNA polymerase activity. These results demonstrated a defect in the 140,000-dalton DNA polymerase of cells infected with Ad5*ts*149, but did not formally prove that the polymerase is virus-encoded. To establish this point, we reexamined the coding potential of the E2B region. RNA isolated from infected cells at 8 hours postinfection was shown to contain mRNA complementary to viral *l*-strand DNA in the region containing the N-group mutants. This mRNA, when translated in a cell-free system, produces proteins of 140,000 daltons and

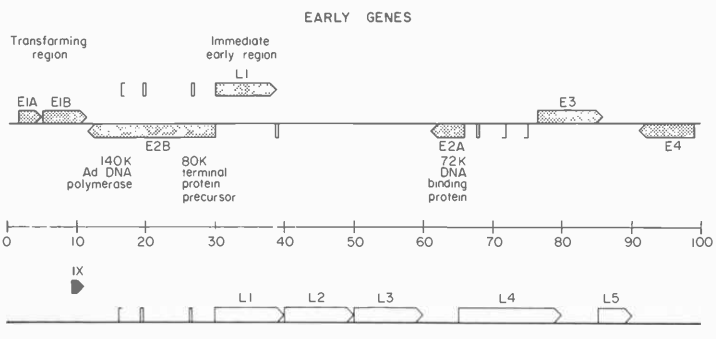


Figure 1
Topography of the adenoviral genome. The diagram shows the distribution of the main early (stippled), intermediate (filled in), and late (open) gene blocks along the viral DNA. Also indicated are the protein products of some of the early genes and their functions. Square brackets and narrow boxes mark the positions of promoters and leader segments.

80,000 daltons; the latter had previously been identified as pTP. The mRNA encoding the 140,000-dalton protein was mapped more closely by hybridization to small segments of viral DNA cloned into M13 phage DNA. The results showed that the protein maps to the left of coordinate 24, leaving little room to doubt that the 140K protein is the virus-encoded DNA polymerase. Corroborating evidence comes from the DNA sequence of this region, which shows an open reading frame capable of accommodating a protein in excess of 120,000 daltons.

Other proteins are also produced from the E2B region (75K and 105K) and there seems to be temporal control of the synthesis of mRNAs for these proteins. These features raise the possibility that additional viral factors may be involved in replication. Furthermore, the purified Ad-DNA polymerase and pTP do not support a significant amount of pTP-dCMP complex formation when incubated with Ad2 DNA-protein complex as template, but full activity is restored when extracts of uninfected HeLa cell nuclei are included in the reaction. This finding does not support the idea that additional viral products, including the E2A DBP, are essential; rather, it suggests that a cellular factor is required for pTP-dCMP complex formation and initiation of DNA replication. The nature of this factor, as well as of the adenoviral DNA template about the origin of replication, is addressed in the report of the DNA Synthesis Section which was instituted in the middle of the year to pursue these questions in more detail.

In summary, three viral proteins are required for the synthesis of adenoviral DNA: the 72,000-dalton single-strand DBP, the 80,000-dalton terminal protein precursor, and the newly discovered 140,000-dalton DNA polymerase activity. These proteins are synthesized from a single complex transcription unit or gene family on the adenoviral genome. How the process is regulated during the infection cycle, and how the Ad-DNA polymerase is related to cell transformation are intriguing open questions.

Small Viral RNAs and RNPs

A.M. Francoeur, M.B. Mathews

In previous reports, we have described our studies of the structure and properties of the two small (~160-nucleotide) noncoding adenoviral VA-RNAs. Interest in these species has quickened with the discovery that they interact with viral mRNA, on the one hand, and with a host cell protein on the other. We have continued to characterize these interactions as an approach to the function of the species involved.

Sera from patients with the autoimmune disease systemic lupus erythematosus often contain antibodies that react with cellular RNPs. One such antibody specificity, the anti-La class, recognizes particles from adenovirus-infected HeLa cells that contain the VA-RNAs (Fig. 2). These particles can assemble in vitro in a cell-free transcription system from HeLa cells that also acts as source of the La antigen. Using variant forms of the gene for VA-RNA$_1$ as transcription template, we have examined the RNA requirements for particle formation. Development of a reconstitution assay, in which preformed

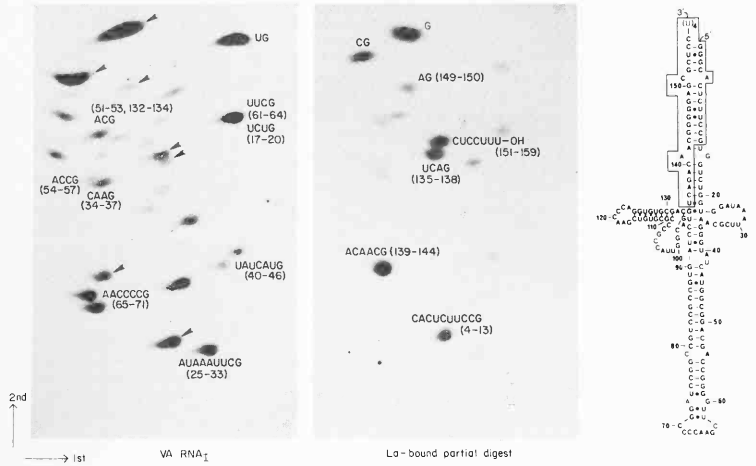


Figure 2
The binding of VA RNA to the lupus antigen La. After reconstitution of the ribonucleoprotein particle with fragmented VA RNA, the bound fragments were characterized by fingerprinting (*center panel*). The origin of the fragments is indicated on the illustration of the intact, base-paired VA RNA molecule (*right panel*) by the boxed-in portion. For comparison, the fingerprint of the entire molecule is shown in the left panel.

and fragmented VA-RNA can bind the antigen, allowed further refinement of the analysis. Together, these experiments define the termini of the VA-RNAs as the site of La antigen binding. Since the termini of RNAs (such as the VA-RNAs) that are synthesized by RNA polymerase III present common features, it is not surprising that the La antigen also recognizes other molecules–such as 5S RNA and tRNA—which are also made by the same polymerase.

The antigen binds to the VA-RNAs at the time of transcription or very soon afterward, but does not seem to be required for transcription. Neither addition of antibody to the transcription system, nor use of immobilized antibody to deplete the extract of the La antigen, had any effect on synthesis of VA-RNA (or other polymerase III products), although, as expected, the RNAs failed to assemble into RNPs in these conditions as no La antigen was available. This result implies that the role of the antigen is subsequent to RNA synthesis.

To explore its role further, we have begun to characterize and purify the protein. We have performed immunoprecipitation experiments with several independent human anti-La sera and ^{35}S- or ^{32}P-labeled HeLa cell extracts, and have examined the proteins by both one-dimensional and two-dimensional gel electrophoresis (some of the latter in collaboration with Dr. J. Garrels). These results identify the La antigen as a family of cellular phosphoproteins of apparent molecular weight 45,000 daltons. This conclusion is supported by data obtained with the Western gel transfer technique. Using a recently developed rapid assay for RNP formation in concert with conventional chromatographic methods, we have succeeded in purifying the La antigen about 1000-fold. The enriched fractions have been injected into animals for antibody production, and in parallel are being analyzed for enzymic activities.

Turning to the mRNA-binding properties of the VA-RNAs, we have begun to characterize in detail the sites of interaction between the two RNA species with methods similar to those employed in studies of the La antigen-VA-RNA interaction. Preliminary findings suggest involvement of the 5′ ends of the mRNA and VA-RNA molecules, but further work is needed to define the nucleotide sequences precisely.

The Stress Response of Mammalian Cells

G.P. Thomas, M.B. Mathews

Human cells, and cells of most organisms, respond to metabolic stress of various kinds in a way that is closely akin to the heat-shock response first studied in *Drosophila*. Having established the kinetics and some of the mechanistic requirements of the induction of stress proteins in HeLa cells by amino acid analogs, our attention this year has been focused primarily in two areas: the isolation and construction of suitable gene probes and their characterization, and investigation of the translational controls operating in stressed cells.

Last year we described the isolation of bacteriophage clones containing some or all of the gene for the 90,000-dalton (90K) stress protein. We have since then begun to analyze the organization of coding sequences using both S1 nuclease analysis and hybridization selection. As a first step, an unambiguous restriction map was derived using fragments subcloned into pBR322 as probes for Southern blots of restricted phage DNA. By hybridization selection, two noncontiguous regions of the DNA were found to select the 90K mRNA and to make nuclease-resistant hybrids with RNA from stressed cells. This presumably implies that the mRNA for the 90K protein is spliced, as is its homolog, the 83K protein mRNA in *Drosophila*, where it has been found to be the only heat-shock protein mRNA that is spliced. More intriguing is the finding that all regions of the 90K clone also select the mRNA encoding the 72K stress protein, the other major induced protein of mammalian cells, if hybridization is conducted at higher temperature. This is true whether hybrids are formed in solution or on filters, and whether the complete phage DNA or plasmid subclones from it are used. The possibility that these two genes contain common sequences is under investigation. From a cDNA library constructed using stressed-cell RNA, a number of clones displayed preferential hybridization to a stressed-cell probe as against an unstressed-cell probe. Of these, a small number also hybridized with a probe for the *Drosophila* 70K stress protein (the homolog of the mammalian 72K stress protein). These clones have proven capable of selecting the mRNA for the human 72K stress protein, as have other clones that failed to hybridize with the insect probe. Two of the 72K cDNAs have different restriction patterns, although we do not yet know whether they are copies of different mRNAs or of parts of the same mRNA. These are currently being used to isolate genomic clones to determine how many genes exist in human cells (in *Drosophila* there are many copies of the 70K gene as well as multiple related but not inducible genes) and to obtain pure preparations of 72K mRNA for use in translational control experiments.

Our earlier studies had indicated that preexisting mRNAs are rendered nonfunctional in stressed cells, yet retain both their polysomal location and the ability to be translated in cell-free systems. We interpret this as an indication of translational control at the level of polypeptide elongation, a situation for which no obvious precedents are available. The normal mRNAs can be released from polysomes by treatment with either EDTA or puromycin, but are not utilized for translation when polysomes are incubated intact in a rabbit reticulocyte system. Thus, the discrimination between the normal and stress protein is rather stable, implying that some integral or tightly associated component of the ribosomes or mRNPs is responsible.

Immediate goals for the future include the development of initiating cell-free systems with which to assess utilization of particular classes of mRNA and comparisons of the protein components of the various RNP and ribosomal structures. In addition, we are presuming a more detailed analysis of the requirement for RNA synthesis during establishment of the response.

Publications

Asselbergs, F.A.M. and M.B. Mathews. DNA directed synthesis of adenovirus polypeptide IX in *Xenopus* oocytes does not require viral DNA sequence downstream from the poly(A) addition site. (Submitted.)

Asselbergs, F.A.M., M.B. Mathews, and J.E. Smart. 1982. Structural characterization of the proteins encoded by adenovirus early region 2A. *J. Mol. Biol.* (in press).

Asselbergs, F.A.M., J.E. Smart, and M.B. Mathews. 1982. Analysis of expression of adenovirus DNA (fragments) by microinjection in *Xenopus* oocytes: Independent synthesis of minor or early region 2A proteins. *J. Mol. Biol.* (in press).

Feramisco, J.R., D.M. Helfman, K. Burridge, J.E. Smart, and G.P. Thomas. 1982. Coexistence of vinculin and a vinculin-like protein of higher molecular weight in smooth muscle. *J. Biol. Chem. 257:* 11024.

Francoeur, A.M. and M.B. Mathews. 1982. Interaction between VA RNA and the lupus antigen La: Formation of a ribonucleoprotein particle *in vitro*. *Proc. Natl. Acad. Sci. 79:* 6772.

Francoeur, A.M., J.I. Garrels, and M.B. Mathews. Purification and characterization of the lupus antigen La. (In preparation.)

Helfman, D.M., J.R. Feramisco, J.C. Fiddes, G.P. Thomas, and S.H. Hughes. 1983. Identification of clones that encode chicken tropomyosin by direct immunological screening of a cDNA expression library. *Proc. Natl. Acad. Sci. 80:* 31.

Hughes, S.H., J. Sorge, G.P. Thomas, J.R. Feramisco, L.T. Chow, and J.I. Garrels. 1982. Approaches for isolating genes encoding the cytoskeletal proteins and for reintroducing the genes into cultured cells. *Cold Spring Harbor Symp. Quant. Biol. 46:* 507.

Matsumura, F., J.J.-C. Lin, S. Yamashiro-Matsumura, G.P. Thomas, and W.C. Topp. Differential expression of tropomyosin forms in the microfilaments isolated from normal and transformed rat cultured cells. (In preparation.)

Smart, J.E. and B.W. Stillman. 1982. Adenovirus terminal protein precursor: Partial amino acid sequence and site of covalent linkage to virus DNA. *J. Biol. Chem. 257:* 13499.

Stillman, B.W. and F. Tamanoi. 1982. Adenovirus DNA replication: DNA sequences and enzymes required for initiation *in vitro*. *Cold Spring Harbor Symp. Quant. Biol. 47:* 741.

Stillman, B.W., F. Tamanoi, and M.B. Mathews. 1982. Purification of an adenovirus coded DNA polymerase that is required for initiation of DNA replication. *Cell 31:* 613.

Thomas, G.P. and M.B. Mathews. 1982. Control of polypeptide chain elongation in the stress-response: A novel translational control. In *Heat shock: from bacteria to man* (ed. M.J. Schlesinger et al.), p. 207. Cold Spring Harbor Laboratory, New York.

——. Alterations of transcription and translation in Hela cells exposed to amino acid analogues. (Submitted.)

Thomas, G.P., J.I. Garrels, and M.B. Mathews. Differential induction of stress response proteins in Hela cells and chicken fibroblast. (Submitted.)

Thomas, G.P., W.J. Welch, M.B. Mathews, and J.R. Feramisco. 1982. Molecular and cellular effects of heat shock and related treatments of mammalian tissue culture cells. *Cold Spring Harbor Symp. Quant. Biol. 46:* 985.

Welch, W.J., J.I. Garrels, J.J.-C. Lin, G.P. Thomas, and J.R. Feramisco. 1983. Biochemical characterisation of the mammalian stress proteins and identification of two stress proteins as glucose- and Ca^{++}-ionophore regulated proteins. *J. Biol. Chem.* (in press.)

DNA SYNTHESIS

F. Tamanoi, B.W. Stillman, *M. Dermody-Weisbrod, P. Lalik, D. Taylor, M. Walsh, E. Woodruff*

The DNA Synthesis laboratory came into existence during the past year; its major interest is to understand the enzymatic mechanism of DNA replication in eukaryotes. The recent development of cell-free extracts that are capable of replicating DNA has greatly facilitated such studies. Our work on DNA replication, which grew from the study of adenoviral DNA replication that was begun in the Protein Synthesis Section, has already contributed to the discovery of a novel DNA polymerase and a new mechanism for the priming of DNA synthesis at the origin of replication. We intend to exploit the adenoviral DNA replication system further and also to expand the work to cellular DNA replication.

Virus-encoded Proteins Required for DNA Replication

As described in last year's report (Protein Synthesis Section), we have characterized two virus-encoded proteins that are required for DNA replication in vitro: the E2A single-stranded DNA binding protein (DBP) (72,000-dalton [72K]) and the E2B terminal protein precursor (pTP). By aligning the partial amino acid sequence of pTP (performed in collaboration with the Protein Chemistry Section) to the DNA sequence of the E2B region (determined by the Nucleic Acid Chemistry Section), it was demonstrated that the coding region for pTP was between 28.9 and 23.4 map units on the genome. Galos et al. (*Cell 17:* 945 [1979]) had previously mapped the N complementation group of adenovirus type-5 (Ad5) temperature-sensitive mutants to 18.0–22.5 map units on the viral genome, which lies outside the region encoding pTP. These mutants are defective for replication of viral DNA in vivo and also for transformation of rat embryo cells. These combined results suggested that a third virus-encoded protein, other than pTP and the 72K DBP, was involved in the replication of adenoviral DNA and that this protein was also encoded by region E2B.

As a first approach to identify the gene product defined by the N complementation mutants (e.g., Ad5*ts*149), nuclear extracts were prepared from wild-type Ad2- and Ad5*ts*149-infected HeLa cells grown at the nonpermissive temperature of 39.5°C. The extracts prepared from wild-type-infected cells supported adenoviral DNA synthesis, whereas extracts prepared from Ad5*ts*149-infected cells did not. Furthermore, the Ad5*ts*149 extracts were also defective for the formation of a covalent linkage between pTP and dCMP, which is the proposed initial step in the priming of DNA synthesis at the replication origin. By complementation of the defective Ad5*ts*149 extracts with fractions derived from wild-type Ad2-infected HeLa cell extracts, a 140,000-dalton (140K) protein was purified and shown to contain DNA polymerase activity. The 140K DNA polymerase activity copurified with the pTP, and both proteins were required for the pTP-dCMP complex formation (also demonstrated by Enomoto et al., *Proc. Natl. Acad. Sci. 78:* 6779 [1981]; Lichy et al., *Proc. Natl. Acad. Sci. 79:* 5225 [1982]).

The purified 140K DNA polymerase and pTP were unable to link dCMP covalently (which is the first nucleotide in the nascent DNA chain) to the pTP when Ad2 DNA-protein complex was used as a template DNA. However, the activity could be fully restored by addition of either nuclear extracts prepared from Ad5*ts*149-infected or uninfected HeLa cells. Thus, at least one cellular factor is required for the initiation of adenoviral DNA replication in vitro. Recently, Nagata et al. (*Proc. Natl. Acad. Sci. 79:* 6438 [1982]) have purified a nuclear factor that stimulates the initiation reaction, but they have not yet assigned any enzymatic function to this protein.

The Ad5*ts*149 mutation maps within the E2B region of the genome (Galos et al., *Cell 17:* 945 [1979]), and we had previously shown that this region encodes proteins of 105K and 75K, in addition to pTP (1980 Annual Report). However, since the DNA sequence of the E2B region of the genome shows a large open translational reading frame that might encode the DNA polymerase, we reexamined the coding potential of the E2B region. In collaboration with M. Mathews (Protein Synthesis Section), RNA was prepared from Ad2-infected cells at various times postinfection and selected by hybridization to cloned fragments of adenoviral DNA, followed by translation in vitro. Substantial amounts of a 140K protein, as well as three- to fourfold higher amounts of the 80K pTP were synthesized from mRNA isolated at 8 hours postinfection, after addition of 10 μM anisomycin 3 hours after infection. At this time postinfection, very little mRNA synthesizing the 75K and 105K proteins was observed. RNAs for the 140K and 80K proteins were selected by all cloned DNAs tested that originated from the region between 18 and 24.5 map units. However, only the 80K pTP mRNA, and not the 140K mRNA, hybridized to cloned DNA fragments that lie to the right of 25 map units. In addition, both proteins were translated from mRNA selected by M13 bacteriophage DNAs containing cloned *l*-strand DNA but not *r*-strand DNA, demonstrating that these proteins are indeed encoded by early region 2B on the viral genome.

The 140K protein synthesized by cell-free translation of mRNA migrated on SDS-polyacrylamide gels with [^{35}S]methionine-labeled 140K DNA polymerase that had been purified from wild-type Ad2-infected HeLa cells. The combined data de-

scribed above support the conclusion that adenovirus encodes a 140K DNA polymerase from early region 2B on the genome that is required for the initiation of DNA replication by formation of a protein (pTP)-deoxynucleotide primer. It remains to be determined whether the adenoviral DNA polymerase catalyzes complete synthesis of viral DNA or whether a cell DNA polymerase is also required.

Thus, adenovirus encodes at least three proteins that are required for DNA replication, all of which are synthesized from early region 2 on the viral genome. The adenoviral single-strand DBP (72K) is encoded by early region E2A (Fig. 1) and is required for elongation of DNA replication. The mRNA for this protein is synthesized at both early and late times from two promoters active at the respective times after infection. The pTP and DNA polymerase are both encoded by early region E2B, and mRNA for these proteins appears to be synthesized from the early promoter. Since these latter proteins are required for initiation of DNA synthesis, the rate of DNA synthesis in adenovirus-infected cells may be regulated at the level of transcription or processing of E2B RNA.

The addition of purified single-stranded DBP, pTP, and the adenoviral DNA polymerase to nuclear extracts prepared from uninfected HeLa cells promotes specific initiation and elongation of adenoviral DNA synthesis in vitro. This suggested that these three virus-coded proteins are the only such proteins directly required for DNA replication in vivo. However, Martin et al. (*J. Gen. Virol. 41:* 303 [1978]) have reported the isolation of a temperature-sensitive mutant of Ad2 that could complement mutants in the DBP (Ad5*ts*125) and the DNA polymerase (N complementation-group mutants). The mutant, Ad2*ts*111, maps within the left 30% of the adenoviral genome (D'Halluin et al., *J. Virol. 41:* 401 [1982]) and is defective for the replication of viral DNA in vivo as well as causing degradation of both cell and viral DNA at the nonpermissive temperature (D'Halluin et al., *J. Virol. 32:* 61 [1978]).

We have prepared nuclear extracts from Ad2*ts*111-infected HeLa cells grown at 39.5°C (nonpermissive temperature) and demonstrated that these extracts are defective for the replication of added adenoviral DNA. The block to replication in vitro is not at the stage of initiation of DNA replication, since the Ad2*ts*111 nuclear extracts are capable of forming the covalent linkage between pTP and dCMP. This latter result indicates that these extracts contain functional pTP and 140K DNA polymerase activity. Complete DNA synthesis can be restored to Ad2*ts*111-defective extracts by addition of the purified single-strand DBP isolated from wild-type-infected cells. Since the mutation in Ad2*ts*111 maps outside the gene for the DBP, this result suggests that another adenoviral protein affects the activity of the DBP in vivo. We are currently investigating the biochemical nature of this block to DNA synthesis and its possible link to the DNA degradation phenotype of this mutant, as well as precisely mapping the mutation (in collaboration with T. Grodzicker, Tumor Virus Section).

HeLa Cell Proteins

For the identification of the virus-encoded proteins that are necessary for DNA replication in vitro, the use of mutants that affect DNA replication in vivo has been invaluable. Unfortunately, such a genetic approach is not practical for the identification of cellular enzymes required for the replication of adenoviral DNA, therefore we have begun to fractionate extracts prepared from uninfected HeLa cells and have identified three fractions derived from nuclear extracts that are either necessary for, or stimulate the rate of adenoviral DNA synthesis in

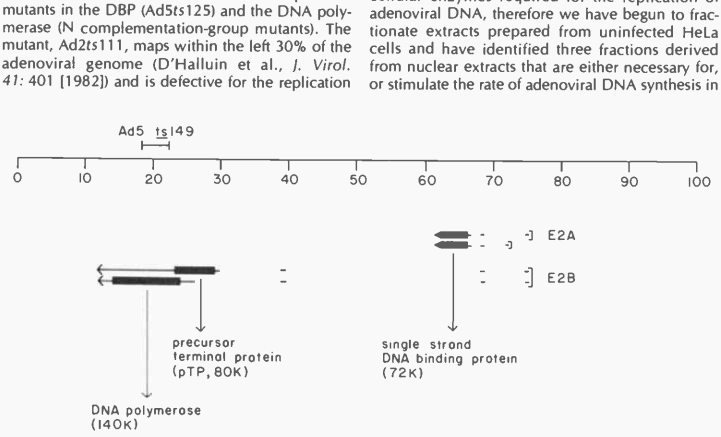


Figure 1
A map of adenovirus early region 2 showing the structure of the mRNAs. The DNA replication proteins encoded by this region are shown, as is the map location of the Ad5*ts*149 mutation used to identify the virus-encoded DNA polymerase.

vitro. One such factor that is currently under investigation is required for completion of full-length DNA chains. As mentioned above, another nuclear factor is required for initiation of DNA synthesis in vitro and has been purified by Nagata et al. (*Proc. Natl. Acad. Sci. 79:* 6438 [1982]). It is hoped that in the near future, the replication of adenoviral DNA will be accomplished with purified proteins and that we can then learn the precise nature of eukaryotic DNA synthesis.

Origin of Adenoviral DNA Replication

In many systems, specific DNA sequences define the region where initiation of DNA replication takes place. This has been demonstrated by constructing small deletions or base changes at the origin that destroy the initiation of replication. From the study of prokaryotic systems, especially that of the small single-stranded DNA phages, it has become clear that the origin serves as a site where enzymes involved in initiation form a complex, or a so called replisome.

In last year's report, we described the development of an in vitro system to search for DNA sequences required for the initiation of adenoviral DNA synthesis. Briefly, the initiation is carried out on a cloned origin of adenoviral DNA replication using a partially purified enzyme fraction. The cloned DNA has to be linearized in such a way that the adenoviral DNA sequence is located at the end of the linear DNA molecule. The initiation on such a linear DNA occurs at the correct initiation site as well as at an additional site four nucleotides from the correct site. This system has been exploited to search DNA sequences that are required for the formation of the pTP-dCMP primer complex.

First, a set of deletions beginning from an internal *Pvu*II site at 453 nucleotides and extending toward the terminus was created by nuclease BAL31 digestion. Also, exonuclease III and S1 nuclease were used to create fine deletions. Examination of their template activity has shown that all the plasmid DNAs that contain adenoviral DNA longer than 20 nucleotides support the pTP-dCMP complex formation, whereas those that contain 14 nucleotides or less did not. Second, a set of mutants was constructed that created deletions from the end of adenoviral DNA. However, this time, the template activity was lost completely, even when a few nucleotides were removed. A plasmid DNA containing 20 nucleotides of adenoviral DNA was capable of supporting a limited amount of DNA chain elongation. Therefore, the terminal 20 nucleotides define a minimum sequence required for the initiation in vitro (Fig. 2).

Comparison of the terminal sequence of different serotypes of adenovirus has revealed conserved sequences (see 1981 Annual Report). The inverted terminal repeat could be divided into a terminal A·T-rich domain and a G·C-rich domain and within the terminal A·T-rich domain, the first 22 nucleotides are highly conserved. Within this region, there is a perfectly conserved sequence that extends from bases 9 to 18 from the end of the DNA.

We have begun to make base changes within the origin of adenoviral DNA replication using chemically synthesized DNA (see below and Fig. 3). As a first trial, oligonucleotides corresponding to the terminal 20 nucleotides of both the *l* and *r* strands of adenoviral DNA have been chemically synthesized. The DNA contained base changes in the perfectly conserved region: GG instead of TA. The single-stranded oligonucleotides were annealed, linkers *Eco*RI and *Sal*I were ligated to the ends, and the DNA was then cloned into the *Eco*RI and *Sal*I sites of pAT DNA. DNA sequencing of the resulting plasmid DNAs confirmed the mutant sequence. When the template activity of this mutant DNA was examined, the activity was reduced to less than 30% of the wild-type DNA. This indicated that the conserved sequence is playing an important role in the initiation of adenoviral DNA replication. We plan to use this method further to create mutations at other locations within the origin.

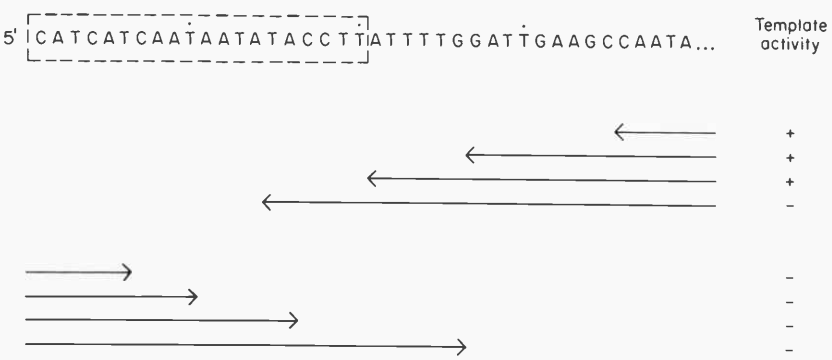


Figure 2
The nucleotide sequence of the 40 bases at the 5′ end of adenoviral DNA and the position of deletions created within the origin. The ability of each cloned DNA to act as a template for the initation of DNA replication in vitro is also indicated. The boxed area shows those nucleotides that constitute the minimum origin of DNA replication.

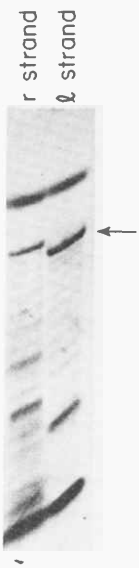


Figure 3
Oligonucleotides (20-mer) were chemically synthesized de novo by the phosphite triester method and then separated by electrophoresis in a polyacrylamide-urea gel. The photograph shows the oligonucleotides identified by the UV shadowing technique that contained base changes within the terminal 20 bases of each strand of adenoviral DNA. They are used for site-directed mutagenesis of the adenoviral origin of DNA replication.

Oligonucleotide Synthesis

The ultimate aim in investigating regulatory sequences such as replication origins is to find out how proteins recognize specific DNA sequences and whether this involves the recognition by the protein of a subset of bases in the DNA sequence. The most straightforward approach to this problem is to synthesize DNA chemically with base alterations. The functional origin of adenoviral DNA replication defined by us is 20 nucleotides, which is in a range that can easily be synthesized. Therefore, during the last year we have set up a system to synthesize DNA chemically.

Phosphite triester and phosphate triester methods are the two most common methods for chemically synthesizing DNA. However, each method has some drawbacks. The phosphite triester synthesis is fast and time saving, but the reaction is very sensitive to moisture and some phosphoramidites used as building blocks are unstable. On the other hand, the phosphate triester synthesis is less sensitive to moisture but it is time consuming. We have tried to solve the stability problem of the phosphoramidites by using different derivatives. Diethyl, morpholino, and di-isopropyl derivatives were tested and it was found that the di-isopropyl derivatives provided the best results. For the phosphate triester method, we have been examining a modified method recently reported by Efimov et al. (*Nucleic Acids Res. 10*: 6675 [1982]). This method is very attractive since the reaction is rapid and uses acetonitrile as the only solvent.

We now have the capability to produce several hundred micrograms of oligomer in a matter of hours and these oligomers have become important tools for mutagenizing DNA, priming DNA sequencing reactions, and for the synthesis and screening of cDNA libraries.

Publications

Smart, J.E. and B.W. Stillman. 1982. Adenovirus terminal protein precursor. Partial amino acid sequence and site of covalent linkage to virus DNA. *J. Biol. Chem. 257:* 13499.

Stillman, B.W. and F. Tamanoi. 1983. Adenovirus DNA replication: DNA sequences and enzymes required for initiation *in vitro*. *Cold Spring Harbor Symp. Quant. Biol. 47:* 741.

Stillman, B.W., F. Tamanoi, and M.B. Mathews. 1982. Purification of an adenovirus coded DNA polymerase that is required for initiation of DNA replication. *Cell 31:* 613.

Stillman, B.W., W.C. Topp, and J.A. Engler. 1982. Conserved sequences at the origin of an adenovirus DNA replication. *J. Virol. 44:* 530.

Tamanoi, F. and B.W. Stillman. 1982. Function of adenovirus terminal protein in the initiation of DNA replication. *Proc. Natl. Acad. Sci. 79:* 2221.

Tamanoi, F. and B.W. Stillman. 1983. A specific DNA sequence is required for initiation of adenovirus DNA replication *in vitro*. *Proc. Natl. Acad. Sci.* (in press).

Movable Genetic Elements

This section is comprised of scientists who are interested in mechanisms by which genetic rearrangements occur and affect gene expression. Studies on transposable genetic elements have led to the establishment of two basic principles: (1) DNA can undergo rearrangements at a frequency higher than DNA replication errors in all organisms. (2) In many cases, unstable phenotypes result from DNA rearrangements. Perhaps within the next few years many of the interesting genetic mysteries noticed by geneticists for decades will be explained on the basis of these two principles.

Our section is devoted to two main research activities: Work on prokaryotic transposable genetic elements, with bacteriophage Mu as a model system, and work on DNA transposition in eukaryotic systems with mating type genes in yeast as a model system. Other research projects involving various microbial, plant, or mammalian systems are added to this section when necessary and appropriate. During the past year, this section included studies on transposition of Tn*3* (a transposon for ampicillin resistance) and the DNA rearrangements that affect the pilus antigen expression in *Neisseria gonorrhoeae*.

The two main model systems under study in our section represent two fundamental aspects of the biology of movable genetic elements. Mu DNA transposition represents DNA rearrangements and genetic variation at the *population level* (the underlying principle being that genetic variation can occur abruptly and at a high frequency in a population). Switching of mating-type genes represents DNA rearrangements at the *developmental level* (the underlying principle being that a change in gene organization at a given stage may alter gene expression and thus affect developmental patterns). These two aspects are at the center of genetics today, and we hope that by concentrating on the two model systems we can advance our knowledge of the causes and effects of genetic variation.

INSERTION ELEMENTS AND PLASMIDS

A.I. Bukhari, R. Harshey, *L. Dalessandro, M. Goradia, Z. Manzoor, C. Monaghan, G. Penzi, S. Silberstein, B. Vogel*

During the past year, we continued our studies on the mechanisms by which bacteriophage Mu DNA undergoes transposition and causes various DNA rearrangements. We also studied the expression of the DNA modification function, *mom*, of Mu.

Products of Mu DNA Transposition

R. Harshey

Studies on the transposable bacteriophage Mu have demonstrated that, upon induction of a Mu prophage, replication is associated with transposition. Similar conclusions have been reached from studies of other transposable elements. Different elements differ in the nature of the final product of transposition. Some give cointegrates (replicon fusions) in which two circular genomes fuse to generate a structure containing two copies of the element, one at each junction of the two fused replicons. Others give simple insertions in which the donor and recipient genomes are not found joined (Fig. 1). In some transposons, such as Tn*3*, cointegrates appear to be intermediate products that are resolved into separate replicons by a site-specific recombination event mediated by a resolvase that acts on a site within the element after replication and transposition. Other transposons do not appear to have internal resolution systems, but curiously one still observes that these transposons give both kinds of products. The *A* and *B* genes of bacteriophage Mu code for functions that mediate its transposition. The *A* function is absolutely required for transposition and related DNA rearrangements. The B^- mutants can promote DNA rearrangements but at greatly reduced frequencies. There appear to be two pathways for Mu transposition—one that normally operates during prophage induction and lytic growth and produces cointegrates and a second that operates during the events leading to lysogeny and gives simple insertions.

Since integration of Mu during lysogeny is not dependent on *B* function, we decided to determine the nature of transposition products in its absence. We find that under these conditions the majority of end products of transposition are simple insertions. This is in striking contrast to the situation in which the majority of the products are cointegrates in the presence of both *A* and *B* gene products. There are three possible relationships between simple insertions and cointegrates: (1) Simple insertions arise by a resolution of cointegrates after completion of replication, as in the case of Tn*3*. (2) Simple insertions and cointegrates are alternative products of a replication pathway that diverges before replication is complete. (3) There are two mechanistically different pathways for transposition—one involves replication of the transposable element and always generates cointegrates, the other may involve excision of the element before or after replication with subsequent integration of this excised DNA into a new target site to give simple insertions. This scheme is reminiscent of the suicide models proposed for transposition. The first possibility was tested by looking for a recombination event between the two direct repeats of a mini-Mu*kan* cointegrate carried on an F *pro*⁺*lac* episome in the presence of large amounts of the *A* protein. It was found that the cointegrate did not resolve to give simple insertions. The other two possibilities remain to be tested. Since we now have conditions for generating simple insertions almost exclusively, we can begin to study the actual mechanism involved in their formation.

The developmental regulation of the transposition proteins *A* and *B* is unclear. From our results reported here, it is tempting to speculate that during lysogenization only the *A* protein is synthesized. Alternately, an early abundance of *A* protein could be ensured if the *A* protein were packaged into phage particles and injected along with the DNA.

Infecting Bacteriophage Mu DNA Forms of a Circular DNA-Protein Complex

R. Harshey

Upon superinfection of immune (lysogenic) cells with bacteriophage Mu, a form of Mu DNA accumulates that sediments about twice as fast as the linear phage DNA marker in neutral sucrose gradients. This form is also detected upon infection of sensitive cells with Mu. We have purified it and examined its physical nature. Under the electron

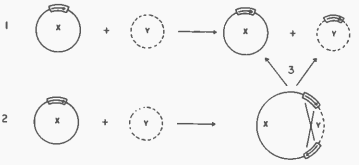


Figure 1
Alternate pathways of transposition. 1. Simple transposition without the intermediate step of cointegrate formation. 2. Cointegrate formation. 3. Resolution of cointegrates to give transposition products.

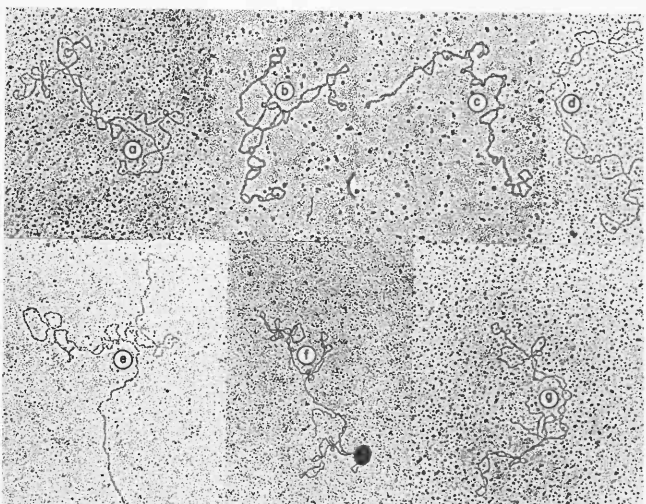


Figure 2
Electron micrographs of Mu DNA-protein complex. (*a–d*) Supertwisted molecules:0, (e–g) partially supertwisted molecules with open ends; (*h–i*) open circular molecules.

microscope, it appears circular and supertwisted. Upon treatment with Pronase, phenol, or SDS, however, it is converted to a linear Mu-length form, indicating that the circle is not covalently closed. The linear DNA still has heterogeneous host sequences at its termini. The circular DNA is resistant to the action of *E. coli* exonuclease III and T7 exonuclease, but becomes sensitive to these nucleases after treatment with Pronase showing the presence of a protein that binds noncovalently to the ends of the DNA to circularize it as well as protect it from digestion with exonucleases. The complex is resistant to high salt (up to 6 M NaCl), but can undergo transitions between forms that are partially open, open circular, linear and circular dimers, and trimers. Examination of DNA from mature phage particles reveals that a circular DNA species is present in at least 0.1–1% of the population. The purified complex is extremely efficient in transfection of *E. coli* speroplasts. We estimate the molecular weight of the protein in this DNA-protein complex to be approximately 64,000 daltons and suggest that this complex might represent the integrative precursor of infecting Mu DNA.

Integration of Infecting Mu DNA

G. Chaconas, D. Evans

We have previously presented evidence that during both the lysogenic and the lytic cycle, but not after prophage induction, the integration of Mu follows the simple insertion mode rather than cointegrate formation (Chaconas et al., *J. Mol. Biol. 150:* 341 [1981]).

E. Ljungquist, while at Cold Spring Harbor Laboratory, had previously shown that ^{32}P-labeled Mu DNA gets integrated into the host DNA after infection. G. Chaconas, now at the University of Western Ontario, extended these studies by showing that both strands of infecting Mu DNA are integrated into the host DNA. His experiments involved separation of Mu DNA strands by agarose gel electrophoresis in the presence of poly(UG). ^{32}P-labeled Mu DNA integrated into the host DNA was isolated and hybridized to the separated Mu DNA strands. Both strands hybridized with the integrated Mu DNA. These studies were completed by George Chaconas in his new laboratory at Western Ontario. He used λ phages carrying various regions of Mu to

conclude that there is no strand preference in the integration process and there is no site-specific resolution site in Mu. The simple insertions that are the end products of Mu DNA integration following infection appear to be generated independently rather than by the resolution of cointegrate structures.

The *mom* Function of Mu

C. Monaghan, M. Goradia

Transcription of the *mom* gene apparently requires an active *dam*$^+$ function of the host. We have shown that this requirement for *dam*$^+$ can be circumvented by manipulating sequences upstream from the *mom* gene. Apparently, *dam*$^+$ methylation of a specific region is required for transcription. It also seems that the regulatory sequences of the *mom* gene overlap the *gin* gene, and alterations in this region have a dramatic effect on the *dam*$^+$ requirement for gene expression. Under normal circumstances *dam*$^+$ methylation is necessary, but not sufficient for, *mom* gene expression. This follows from the observation that *mom* is not expressed in plasmids containing a cloned *mom* gene. However, infection with a *mom*$^-$ phage results in transactivation of the *mom* gene present on the plasmid. Our results on in vitro transcription with methylated Mu DNA and purified RNA polymerase (a gift of Dr. John Dunn) have shown that whereas the *gin* gene is transcribed at a low efficiency in the in vitro system, the *mom* gene transcript is not detectable at all. Evidently, this result reflects the requirement for the *trans*-acting function for synthesis of a *mom* transcript.

We have now identified the *mom* gene product as a 27,000-dalton protein encoded in the β segment of Mu. This protein cannot ordinarily be detected in minicells; it can be seen only if the *mom* gene is expressed under the control of a different promoter, such as the *lac* promoter or the Mu early promoter. It is for this reason that the *mom* protein was not identified earlier. These studies have also revealed a 24–25K protein that was previously identified as the *R* gene product and then as the *gin* gene product (Giphart-Gassler et al., *J. Mol. Biol. 145*:139 [1981]). However, our speculation is that it is the *trans*-acting function.

Publications

Bukhari, A.I. 1982. Self-copying DNA programs. In *Physics and contemporary needs* (ed. Riazuddin et al.), vol. 6. Plenum Press, New York.

——.1983. Transposable genetic elements: The Mu paradigm. *American Society for Microbiology News* (in press).

Bukhari, A.I. and H. Khatoon. 1982. Low level and high level DNA rearrangements in *Escherichia coli*. In *Molecular and cellular mechanisms of mutagenesis* (ed. J.F. Lemontt and W.M. Generoso), p. 235. Plenum Press, New York.

Chaconas, G., D.L. Kennedy, and D. Evans. 1983. Predominant integration end-products of infecting bacteriophage Mu DNA are simple insertions with no preference for integration of either Mu DNA strand. *Virology* (in press).

Harshey, R.M. 1983. A switch in the transposition products of Mu DNA mediated by proteins: Cointegrates versus simple insertions. *Proc. Natl. Acad. Sci.* (in press).

Harshey, R.M. and A.I. Bukhari. 1983. Infecting bacteriophage Mu DNA forms a circular DNA-protein complex. *J. Mol. Biol.* (in press).

Harshey, R.M., R. McKay, and A.I. Bukhari. 1982. DNA intermediates in transposition of phage Mu. *Cell 29*: 561.

Hattman, S., M. Goradia, C. Monaghan, and A.I. Bukhari. 1983. Regulation of the DNA modification function of bacteriophage Mu. *Cold Spring Harbor Symp. Quant. Biol. 47*: 647.

YEAST AND PLANT GENETICS

J. Hicks, A.J.S. Klar, J.N. Strathern, R. Malmberg, S. Bonitz, *J. Abraham, D. Beach, P. Creatura, S. Dellaporta, J. Ivy, M. Kelly, G. LoFranco, C. McGill, J. McIndoo, D. Prudente, R. Schwarz, C. Stephens, S. Weisbrod, J. Wood*

Two new staff members joined the Yeast/Plant Genetics Group in 1982, each bringing a new project to expand further the range of genetics studies in the Delbrück Building. Dr. David Beach, who had previously developed a DNA transformation system in the fission yeast *Schizosaccharomyces pombe* while at the University of Sussex, England, initiated a molecular study of the mating-type switching system in that organism. Later in the year, Dr. Susan Bonitz joined the group as a visiting staff scientist employed by Exxon Research and Engineering Company as part of our joint research program. Dr. Bonitz will continue her work on the processing of mRNA species in the yeast mitochondrion. These projects now coexist with our ongoing studies of mating type in *Saccharomyces cerevisiae* and our expanding plant genetic studies, which now include tomato as well as tobacco cell culture and transposable elements in corn.

Control of Mating Type in *Saccharomyces* Yeasts

J. Strathern, C. McGill, A. Klar, D. Prudente, J. Abraham, M. Kelly, S. Weisbrod, J. Hicks

Changes of mating type in *Saccharomyces* involve the activation of a regulatory gene (either the *MAT***a** or *MAT*α allele of the mating-type locus) by a transposition from storage sites at which they are not expressed to a unique site, *MAT*. The copy of the donor is substituted for the resident allele, which is lost. The end-product description of this process is well documented both in terms of genetic and physical proofs. This simple statement of the mechanism of mating-type differentiation suggests several questions: What are the cell-type-specific genes that are regulated by *MAT***a** and *MAT*α? What are the products of the *MAT* alleles and how do they control the expression of cell-type-specific genes? How are the silent genes kept from being expressed? How are copies of the donor loci made, transposed, and substituted into *MAT*? We and others have made substantial progress in each of these areas.

The specific DNA rearrangement associated with mating-type switching in *S. cerevisiae* is the best-characterized example of such a mechanism regulating cell type in a eukaryote. The high efficiency with which it occurs, the availability of mutants defective in switching, the fact that the donor and recipient loci have been cloned (and work on plasmids), and the amenability of yeast to biochemical as well as genetic manipulation all suggest that research in this area will continue to contribute to basic knowledge about how such mechanisms can be used for differentiation.

Cloning and molecular characterization of the mating-type cassettes led to the elucidation of the physical structure diagramed in Figure 1. Each transcription unit consists of homologous DNA segments common to all three loci flanking a sequence specifying the **a** gene or α gene transcripts. In homothallic switching, this sex-specific sequence is transposed from one of the silent loci to the *MAT* locus where it is then expressed.

One of the most novel aspects of the mating-type regulatory scheme is the mechanism by which the normally silent storage cassettes at the *HML* and *HMR* loci are controlled (see Fig. 1). Each silent cassette contains a fully functional set of mating-type information that is under negative transcriptional control through the action of the *MAR/SIR* gene products. As described in last year's report, we have identified sites present at the silent loci (and not present at *MAT*) that are necessary in *cis* for *MAR/SIR* control to be exerted. These sites differ in their activity and have been designated E (for Essential) and I (for Important), because deletion of the E site results in complete loss of control (and, thus, full expression of the cassette) whereas deletion of the I site results in only partial expression of the affected locus. Furthermore, we have shown that the E and I sites are coincident with sites allowing autonomous replication of plasmid DNA molecules. The role of these autonomous replication sites (ARS) in gene control, or even chromosomal DNA replication, is not yet clear. However, possible interactions between the replication activity and the action of the *MAR/SIR* regulatory genes are under examination.

Chromatin Structure of the Cassettes

Last year we also reported that the regulation of the silent loci was reflected in changes in the chromatin structure of these loci. By probing the chromatin of the mating-type loci with the enzymes DNase I and micrococcal nuclease, Kim Nasmyth found that some of the sites within *HML* and *HMR* that are hypersensitive to cleavage by these nucleases shift in location and intensity when the nuclease cleavage patterns of chromatin from Mar$^+$/Sir$^+$ and Mar$^-$ cells were compared. No such shift was seen in the chromatin of the *MAT* locus isolated from the two types of cells. The differences seen in the chromatin at the silent loci appear to be confined to the sequences lying between the E and I regions, which suggests that Mar/Sir regulation may involve the creation of a "domain" of altered chromatin structure that is bounded by E and I. Nasmyth also found that regulation is associated with a change in

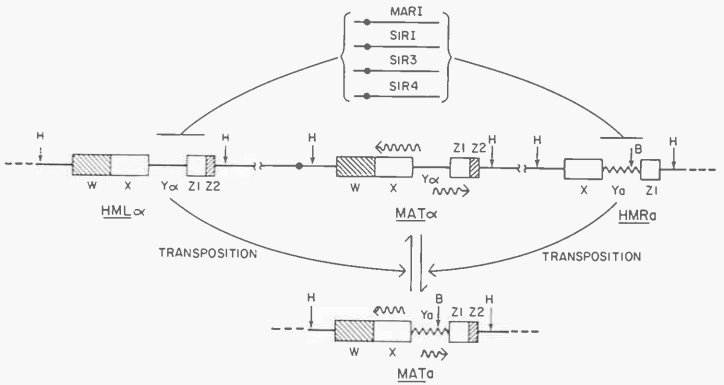


Figure 1
Diagram of the mating-type casettes on chromosome III. W, X, Y, Z1, and Z2 represent regions defined by homology between *MAT* and the *HML*, or *HMR* loci. The W region is 723 bases in length and is found at *MAT* and *HML*; the X region is 704 bases in length and is found at *MAT*, *HML*,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

the linking number of the DNA, in that plasmids carrying *HML* or *HMR* are more negatively supercoiled when purified from a Mar⁺/Sir⁺ strain than when isolated from a Mar⁻ strain. Nasmyth and Judy Abraham have demonstrated that, at *HMR*, the supercoil shift requires the presence of the E regulatory region; deletion of the I region does not appear to alter the shift substantially (see Fig. 2). The cause of the change in linking number, and the role that the change plays in the regulation process, has not yet been determined.

In Strains Undergoing Switching, A Double-stranded Cut Exists in the MAT *DNA*

As part of our investigations of how the actual replication-transposition-substitution process occurs, we have identified intermediates in the pathway. We looked for DNAs that do not correspond to normal genomic fragments and that are unique to strains undergoing homothallic switching. These studies did not identify any candidate for a diffusible copy of the donor cassette unattached to the chromosomal DNA. We did find that about 2% of the *MAT* DNA has a double-stranded cut, and we localized this switching-specific cut to the YZ junction.

To determine the role of this double-stranded cut in homothallic switching, we asked two questions: (1) Is this cut associated with initiation or resolution of the switching process? Because we observed cutting at *MAT* in strains that do not have *HML* or *HMR*, we conclude that the cutting does not reflect a resolution process. (2) Is the ability to make this double-stranded cut essential to homothallic switching? We addressed this question by in vitro mutagenesis of the *MAT* locus and then testing the variants for their activity as substrates for switching. We reported that deletion of most of the Y region does not affect the ability of the plasmids to be switched (*mata*⁻ to *MAT*α). However, deletions that cross the YZ boundary abolish switching. These observations established that there is a site near the YZ boundary that is required for switching, and suggest that an endonuclease specific to homothallically switching cells cuts at a site near the YZ boundary as an initiation step in switching. These studies have identified no unique site other than the YZ junction required for switching.

A more general technique for the examination of mutations has been made available by the observation that transformation of yeast with a linear, gapped molecule results in repair of the gap (Orr-Weaver et al., *Proc. Natl. Acad. Sci. 78:* 6354 [1981]). An integrating yeast cloning vector was cut in such a way as to remove a piece of yeast DNA. The resulting linearized, gapped plasmid was then used to transform yeast. Orr-Weaver et al. (*Proc. Natl.*

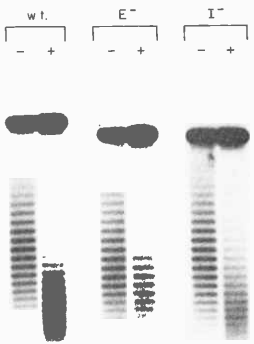


Figure 2
Comparison of supercoil densities of plasmids isolated from a Mar⁺/Sir⁺ strain (+ lanes and a Mar⁻strain (− lanes). Plasmids were subjected to electrophoresis through a 0.7% agarose gel containing 2.5 μg/ml chloroquine, and the gel was then blotted to nitrocellulose and probed with ^{32}P-labeled pBR322 DNA. The first two lanes show the results for a plasmid carrying a wild-type copy of *HMR*, the middle two lanes show the same plasmid except containing a deletion of the E region, and the last two lanes show the results for an I deletion plasmid. The dark band near the top of each lane is presumed to be nicked open-circle DNA.

Acad. Sci. 78: 6354 [1981]) reported that the transformation rate was higher than for circular plasmids and the transformants had an integrated copy of the clone in which the gap had been repaired by duplicating host sequences. Obviously, if this procedure were applied to transformation of a mutant strain with a gapped wild-type clone, the result would be an integrated vector in which mutant host sequences have replaced wild type. The mutant allele can then be isolated from this structure as described above.

We used a related gapped molecule technique to recover alleles of *MAT*. Starting with the *MAT Hin*dIII fragment in YEp213, we constructed a deletion of the cassette that left only unique DNA from each side of *MAT* separated by a *Xho*I site. Transformation of a *MAT*a strain with this plasmid linearized by *Xho*I restriction resulted in repair of the gap to yield YEp213:MATa. This replicating plasmid was readily recovered from a DNA preparation by transformation of *E. coli* without restriction or ligation. With this tool, we can quite simply recover *MAT* alleles from any transformable *leu2*⁻ strain. We have designated this *MAT* recovery plasmid the "golden retriever" and have used it to recover a series of mutations affecting cassette switching, as described in a subsequent section.

Introduction of Mutant Alleles into the Genome

A particularly useful feature of the properties of transformation of yeast with linear DNA is that it provides the opportunity to isolate or generate mutant alleles and to replace the wild-type loci in the genome with those mutant alleles. Thus, the behavior of in vitro-manipulated precise mutations can readily be tested in vivo. For recessive mutations generated in vitro, it is important to be able to replace the wild-type allele with the new mutation. Several methods may be used to "transplace" wild-type loci with the mutant variants. One such method, first suggested by Rodney Rothstein (*Methods Enzymol. 101:* 202 [1983]), is based on the recombinogenic property of cut DNA (Orr-Weaver et al., *Proc. Natl. Acad. Sci. 78:* 6354 [1981]). In one approach, a selectable yeast marker is inserted into the cloned mutant allele. The hybrid yeast fragment is then cut out of the vector and transformed into yeast selecting for the marker. For example, we transformed yeast with the *HML BamH*I fragment in which an internal restriction fragment carrying the *HML* cassette was replaced by *LEU2*. The Leu⁺ cells produced by transformation with this linear DNA had *HML* deleted and replaced by *LEU2* (A. Klar, unpubl.). Likewise, an inverted *HML* locus has been placed on the chromosome.

Another variation of this scheme circumvents the need for insertion of the selectable marker into the mutant allele. Instead, the cells are transformed with a mixture of linear yeast DNA containing the mutant allele and a circular replicating plasmid that can be selected. The transformants containing the replicating plasmid are screened for "cotransformants" that exhibit the mutant phenotype. We successfully used this procedure to introduce deletion mutations into *MAT*. We observed that nearly 1% of the transformants with the selectable plasmid (YEp13) had undergone a transplacement event replacing the wild-type *MAT* allele with the deletion variant.

The Pedigree of Death

Last year we described the "pedigree of death" that results from the introduction of the *HO*-dependent double-strand cut in strains that cannot complete the switching process because they are deleted for both donor (*HML* and *HMR*) cassettes (Fig. 3). These strains (genotype *hmlΔ* [deletion] *MAT*a (or *MAT*a] *hmrΔ HO*) produce dead cells nonrandomly in a cell lineage (Fig. 3). This pedigree of death follows the rules defined for *MAT* switching in standard strains (Hicks and Herskowitz, *Genetics 83:* 245 [1976]; Strathern and Herskowitz, *Cell 17:* 371 [1979]).

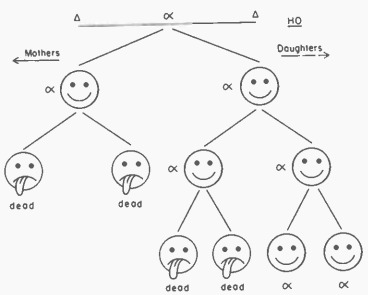


Figure 3
Pedigree of death. Strains with deletions of *HML* and *HMR* produce dead cells in a predictable fashion.

The competent (mother) cells, those that would have normally switched in standard strains, produce two dead cells. The incompetent (daughter) cells always produce a pair of healthy cells. Therefore, we presume that *MAT* in these strains is efficiently cut in cells competent to switch, thus causing lethality. Clearly, lethality results from break at *MAT* since the control *hmlΔ MATα-inc hmrΔ HO* and *hmlΔ matΔ hmrΔ HO* strains do not produce dead cells. Because this lethality occurs in the absence of the *HM* loci, it is therefore likely that the *MAT* cut is the initiating event of transposition. Thus, every time a switch occurs, it does so by making a break. Since only about 2% of the DNA is cut in exponentially growing cells, the cut must be transient and should be "healed" by switching in standard strains.

Selection of Mutations

Because the competent (mother) cells efficiently produce dead cells in the *hmlΔ MATα hmrΔ HO* strains, such strains grow slowly, essentially in a linear fashion. This growth pattern provides a novel genetic selection scheme for isolating mutations in the switching pathway. Any mutation that blocks switching would allow the cells to grow logarithmically, and therefore can be easily identified because of the larger colony size on the solid medium. At least two classes of mutants, those affecting the *MAT* site and those affecting the activity that catalyzes the break are expected to arise. One class of these mutations isolated (9 of 15 independent isolates) resulted from mutations mapping at *MAT* and the other (6 of 15) mapped at *HO*. The mutants characterized thus far that map at *MAT* fall into two categories. One mutant, which has an a-like mating phenotype, resulted from large deletion of *MAT*, whereas the other eight mutants are sterile but exhibit no gross changes at the DNA level. Several of the mutants have been shown to be defective as substrates for cutting in vivo. Since the cut site falls within the α1 gene-coding region and the $\alpha 1^-$ mutants are sterile, it is likely that these sterile mutants represent mutations of the cut site. We have cloned the mutant loci and have sequenced across the YZ junction. The sequences of these mutant loci are shown in Figure 4 and coincide with the site of cutting defined by Kostriken and Heffron using an in vitro assay for cutting at the YZ junction. Knowledge gained from the analysis of these, and additional mutants presently being isolated, will help us define precisely the *MAT* sequences required for cutting.

The Pedigree of "Lingering Death"

In standard strains the transposition of mating-type cassettes is unidirectional: Only *MAT* switches while the *HM* loci act as donors. The *HM* loci themselves remain unaltered. In Mar^+ strains, the same YZ junction sequence is not cut. However, we have shown previously that the *HM* loci switch as efficiently as *MAT* in Mar^- strains (Klar et al., *Cell* 25: 517 [1981]). If presence of the double-strand break defines the recipient locus, then, in Mar^- cells the *HM* loci should also experience the double-strand break.

To test this prediction, cell lineage studies were conducted on a strain with the genotype *HMLα matΔ*

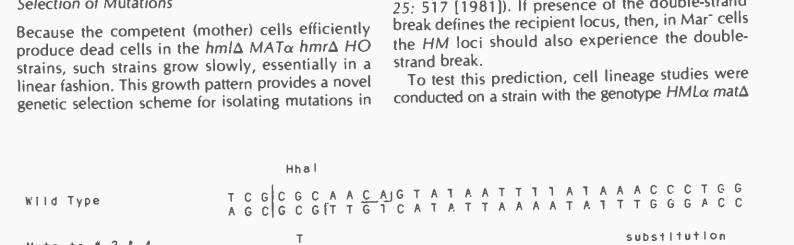


Figure 4
Sequence change of "fast growing" *MAT-Inc* mutants.

hmrΔ mar1 HO. This genotype allows us to focus on the behavior of a single *HM* locus during switching. Because of the expression of the *HMLα* information, this strain expresses an α phenotype. This strain produces **a**-like cells in a cell lineage that follow the same rules defined for *MAT* switching. The **a**-like cells we presume result from a double-strand break at the *HMLα* locus. Such cells in the absence of any functional *MAT* information are supposed to behave as **a**-like. Interestingly enough, these **a**-like cells grow to make a microcolony containing about 20–32 cells, which are all dead at that stage. No essential genetic loci exist distal to *HML*. We rationalize that the double-strand break at *HML* in *HMLα matΔ hmrΔ mar1 HO* cells is not lethal immediately, the "lingering death" may ensue from the broken end (which lacks a telomere) during subsequent rounds of growth. DNA isolated from a strain with the genotype *HMLα matΔ hmrΔ mar1 HO* was shown to contain a cut at the YZ junction of *HML*.

Cell-cycle Enrichment

Although we have been interested in the timing and cell-cycle regulation of the switching process for many years, we will dwell here only on its usefulness as a potential means of enriching for or trapping switching intermediates that would be undetectable in exponentially growing cultures. The observation that switches occur in pairs led to the suggestion that it was an event that affects both strands of the DNA prior to DNA synthesis, and then was replicated and segregated into both daughter cells. The ability to detect the double-stranded cut at *MAT* YZ does not depend on mating (a G_1 event) and hence can be monitored throughout the cell cycle.

Our published studies have shown that saturated cultures in which most cells are in the G_1 phase of the cell cycle do not exhibit the *HO* cut. We have extended these studies to show that cells arrested in G_1 by α-factor also lack the cut, whereas cells allowed to proceed into the DNA synthetic phase before being arrested by treatment with hydroxyurea have enhanced levels of cut chromosomal DNA (Fig. 5).

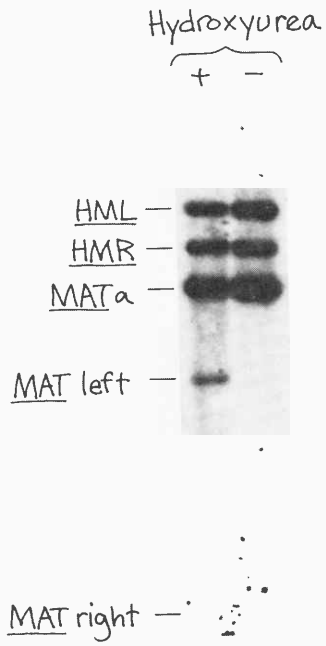


Figure 5
Southern blot of yeast DNA digested with *Hin*dIII. Fragments (*MAT left*, *MAT right*), derived by *HO*-cutting in addition to the restriction digest. Note that *HO*-cutting is enhanced in the strain treated with hydroxyurea.

Isolation and Characterization of HMLα Minichromosomes

Differences in chromatin conformation of the silent mating-type cassettes have also been observed, in this case in superhelical density (K. Nasmyth and J. Abraham, in prep.) and nuclease digestion patterns (Nasmyth, *Cell 30:* 567 [1982]). These differences have been shown to be mediated through the action (directly or indirectly) of *MAR/SIR*. Thus, it seems reasonable that the *MAR/SIR* gene products themselves, or proteins that *MAR/SIR* regulate or add specificity to, interact with the chromatin structure to repress transcription of the silent genes. This can best be investigated by purifying the *MAR/SIR* gene products and isolating the silent cassettes as repressed chromatin. This would enable us (1) to determine if the *MAR/SIR* product(s) directly interact with the silent genes, and (2) to provide information on the effect of regulatory proteins on chromatin conformation.

When a plasmid is introduced into a cell by transformation, the DNA is assembled into a nucleosome conformation that is indistinguishable from normal chromatin (Livingston and Hahn, *Proc. Natl. Acad. Sci. 76:* 3727 [1979]; Nelson and Fangman, *Proc. Natl. Acad. Sci. 76:*6515 [1979]), resulting in a minichromosome. When a silent cassette containing the α allele is introduced in this fashion into a *MAR/SIR*$^+$ heterothallic **a** cell, the resulting minichromosome is regulated (repressed), as judged by a mating-type test, at nearly the same level as the chromosomal gene. In one case, a plasmid (*Bam*26) containing the *HMLα* cassette on a 6.5-kb *Bam*H1 fragment, the *LEU2* gene and p*BR*322 when transformed into a cell (DC67) lacking the endogenous plasmid 2μ, results in a plasmid copy number of approximately 150 per cell, all of which are regulated. The mechanism responsible for this extremely high copy number is not clear and is under investigation. Two chromosomal ARS elements are present at both the *HML* and *HMR* loci and are coincident with the E and I control sites. The *Bam*26 minichromosome can be extracted by gentle lysis of Ficoll-purified nuclei followed by sedimentation on a 10–30% linear sucrose gradient with 0.2 mM EDTA. The gradients are easily assayed by dot blotting for the presence of the plasmid DNA, and the proteins on the mini-

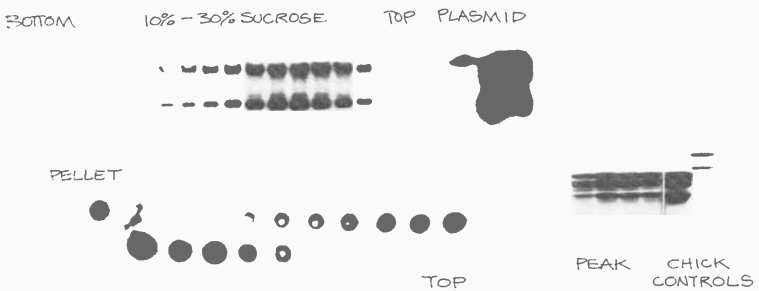


Figure 6
Sucrose gradient purification of *HMLα* minichromosomes from wild-type yeast.

chromosome can be visualized on an SDS acrylamide gel (Fig. 6). The material sedimenting at approximately 75S contains 90–95% of the minichromosomes (plus ribosomal complexes as the major nonchromatin contaminant). Greater than 90% of the proteins associated with the minichromosome are the four inner histones, H3, H4, H2a, and H2b. Topoisomerase I can be enzymatically detected cosedimenting with the peak of the minichromosomes. A number of standard techniques (see below) are presently being used to compare the proteins associated with the *HML* minichromosomes in wild-type and Mar$^-$/Sir$^-$ strains.

CVO3Bam26 has been introduced into two mutant strains, carrying either a *mar1* or a *sir4* defect. In *mar1*$^-$, the plasmid also remains episomal and has a high copy number but is mitotically unstable and segregates at a rate of approximately 25% per generation. However, when minichromosomes are prepared from the *mar1*$^-$ strain by techniques similar to those used for the wild-type strain, a very poor yield is obtained (less than 5% compared with the wild type). To try to increase the yield, increasing amounts of nonionic detergents and NaCl were added at the chromosome-releasing step. Using 2 M NaCl and 1% Triton X-100, the minichromosome is still pelleted with the bulk of the nuclear DNA. It can be released with 1.0% SDS or by nicking with DNase I. This behavior satisfied the operation definition of attachment to the nuclear matrix in *mar1*$^-$ strains. The inability to release the CVO3Bam26 plasmid from the nuclear scaffold in *mar1* strains posed a problem in that it seemed impossible to compare minichromosomes from wild-type and mutant strains.

Evidence from a number of other labs (Robinson et al., *Cell 28:* 99 [1982]) suggests that transcribed genes are more closely associated with the nuclear scaffold than transcriptionally inert genes. That is, after the histones are removed with 2 M NaCl and the histone-depleted nucleoid is purified, the active genes are *less* accessible to digestion by DNase I than are the inactive genes. Preliminary results indicate that this is also true for the yeast mating-type genes. The silent copies of mating type are more easily digested with DNase I in wild-type cells than is the transcribed *MAT* locus, while the digestion rates are indistinguishable in *mar1*$^-$ cells.

The proposal that the reason for the inability to release the minichromosome in *mar1*$^-$ strains has to do with its expression suggested that a general inhibitor of transcription would result in the release of CVO3Bam26 in these strains. Recently, it has been demonstrated that the heat-shock response in yeast is tightly controlled at the transcription level (McAlister and Finkelstein, *J. Bacteriol. 143:* 603[1980]). During heat shock, the predominant heat-shock messages are transcribed at a high rate to the detriment of most other messages coding for non-life-threatening functions. We found that when the *mar1*$^-$ stain containing CVO3Bam26 is grown for 3 hours at 40°C, the minichromosome can be released from lysed nuclei and can be isolated in the same manner as in the wild-type case, indicating that transcription control during heat shock in yeast may involve release from the nuclear matrix (S. Weisbrod, unpubl.).

Fission Yeast Mating-type Switching
A. Klar, D. Beach

To determine generalities of the principles discovered in the *MAT* switching system of *S. cerevisiae*, we have begun studies on an unrelated fission yeast, *Schizosaccharomyces pombe*. This organism, although morphologically and genetically distinct, switches its mating type at high frequency in a manner analogous to that in *S. cerevisiae*. Previous work on the fission yeast mating-type switching has been limited to genetic analysis, but with the development of transformation in this organism it has become possible to clone *S. pombe* genes easily and to proceed with a molecular investigation of the many interesting genetic phenomena found in this system.

Haploid fission yeast grows by division in rich medium, but as this is depleted, particularly of a nitrogen source, cells cease division and conjugate with those of opposite mating type, h^+ or h^-. The diploid thus formed directly enters a round of meiotic division that leads to the formation of a four-spored ascus. Although expression of sexual functions is apparently limited to conditions of nutritional deprivation, switching of mating-type occurs during exponential growth.

Using techniques analogous to those employed in the isolation of the mating-type cassettes from *S. cerevisiae*, we have cloned the mating-type information from *S. pombe* and found it to be arranged in three distinct copies. We presume that these elements are functionally similar to the three cassettes in *S. cerevisiae* but there is no evidence for DNA sequence homology.

Isolation of DNA fragments containing the mating-type genes has allowed the structure of the locus to be investigated in wild-type strains and in the many mutants that arise at high frequency as the result of abnormal switching events. Figure 7 shows the three copies of mating-type information; the expressed copy at *MAT1* and the store of silent-plus information *MAT2-P* and silent-minus information *MAT3-M*. The mating type of the cell is determined by which of P (for Plus) or M (for Minus) information is the temporary resident at *MAT1*. Unlike in *S. cerevisiae*, *MAT1*, *MAT2*, and *MAT3* in *S. pombe* are a tightly linked cluster of genes. The cassettes are flanked in each case by two regions of homology, H1 (~90 bp) and H2 (~240 bp), detected by means of heteroduplex analysis (Fig. 8). Interestingly, a surprisingly large percentage of the chromosome (~25%) is found to contain a double-stranded cut in the H1 region at *MAT1*. This cut is much reduced in switching defective (swi^-) strains and is probably involved in initiation of the recombination event of a switch. The presence of such a high percentage of broken chromosome suggests the existence of a special mechanism stabilizing the cut ends.

Switching gives rise to many abnormal events such as deletions and insertions. Deletions fusing *MAT2-P* with *MAT3-M* generate a stable minus strain (h^{-S}), whereas transposition of both *MAT2-P* and *MAT3-M*, and the sequences in between, into *MAT1* generates heterothallic h^{+N} and h^{-U} strains. The latter strains, at frequencies less than 10^{-4}, revert back to homothallic strains. The *MAT2-P* and *MAT3-M* information can be activated in situ by fusions to *MAT1* resulting in deletions of the internal sequences. This property allowed us to orient and order these determinants on chromosome II.

Northern blots have been used to analyze the transcripts of the mating-type locus. Neither *MAT2-P* nor *MAT3-M* produce transcripts. *MAT1-M* has one constitutive transcript, whereas *MAT1-P* has two transcripts. One of these is constitutively expressed but the other is induced only during conditions of nitrogen deprivation.

Future work on the fission yeast mating-type system will be twofold. First, it will provide a model system in which to study eukaryotic recombination. The recombination event takes place at almost every cell division and is initiated and resolved in precisely known regions (H1 and H2). Furthermore, there are now numerous mutants affecting switching; these may allow analysis of intermediate steps in the process of recombination.

The second area of interest is the role of the mating-type gene products in the control of mating and sporulation. Use will be made of mutants that derepress the cell for the normal requirement of heterozygosity at the mating-type locus for sporulation. Such mutations may lie in genes acting just downstream of the mating-type genes in the control of the transition from mitotic growth to meiosis.

Mutant Selection in *Nicotiana*

R.L. Malmberg, J. McIndoo

Our major results for this year derive from selection and characterization of a number of new *Nicotiana tabacum* (tobacco) cell culture mutants. The goals of this project have been (1) to increase the number of characterized, selectable mutants, and (2) to perform a mutational analysis of the polyamine synthesis pathway. We have done this by developing a UV light mutagenesis procedure and a selection system based on specific inhibitors of the pathway. The polyamine synthesis pathway was chosen

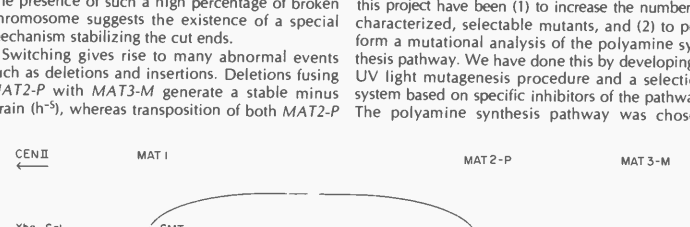


Figure 7
Restriction map of the *S. pombe* mating-type genes. Open and solid blocks represent regions of DNA homology. SMT is a site where a double-stranded DNA break is found in vivo.

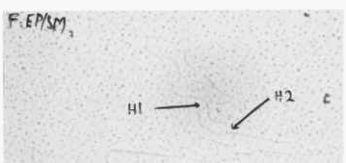


Figure 8
MAT1-P × *MAT3-M* heteroduplex.

because mutants we had previously obtained in the pathway showed a developmental switch in flower morphology in regenerated plants. This posed the questions of whether or not the mutation in the polyamine pathway was directly correlated with the developmental phenotype, or if the switch was the result of some ill-defined pleiotropic interaction. We have now isolated a number of new mutants resistant to polyamine synthesis inhibitors. One of these has a developmental switch in flower morphology related to, but even more dramatic than, the lesions observed in our first temperature-sensitive line.

The original mutant, *ts4*, was isolated in a negative selection for temperature-sensitive growth from a haploid tobacco cell line. A second-site revertant, *Rt1*, was obtained from *ts4*; since tobacco is amphidiploid in origin, the reversion could have come from some event at the homologous locus to *ts4*. These mutants have been shown to be defective in polyamine biosynthesis, and to have low intracellular levels of spermidine and spermine, although we have not been able to assign a structural gene to either. The *ts4* mutant also has the property that it produces a polypeptide of *M*, 30,000 and pI 5.3 which is not found in either wild-type or *Rt1* protein preparations. While analyzing the effects of a polyamine synthesis inhibitor on wild-type cells, we noticed that the same polypeptide is overproduced. Blocking the wild-type pathway with the inhibitor produces a phenocopy of the *ts4* protein. The inhibitor used was methylglyoxal-*bis*(guanylhydrazone) (MGBG, a spermidine analog) which affects *S*-adenosylmethionine decarboxylase, the second and third enzymes of the pathway. This effect is not seen with difluormethylornithine (DFMO, an ornithine analog) an inhibitor of ornithine decarboxylase, the first enzyme in the pathway. Subsequently, we have shown that the polypeptides from induced wild type and from mutant are identical by analyzing peptide maps resolved by HPLC. In collaboration with John Smart (Biogen, Cambridge, Mass.) we are sequencing the polypeptide.

The mutants *ts4* and *Rt1* had previously been regenerated from cell cultures into whole plants. The *ts4* plants are very small, grow poorly, are light green, and never flower. In contrast, *Rt1* plants are dark green, compact, and flower with the switch of anthers into petals. Approximately once per leaf on *Rt1* plants, a light green sector appears, which can be excised and cultured and shown to be temperature sensitive. A mitotic event is occurring at high frequency on these leaves to separate the two mutants, revealing *ts4*. The major question posed by the existence of these mutants is thus, Is the switch from anthers into petals a direct consequence of the lesions in polyamine synthesis?

Because of the phenocopy effect, this year we concentrated on selection and analysis of MGBG-resistant tobacco lines, after UV light mutagenesis; more recently, we have also obtained some DFMO-resistant lines. These mutants were mostly selected from a diploid line of cultivar Xanthi; partway through the selection process, we developed a haploid line, and some selections have been performed with it. From the MGBG diploid selection, we obtained roughly 190 colonies, from which it now appears that about 24 will prove resistant, representing about 20 independent events. The mutagenesis protocol was highly successful. Plants have been regenerated from several of the MGBG-resistant cell lines, and two of these strongly resemble the *Rt1* plants—they are normal colored but dwarfed. The dwarfism exhibited may be a direct consequence of the polyamine phenotype, since I. Bernal-Lugo, J. Varner, and D. Ho (pers. comm.) have shown that gibberellic acid treatment of tissues causes pronounced increases in polyamine levels, even prior to the well-documented α-amylase induction; of course, dwarfism can be a consequence of lack of gibberellic acid. Thus, the short internode length of the new MGBG-R mutants with the same effect in the *Rt1-ts4* mutants may be a strong confirmation of the role of polyamines in the gibberellic acid response of plants.

One of the MGBG-resistant lines, *Mgr3*, has flowered, revealing an extraordinary developmental change in flower morphology. The flowers appear normal at first, but then the ovary swells greatly, bursting the corolla and killing the five anthers. The stigma elongates until it exerts from the corolla as much as 5 mm. The stigma surface is divided into quadrants instead of halves, and after a short period of time, the surface turns black. Dissection of the ovaries reveals the source of the dramatic swelling. In a normal tobacco plant, there are usually found hundreds of ovules, white discs, implanted on the placental wall. In *Mgr3*, almost all of the ovules are converted into anthers. Upon dissecting the ovary wall, one is suddenly confronted with hundreds of anthers, appearing like green spaghetti (Fig. 9). This is a female-to-male developmental-switch mutant. The implications of this are that polyamine synthesis is intimately involved with flower morphology, specifically anther development, since the two regenerated mutants, *Rt1* and now *Mgr3*, both have altered gametogenesis.

The ovules-to-anthers phenotype is surprising, even given that a female-to-male switch would occur. Ovules and anthers are not equivalently advanced

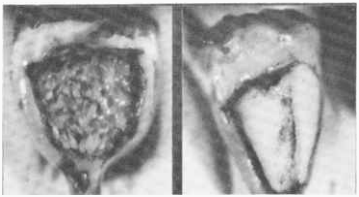


Figure 9
Dissected ovaries of wild-type *Nicotiana tabacum (left)* and mutant *Mgr3 (right)*. The ovaries were removed from the flower and then a triangular flap of the ovary wall was removed. (Photomicrography performed with the assistance of Sue Hockfield and Jorgen Johansen.)

structures. The female equivalent of the anther is the whole ovary/stigma structure. Thus, this mutant reveals that a developmental switch can occur that does not just replace the ovule with the homologous male organ, but also resets the developmental program to an earlier stage.

Critical experiments to perform in the future must include discovering the nature of the biochemical lesion of the *Mgr3* mutant, as well as meiotic genetic analysis. *Mgr3* happens to be the first of the mutants selected to be regenerated into a plant and flower. We are eagerly awaiting the regeneration of more of the MGBG- and DFMO-resistant lines.

Transposable Elements Associated with Aberrant Ratio in *Zea mays*

S.L. Dellaporta, J.B. Hicks, J. Wood

This year our attention focused on the isolation and characterization of Zea *mays* (maize) transposable elements. In collaboration with Dr. John Mottinger (University of Rhode Island), we have been working on the molecular characterization and cloning of elements that are activated by viral infection, specifically, seven spontaneous mutations at the *shrunken* locus in maize that were obtained by Dr. Mottinger. These mutations were found in an F_1 population of 346,201 kernels in the following genetic cross:

sh bz wx/sh bz wx × *Sh Bz Wx/Sh Bz Wx*

where the male parents were the advanced progeny of stocks that were originally infected with barley stripe mosaic virus (BSMV) by Sprague and McKinney (*Genetics 54:* 1287 [1966]). The virus infection resulted in phenotypic ratio distortions of colored and colorless kernels among exceptional ears in F_2 progeny segregating for the anthrocyanin factor *A/a* which they termed aberrant ratio or AR. These distortions found in F_2 populations were associated with BSMV, wheat streak mosaic virus, or lily corn fleck virus infection of a male parent homozygous dominant for aleurone color loci crossed to an uninfected female homozygous recessive for a. Subsequently, AR was described in F_2 populations segregating for *Pr/pr* and *Su/su* that have in common a virus-infected male progenitor. Genetic studies by others have since demonstrated that many of the apparent ratio distortions at *A1* can be explained by segregation of alleles at complementary loci such as *A2*, *C*, *C2*, and *R*. However, the apparent transposition of AR from one locus to another and the apparent ability to induce mutations at epistatic loci suggested that spontaneous mutations in AR lines could be directly or indirectly virally related.

Our investigation began by examining the DNA of the original seven *sh** mutants by Southern hybridization analysis and has continued with isolating genomic clones of progenitor and mutant alleles. This work has identified three different isoalleles of *Sh*, distinguished by restriction site polymorphisms, that were segregating in the progenitor and tester strains used in these experiments. After characterizing the progenitor alleles, we were able to show that all of the *shrunken* mutations isolated in the AR stocks were associated with DNA rearrangements, which in two cases have been identified as DNA insertions. We have begun to clone both Sh^A and Sh^B progenitor and mutant alleles for a detailed molecular analysis. At the time of this writing, one progenitor Sh^B and two mutant alleles, *sh-5586* and *sh-5582*, have been cloned from genomic λ libraries in EMBL4. Most of the information we have is on *sh-5586*, its progenitor Sh^B allele, and *sh-5582*.

An 11-kb R1 fragment containing the Sh^B structural gene and a 14.3-kb R1 fragment containing the mutant Sh^B allele *sh-5586* have been obtained. Restriction mapping of both fragments has confirmed that the *sh-5586* mutation is the result of an insertion of 3.3 kb of DNA into the 0.7-kb *Hin*dIII fragment of Sh^B. This region is covered by the cDNA probe, and therefore, the insertion most likely resides within the *Sh* structural gene.

By hybridization analysis of internal and junction fragment probes of insertion 86 to maize genomic DNA, we have concluded that: (1) the terminal regions of insertion 86 contain a highly repetitive sequence found in all stocks tested; (2) internal regions of insertion 86 vary in copy number from two to three per genome to highly repetitive; (3) the highly repetitive internal regions of the transposon are highly dispersed in size and do not show common restriction sites; (4) a repetitive internal region of insertion 86 does not hybridize to the insertion element found in the *sh-5582* allele.

Insertion 86 is thus a member of a family of dispersed repetitive elements and probably not related to the viral genome. Sequences homologous to insertion 86 are found in three maize lines examined, suggesting that it is a normal constituent of the genome. The lack of homology to the insert at *sh-5582* indicates that more than one family of transposons was activated in the AR stocks.

Publications

Abraham, J., K.A. Nasmyth, J.N. Strathern, A.J.S. Klar, and J.B. Hicks. 1983. Regulation of mating-type informa-

tion in yeast: Negative control requiring sequences both 5′ and 3′ to the regulated region. (Submitted.)

Abraham, J., J. Feldman, K.A. Nasmyth, J.N. Strathern, A.J.S. Klar, J.B. Broach, and J.B. Hicks. 1983. Sites required for position-effect regulation of mating type information in yeast. *Cold Spring Harbor Symp. Quant. Biol. 47:* 989.

Broach, J.R., Y.-Y. Li, J. Feldman, M. Jayaram, J. Abraham, K.A. Nasmyth, and J.B. Hicks. 1983. Localization and sequence analysis of yeast origins of DNA replication. *Cold Spring Harbor Symp. Quant. Biol. 47:* 1165.

Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. Maize DNA miniprep procedure. *Maize Genetic Coop. Newsletter:* (in press).

Hicks, J.B., J.N. Strathern, A.J.S. Klar, and S. Dellaporta. 1982. Cloning by complementation in yeast: The mating type genes. In *Genetic engineering* (ed. J. Setlow and A. Hollander), vol. IV. Plenum Press, New York.

Kassir, Y., J.B. Hicks, and I. Herskowitz. 1982. The *SAD* mutation of yeast is an extra **a** cassette. (In press.)

Klar, A.J.S., J.B. Hicks, and J.N. Strathern. 1982. Directionality of yeast mating-type interconversion. *Cell 28:* 551.

Klar, A., J.G.L. Petersen, T. Nilsson-Tillgren, and R.C. von Borstel. 1983. High frequencies of 4:0 and 0:4 tetrads for loci located distal to the ribosomal DNA of *Saccharomyces cerevisiae*. (Submitted.)

Klar, A.J.S., J.N. Strathern, J.B. Hicks, and D. Prudente. 1983. Efficient production of a ring derivative of chromosome II by the mating-type switching mechanism in *Saccharomyces cerevisiae*. *Mol. Cell. Biol. 3.*

Krul, N., T. Crusberg, B. Lowe, and S. Dellaporta. 1983. Primary and secondary embryogenesis in *Vitis* sp 'Sevall'. (Submitted.)

Malmberg, R.L. and R.J. Griesbach. 1983. Chromosomes from protoplasts—Isolation, fractionation, and uptake. In *Plant genetic engineering* (proceedings of a meeting at Davis, California). (In press.)

Malmberg, R.L. and J. McIndoo. 1983. Developmental switch in *Nicotiana tabacum* flower development caused by resistance to a polyamine synthesis inhibitor. (In preparation.)

——.1983. The selection of polyamine synthesis mutants from *Nicotiana tabacum* cell cultures mutagenized by ultraviolet light. (In preparation.)

Mottinger, J. and S.L. Dellaporta. 1983. Genetic and molecular characterization of *shrunken* mutations in maize from aberrant ratio stocks. (In preparation.)

Strathern, J.N., E.W. Jones, and J.R. Broach, eds. 1982. *The molecular biology of the yeast* Saccharomyces. *Metabolism and gene expression*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Strathern, J.N., A.J.S. Klar, J.B. Hicks, J.A. Abraham, J.M. Ivy, K.A. Nasmyth, and C. McGill. 1982. Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the *MAT* locus. *Cell 31:* 183.

MOLECULAR GENETICS OF PATHOGENIC AND VIRULENCE FACTORS

M. So, E. Billyard, C. Deal, C.H. Lee, K. Messina, E. Segal

Our laboratory is interested in studying the components contributing to pathogenesis and virulence at the molecular and genetic levels. At the moment, we are concentrating on two bacterial systems: enterotoxigenic *Escherichia coli* and *Neisseria gonorrhoeae*.

Enterotoxigenic *E. coli* causes diarrheal disease in both humans and animals. The clinical syndrome of the disease—diarrhea due to an electrolyte imbalance in the upper bowels—is due to the action of one or more toxins produced by the invading bacteria. Two types of toxins are known to exist: (1) LT, a heat-labile toxin immunologically and genetically related to cholera toxin and (2) ST, a heat-stable protein less than 5000 daltons in size. There are two classes of ST toxins: (1) STI or STA is methanol soluble and is assayed in suckling mice and (2) STII or STB is methanol insoluble and assayed in jejunal loops of weaned rabbits or piglets.

STI is well characterized genetically and chemically. The gene encoding STI is part of a transposon flanked by inverted or direct repeats of IS1. The amino acid sequence of STI, deduced from the DNA sequence, indicates that it is synthesized within the cell as a prepeptide with a signal sequence and contains a cysteine-rich region at the carboxyterminal end. Comparison of this and subsequent sequence work by several other laboratories suggests that the Cys-rich region comprises the active moiety. Synthetic peptide work (R.A. Noughton, pers. comm.) indicates that the terminal 18 amino acids are required for full toxin activity. The precise mechanism by which STI induces electrolyte imbalance is still poorly understood. STI has been shown to stimulate guanylate cyclase in vitro, and a model based on the last 18-amino sequence bears striking resemblance to an ionophore (M. Gill, pers. comm.).

The precise role played by STII in pathogenesis is not known. This is due in large part to the cumbersome assay required to detect its activity. Some *E. coli* isolates from diseased piglets produce STII but not LT or STI. However, these strains are unable to cause disease in experimentally infected piglets. *E. coli* isolates from humans were thought to produce STII only. Southern blotting experiments subsequently showed most of them also contain the LT and/or STI gene(s). We have shown that STI and STII are genetically distinct. To understand better the contribution of STII to pathogenesis and virulence, we have cloned an 806-bp fragment of DNA encoding STII from a 60 x 10^6-dalton plasmid into a genetically characterized K-12 strain of *E. coli*. This K-12 recipient now produces STII as assayed in the jejunal loops of weaned pigs. Through subcloning and mutation analysis, we have identified the coding region of the STII insert.

The nucleotide sequence of STII is quite different from that of STI, as our previous work had suggested. The deduced protein sequence is 71 amino acids in length with a signal peptidelike sequence at the aminoterminal end and a series of hydrophobic amino acids at the carboxyterminal end; like STI, it is Cys-rich, although the cysteines are not arranged in as striking a manner.

Our previous work had indicated that the STI gene may be disseminated by transposition. The immediate areas flanking STII do not contain any direct or inverted sequences that are hallmarks of transposable elements. However, the original plasmid from which the STII gene was cloned does contain a 600-bp inverted repeat. This sequence does not appear to be related to IS1, and its relationship to the transposability of STII is now under investigation.

We have used the STII gene as a probe in a set of preliminary colony hybridizations and the data indicate that all STII and only STII producing enterotoxigenic *E. coli* are detected. This observation makes the task of epidemiology of enterotoxigenic *E. coli* easier.

Recently, we have also been studying the genetic regulation of pilin expression in *N. gonorrhoeae*. Pili, comprised of identical 18,000-dalton subunits, are surface proteins that function in colonization. There is no animal model for gonorrhea since man is the only host for this disease. At least 100 serotypically distinct pili are known to exist. The protein sequences of three different pilin subunits indicate that they all share a region of homology located at the aminoterminal end. In addition, any *N. gonorrhoeae* clinical isolate can switch from a pilus$^+$ (P^+) to pilus$^-$ (P^-) phenotype and back again to P^+ at frequencies higher than can be explained by random mutation. We have cloned two expressing pilus genes into *E. coli* K-12; by using them as probes, we were able to show that transcription stops when the cell switches from the P^+ to the P^- state. In addition, genomic rearrangement is involved in this switching process.

Subcloning experiments localized the pilus-coding region to a 1-kb fragment in each of the two inserts of 2 kb and 4 kb. We have completed the sequence of the 2-kb insert. The structural gene contains promoter sequences similar to those known for *E. coli* and other bacterial species such as *S. pneumoniae*. The noncoding region contains interesting direct and indirect repeated sequences, in addition to an extra copy of the region of the pilus structural gene corresponding to the variable region of the protein. We are now sequencing the clones

from a P^- genome derived from the original P^+ isolate, which contains the "expression site," provided us by T.F. Meyer (Max Planck Institute). These experiments should elucidate the sites important in the rearrangement process.

In other experiments, we are also examining the genetic potential of each cell to generate immunologically distinct pili subunits by a detailed set of lineage studies. We have a set of subclones containing only the constant and variable regions and noncoding sequences and are using them as probes to map the region of the *N. gonorrhoeae* genome that functions in pilus expression. Last, we are generating a set of mutants of *N. gonorrhoeae* that fail to switch from $P^+ \rightarrow P^-$ and from $P^- \rightarrow P^+$.

Publications

Lee, C.H., S.L. Moseley, H.W. Moon, C.L. Glyes, and M. So. Characterization of the gene encoding STII toxin and preliminary molecular epidemiologic studies of ETEC producers. (Submitted.)

Meyer, T.F., N. Mlawer, and M. So. 1982. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. *Cell 30:* 45.

BACTERIAL TRANSPOSITION

F. Heffron, *A. Bhagwat, R. Kostriken, C.-H. Lee*

In the last year, my laboratory has focused on two problems:

I. A large amount of data supports the idea that recombination in yeast (and perhaps other organisms) proceeds via an intermediate containing a double-strand break (Orr-Weaver et al., *Proc. Natl. Acad. Sci. 78:* 6354 [1981]; Szostak et al., *Cell* [1982, in press]). Yeast endonucleases are therefore likely to play a role in recombination. Strathern et al. (*Cell 31:* 183 [1982]) have identified a double-strand break at the mating-type locus in yeast cells that are switching mating type. We have established a sensitive endonuclease assay that allowed us to identify an endonuclease (YZ endo) with the properties one would predict. YZ endo makes a double-strand break within *MAT* (the mating-type locus) at a site that corresponds closely to that mapped in vivo by Strathern et al. (*Cell 31:* 183 [1982]). Furthermore, mutations at *MAT* that cannot switch mating type were found to be resistant to digestion by the endonuclease in vitro. YZ endo makes a double-strand break at the sequence

```
C G C A A C A↓G T A A A
G C G T T G T C A T T T
        ↑
```

to generate a four-base 3′ extension terminating in a 3′ hydroxyl in the Z region of the *MAT* locus. Based on deletion studies and studies with synthetic DNA substrates, the enzyme recognizes a short specific sequence contained within the 12 nucleotides shown above. YZ endo makes a concerted double-strand break and does not remain attached to the DNA. It appears to purify as a single protein (or tightly bound complex) as judged by its purification as a single peak of activity on DNA cellulose, DEAE Sephacel, and phosphocellulose column chromotography.

The sequence shown above occurs at least three times in the yeast genome, i.e., once in each cassette of mating-type information; yet, during mating-type interconversion, the double-strand break is observed only at *MAT* and not at either *HML* or *HMR*. Therefore, we were interested in testing if *HML* and *HMR* were not cut by the endonuclease in vivo because the DNA had been covalently modified at the silent cassettes or whether the sites at *HML* and *HMR* were protected by chromatin. We isolated yeast DNA and treated it in vitro with the endonuclease. We found that the two silent cassettes are cut equally as well as *MAT*, thus ruling out covalent modification.

In addition to YZ endo, we have identified a second endonuclease that makes a double-strand break within pBR322 at nucleotide 1330. Like YZ endo, this enzyme cleaves DNA to leave a 4-bp (or 5) 3′ extension; but unlike YZ endo, it is present in all strains of yeast we have tested. We are currently looking for other endonucleases in yeasts and attempting to determine their role in recombination.

II. Tn*3* is a 5-kb bacterial transposon that encodes three genes: (1) a resolvase/repressor, (2) a transposase, and (3) a β-lactamase. In addition to these three genes, it encodes three sites required for its transposition, it is terminated at either end by 38-bp perfect inverted repeats that are required for Tn*3* transposition, and it encodes a resolution site. Transposition of Tn*3* appears to take place by a two-step process; in the first step, the donor and recipient molecules become joined via a direct repeat of Tn*3* (the transposase and terminal repeats are absolutely required for this step). In a second step, the cointegrate is resolved. This step requires the resolvase/repressor and the internal resolution site.

Transposons related to Tn*3* exhibit a phenomenon called transposition immunity first described by Robinson et al. (*J. Bacteriol. 129:* 407 [1977]). A plasmid containing a copy of Tn*3* is immune to further insertions of Tn*3*. It is a particularly interesting phenomenon to study because it works only in *cis*. That is, a plasmid containing Tn*3* is immune to insertion of Tn*3*, but transposition occurs normally to other plasmids in the same cell. Immunity must block some step in transposition because plasmids containing two copies of Tn*3* can be constructed and these are perfectly stable (Robinson et al., *J. Bacteriol. 129:* 407 [1977]). Last of all, we know that transposition immunity is highly specific to the transposon, that is, a plasmid containing Tn*501* and therefore immune to Tn*501* is not immune to Tn*3* (Grinsted et al., *Mol. Gen. Genet. 166:* 313 [1978]). We have determined that tnpR is not required for transposition immunity. By extensive deletion analysis, as well as the chemical synthesis of the terminal 38-bp repeat, we have determined that only the terminal repeat need be present to confer immunity to Tn*3*. In most instances no other part of Tn*3* is required in *cis* for immunity. (We are currently investigating the few exceptions to this rule.)

We believe that transposition immunity can be accounted for by a tracking model. During transposition, the actual integration event in the recipient molecule may be slow or inefficient. As a consequence, an integration complex composed of the transposase joined to the ends of Tn*3* may have a long half life. If the integration complex tracks along a recipient molecule, it would thus have a high probability of scanning the entire recipient molecule before integration. Such a model is supported by transposition specificity studies (Grinsted et al., *Mol. Gen. Genet. 166:* 313 [1978]). A model for immunity could then be that the integration complex dissociates itself when it encounters an immunity

sequence (the terminal 38 bp) on the recipient molecule.

Publications

Kostriken, R. and F. Heffron. Purification of an *HO* dependent endonuclease from *Saccaromyces cerevisiae*. (In preparation.)

Kostriken, R., J. Strathern, A. Klar, J. Hicks, C. Moomaw, and F. Heffron. 1983. Identification and characterization of an *HO* dependent endonuclease from yeast which plays an essential role in mating type interconversion. (Submitted.)

Lee, C.-H., A. Bhagwat, and F. Heffron. 1983. Molecular dissection of Tn*3* transposition immunity. *UCLA Symp.* (In press.)

Vertebrate Gene Structure and Expression

Until recombinant DNA techniques were developed, the structure of vertebrate genes at the DNA level was effectively a black box incapable of being opened up for intelligent dissection. Now virtually any gene can be cloned if we have a trick to select for its presence or if we know a fragment of the amino acid sequence of its protein product or if we can generate an antibody that specifically reacts with the desired protein. The concept that a gene is isolatable and the actual cloning of it, however, are not the same. Much persistent intelligence and experimental skills are more often than not required. To take on the nature of vertebrate genes, we have created a number of research groups of complementary objectives that can fruitfully interact through the sharing of ideas, techniques, and equipment.

This past year saw the six different labs of this section first developing and then exploiting several powerful new general ways for gene cloning. Key to many of our successes was the assistance, both intellectual and practical, given to all by John Fiddes, who for two and a half years led a group focusing on Polypeptide Hormone Genes. Thus, his departure at the year end to help form the new recombinant DNA company, California Biotechnology, Inc., left a gap not easily filled. David Zipser, who for 12 years headed our Molecular Genetics Section, also moved to California where he will be part of a Cognitive Science group at the University of California in La Jolla.

MAMMALIAN CELL GENETICS

M. Wigler, M. Goldfarb, K. Shimizu, *E. Taparowsky, O. Fasano, Y. Suard, D. Birnbaum, L. Edlund, M.A. Ruley, C. Fraser, M. Riggs*

The use of DNA-mediated gene transfer led to the discovery of dominant-acting transforming genes in nonvirally transformed cells (Shih et al., *Proc. Natl. Acad. Sci. 76:* 5714 [1979]). For the past year, our major effort has been the isolation and characterization of transforming genes we previously identified in human neuroblastoma and bladder, lung, and colon carcinoma cell lines (Perucho et al., *Cell 27:* 467 [1981]). Considerable progress has been achieved in isolating the transforming gene common to lung and colon carcinomas. The transforming gene from a neuroblastoma cell line and its normal counterpart from placental DNA have both been fully cloned. Finally, the transforming gene of the bladder carcinoma cell line T24 and its normal counterpart have been cloned, sequenced, and compared, and we understand at the nucleotide level the difference between them. The transforming gene encodes a protein that differs at one amino acid from that encoded by the normal gene (Taparowsky et al., *Nature 300:* 762 [1982]). This difference accounts for its biological activity in the NIH-3T3 focus assay system. How this change in protein structure leads to cellular transformation is now our major concern.

Isolation and Characterization of the Transforming Gene of T24 Bladder Carcinoma Cells

M. Goldfarb, E. Taparowsky, O. Fasano, Y. Suard, M. Wigler

Last year, we reported isolating the transforming gene of T24, a human cell line derived from a bladder carcinoma (Goldfarb et al., *Nature* 296: 404 [1982]). The method of isolation was the tRNA suppressor method developed here by Kenji Shimizu. Hybridization studies of this gene indicated that it was human in origin and that its chromosomal locus displayed a high frequency of restriction fragment length polymorphisms (RFLPs) (Goldfarb et al., *Nature* 296: 404 [1982]). From this observation and blotting to human DNAs, we have been able to infer that this gene is present just once per haploid chromosome complement. This gene has been used as a probe by Ray White and colleagues (Howard Hughes Medical Institute in Salt Lake City, Utah) to demonstrate that hereditary predisposition to colon cancer is *not* linked to this gene (Barker et al. [1983, in prep.]).

Workers from other labs demonstrated that the T24 gene was the human homolog to the viral oncogene *v*-Ha-*ras*, encoding a 21,000-dalton protein (p21) (Der et al., *Proc. Natl. Acad. Sci. 79:* 3637 [1982]); Parada et al., *Nature 297:* 474 [1982]; Santos et al., *Nature 298:* 343 [1982]). This result has been confirmed by us (Shimizu et al., *Proc. Natl. Acad. Sci.* [1983, in press]) and provided the first instance of a gene, previously identified by its presence in oncogenic retroviruses, implicated in human cancer.

Our initial work on this gene has been to determine why it has transforming activity. The suppressor-rescued gene was used as probe to isolate the intact gene from both T24 DNA and placental DNA λ L47.1 libraries. No isolates from the placental DNA were capable of inducing foci on NIH-3T3 cells, whereas all isolates from the T24 library were efficient at focus induction in NIH-3T3. This indicated that the difference between the normal and transforming genes was indeed at the DNA level. By constructing chimeric genes—recombinants between the normal and tranforming genes—we were able to determine that a small 224-bp *Mst*II/*Xba*I fragment of the transforming gene contained the functionally altered sequence. Sequencing this region for the tranforming gene, the normal gene, and a cDNA copy to the transforming gene transcript indicated only one significant base change occurring in the coding portion of the first coding exon whereby the 12th N-terminal amino acid was changed from glycine in the normal to valine in the transforming *ras* p21 (Taparowsky et al., *Nature 300:* 762 [1982]). These sequence changes were also reported by others (Reddy et al., *Nature 300:* 149 [1982]; Tabin et al. *Nature 300:* 143 [1982]). Cotransformation into NIH-3T3 cells of either the normal or transforming gene indicated that indeed the *ras* p21 of T24 was 100–1000 times more efficient than the normal *ras* p21 at inducing the transformed phenotype in NIH-3T3 cells (Taparowsky et al., *Nature 300:* 762 [1982]). Taken together, at the DNA level, the molecular changes resulting in a cancer gene have been defined. Most importantly, these results indicate that, by a very minor alteration, a normal cellular protein can be converted to a transforming protein. Curiously, alterations in the 12th amino acid are also found in the viral *ras* oncogenes, suggesting this amino acid residue plays a critical role in the normal function of this protein. (Similar lesions were found in this gene isolated from the human bladder carcinoma cell line EJ. However, in collaboration with Jorgen Fogh and colleagues at the Sloan Kettering Cancer Research Institute in Rye, New York, we demonstrated that the cell line called EJ probably arose as a subline of T24.)

A complete cDNA to the T24 Ha-*ras* transcript was cloned and sequenced and compared with the genomic Ha-*ras* gene. The human Ha-*ras* gene has at least five exons, and the complete coding sequence is contained in four of these. We have not yet identified the promoter of the Ha-*ras* gene nor

have we located the 5′ untranslated sequences of the mature message. By making genomic/cDNA chimeras, we have established that introns are required for the efficient expression of this gene.

We made comparisons of the complete nucleotide sequence of the human Ha-*ras* gene with the viral Ha-*ras* gene, previously published by others (Dahr et al., *Science 217:* 934 [1982]). This resulted in discovering a remarkable conservation in amino acid sequence (Fasano et al., *J. Mol. Appl. Genet.* [1982, in press]). Although 30% divergence in neutral base positions was observed, only 3 amino acids out of 189 were different. In particular, there was complete conservation at the carboxyl end of the proteins, the regions where the viral Ki-*ras*, another gene in the *ras* family, and the viral Ha-*ras* diverge markedly (Dahr et al., *Science 217:* 934 [1982]; Tsuchida et al., *Science 217:* 937 [1982]). We conclude that this region may distinguish the physiologic functions of the *ras* genes.

Isolation and Characterization of the Lung and Colon Carcinoma Transforming Gene

K. Shimizu, M.A. Ruley, M. Goldfarb, D. Birnbaum, M. Wigler

Previous work identified a large gene of human origin with transforming activity present in several lung and colon carcinoma cell lines (Perucho et al., *Cell 27:* 467 [1981]). Identification was made based on the presence of conserved EcoRI digest fragments containing human repeat sequences in NIH-3T3 cells transformed with DNA from these donors. The first fragment of this gene was cloned by screening λ Charon 4A libraries of NIH-3T3 secondary transformants derived from Calu-1, a human lung carcinoma cell line, with blur 8, a cloned representative of the *Alu* family of human repeat sequences (Shimizu et al., *Proc. Natl. Acad. Sci.* [1983, in press]). Subsequent fragments of this gene were obtained by chromosomal walking (Shimizu et al., and work in progress). The resulting isolation and mapping of over 20 overlapping phage inserts have led to the construction of a composite restriction map. Der et al. (*Proc. Natl. Acad. Sci. 79:* 3637 [1982]) demonstrated that another lung carcinoma cell line, LX-1, carried a transforming gene that was closely homologous to the viral Kirsten *ras* (Ki-*ras*) gene. The Ki-*ras* and Ha-*ras* genes are related members of a diverged family. They encode immunologically cross-reactive 21,000-dalton proteins and share considerable nucleotide homology. Hybridization studies on our cloned gene also indicated its close relation to Ki-*ras*, and its identity to the transforming gene of LX-1. Southern blotting with cloned viral Kirsten sequences as probe has identified the potential coding blocks of this gene. Although the gene product is the same size as the Ha-*ras* gene product, the human Ki-*ras* is 20 times the size (40 kbp) of the human Ha-*ras* gene and contains one more coding block. Preliminary sequence data indicates considerable homology between the second and third coding blocks of human Ki-*ras* and Ha-*ras*. Indeed, they appear to utilize the same splice site. We predict, therefore, that the human Ki-*ras* gene contains either a pseudo-exon or that exon choice exists, possibly in a developmentally regulated fashion, with an alternate third or fourth coding exon. As indicated from our previous studies, we believe these last two exons contain the protein determinants of physiologic specificity.

We have also begun to isolate the human Ki-*ras* gene from a placental DNA library but have not yet done sequence comparison of the normal and transforming genes to determine the potential candidate sites of functionally significant biological alteration.

Work from other labs has implicated this gene in pancreatic carcinoma and osteosarcomas (Pulciani et al., *Nature 300:* 539 [1982]).

Isolation and Characterization of the Transforming Gene of a Human Neuroblastoma Cell Line

K. Shimizu, E. Taparowsky, M. Goldfarb, M. Wigler

Transforming activity was found in the DNA derived from SK-N-SH, a human neuroblastoma cell line (Perucho et al., *Cell 27:* 467 [1981]). This past year, we succeeded in cloning the gene responsible for this activity (Shimizu et al., *Proc. Natl. Acad. Sci. 80:* 383 [1983]) using the methodology of tRNA suppressor rescue that had been developed here by Kenji Shimizu (see last year's report). Hybridization studies indicated that this gene was of human origin, since it had a normal counterpart in the DNA of all human material we examined. Further hybridization studies showed that the transforming gene of SK-N-SH was related to both the Ha-*ras* and Ki-*ras* genes. It encodes an immunologically cross-reactive 19.5-kD protein. Indeed, it constitutes a third member of the *ras* gene family, and all three *ras* genes are about equidistant in nucleotide divergence from each other. We have termed this gene N-*ras*. It is intermediate in size between Ha-*ras* and Ki-*ras*, a biologically active fragment being less than 12 kb and probably less than 9 kb. Its intron/exon structure has not yet been determined nor has sequence comparison to the other *ras* genes begun.

In contrast to T24, SK-N-SH contains both normal and transforming copies of this gene. The homolog cloned from placental DNA is also not active in the NIH-3T3 focus assay. Therefore, the difference between the transforming and normal genes lies at the nucleotide level. We are beginning to perform systematic recombination experiments between the normal and transforming versions of this gene to determine the nucleotide alterations that result in functionally altered biological activity. To facilitate this, we are in the process of isolating cDNA clones of the N-*ras* transcript. Preliminary results from

other labs have begun to indicate that this gene is implicated in a very wide range of tumors.

Other Assays for Transforming Genes

O. Fasano, M. Wigler

It is a significant and curious result that all the transforming genes detectable by us with the NIH-3T3 focus assay are members of the *ras* gene family. On the one hand, this implies that the alteration by mutation of *ras* genes may be an important step in the development of many kinds of human cancer. On the other hand, it may point to a bias in the assay system that readily scores altered *ras* genes but is insensitive to many other tumor genes. For this reason, we have begun to develop another assay system, still utilizing NIH-3T3 cells as recipients for DNA-mediated transfer, but scoring for the transformation event by injecting cells exposed to DNA directly into nude mice. These experiments are being performed in collaboration with Jorgen Fogh of Sloan Kettering Cancer Research Institute, Rye, New York. Preliminary results indicate that this method will be sensitive to genes previously undetectable by the focus assay system.

Publications

Barker, D., M. McCoy, R. Weinberg, M. Goldfarb, M. Wigler, R. Burt, E. Gardner, and R. White. 1983. A test of the role of two oncogenes in an inherited predisposition to colon cancer. (Submitted.)

Fasano, O., E. Taparowsky, J. Fiddes, M. Wigler, and M. Goldfarb. 1983. Sequence and structure of the coding regions of the human H-ras-1 gene from T24 bladder carcinoma cells. *J. Mol. App. Genet.* (in press).

Goldfarb, M., K. Shimizu, M. Perucho, and M. Wigler. 1982. Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. *Nature 296:* 404.

Kwoh, T.J., D. Zipser, and M. Wigler. 1983. Genetic analysis of the cloned chicken thymidine kinase gene by in vitro mutagenesis. (Submitted.)

Shimizu, K., M. Goldfarb, and M. Wigler. 1983. Use of a bacterial tRNA suppressor gene to isolate genetically selectable eukaryotic genes. (In preparation.)

Shimizu, K., M. Goldfarb, M. Perucho, and M. Wigler. 1983. Isolation and preliminary characterization of the transforming gene of a human neuroblastoma cell line. *Proc. Natl. Acad. Sci. 80:* 383.

Shimizu, K., M. Goldfarb, Y. Suard, M. Perucho, Y. Li, E. Stavnezer, T. Kamata, J. Feramisco, and M. Wigler. 1983. Three human transforming genes are distinct members of a diverged gene family related to the ras retroviral oncogenes. *Proc. Natl. Acad. Sci.* (in press).

Taparowsky, E., Y. Suard, O. Fasano, K. Shimizu, M. Goldfarb, and M. Wigler. 1982. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature 300:* 762.

HORMONAL CONTROL OF GENE EXPRESSION

D.T. Kurtz, *D. Danna, L. McCullough, W. Addison, J. MacInnes*

For the past several years, we have been studying the hormonal modulation of the synthesis of a rat liver protein called α_{2u} globulin. α_{2u} represents 1–2% of protein synthesis in the livers of adult male rats. It is induced in vivo by androgens, glucocorticoids, thyroid hormone, and growth hormone, and is strongly repressed by estrogens.

α_{2u} Globulin Gene Structure

Southern blot and kinetic hybridization analysis with a cloned α_{2u} cDNA have indicated that this protein is encoded by a multigene family, with 18–20 copies per haploid genome. Twelve genes have been isolated from a library of the rat genome cloned in λ phage. The α_{2u} structural genes are ~3.5 kb in length and contain six introns. The homology between the different α_{2u} genes extends beyond the 5′ and 3′ ends of the structural gene to encompass a length of 10–12 kb. For the most part, the different genes show extensive homology in exon, intron, and flanking regions. Two of the clones, however, show a complete divergence of sequence in the 3′ portion of the structural gene. This region corresponds to the 3′untranslated region of α_{2u} mRNA. If small segments of DNA from these variant 3′ ends are cloned and used as a probe versus mRNA from rat tissues, no hybridization can be detected, suggesting that these genes are not transcribed in vivo. The divergence at the 3′ end of these genes is the most obvious difference between these variants and the normal genes. Other more subtle sequence differences exist, which will be described below.

α_{2u} Globulin Induction by Glucocorticoids

As we reported previously, the cloned α_{2u} globulin genes, when transfected into mouse L cells, retain their ability to respond to glucocorticoids. Initial experiments were done using the entire λ clone containing the gene. We have subcloned α_{2u} gene fragments containing progressively less flanking sequence and have found that a cloned *Xba–Bam* fragment containing 235 bases of 5′ flanking sequence and approximately 400 bases of 3′ information continues to respond to glucocorticoids when transfected into L cells. The regulatory elements apparently lie quite close to, or in the gene, in agreement with the findings in other laboratories for other inducible genes.

To determine if the ability of the α_{2u} gene to respond to glucocorticoids lies in the 5′ flanking region, a fragment from the promoter region of the α_{2u} globulin gene was ligated to the structural gene for herpes thymidine kinase (TK), using a mutant *tk* gene (obtained from D. Zipser) which had had a *Xho* linker inserted between amino acids 9 and 10 of the protein-coding sequence. The α_{2u}-*tk* hybrid construct should result in a chimeric protein containing the first three amino acids from α_{2u}, three amino acids from the linker, and the remainder from *tk*. The α_{2u}-*tk* hybrids were used to transfect Ltk cells to the tk^+ phenotype. tk^+ clones were isolated and *tk* activity and *tk* mRNA were measured with and without dexamethasone. It was found that both TK enzyme activity and total *tk* mRNA were induced slightly (2–4-fold) by dexamethasone treatment. This is significantly less than the induction seen when the intact α_{2u} gene is used. When the *tk* mRNA produced by these cells was analyzed on Northern blots, two *tk* mRNA species were detected—one of approximately 1250 bases, the size expected for *tk* mRNA produced from the α_{2u} promoter, and another short (~800 base) transcript. This short mRNA, apparently produced from a promoterlike sequence inside the normal *tk* protein-coding region, has been found by other investigators.

The 1250-base *tk* message produced from the α_{2u} promoter is inducible by dexamethasone, whereas the level of the short transcript is unaffected. The 5′ flanking region of the α_{2u} gene apparently contains sequences that are at least in part responsible for glucocorticoid inducibility. Sequence analysis of the 5′ flanking region of α_{2u} shows no obvious homology with other glucocorticoid-inducible genes that have been sequenced.

A clue to the DNA sequences that may be involved in glucocorticoid regulation is provided by the transcriptional behavior of the variant α_{24} genes mentioned above. When these genes are transfected into L cells, they are transcribed at relatively high levels even with no hormone added. The level of α_{2u} mRNA produced by these variant genes is similar to the induced level seen for the inducible genes and is unaffected by glucocorticoid. This constitutive high phenomenon seems to be dependent on the 5′ end of the variant genes: An α_{2u}-*tk* hybrid gene was constructed using the 5′ promoter fragment from λ clone 211, one of the variant genes. This hybrid construct, when transfected into cells, results in constitutive high expression of *tk* mRNA, in the absence of hormones, in contrast to the results using the inducible promoter. (These studies were done in tk^- Rat 2 cells, which do not transcribe *tk* mRNA from the internal promoter mentioned above. All *tk* mRNA in these cells derives from the α_{2u} promoter.) The 5′ flanking sequence of λ clone 211, the constitutive promoter, is identical to the sequence of the inducible promoter from −230 until −88. From this point, there is a sequence of 11

bases in the inducible promoter (−88 to −77) that is absent from the constitutive promoter, where it is replaced by a sequence of five bases. At −76, the two sequences pick up again and are identical through the cap site into the mRNA coding region, with the exception of four bases at −49 to −46, which are altered.

This suggests the possibility that sequences between approximately −90 and −75 may be important in the hormonal response of the α_{2u} gene. Furthermore, the fact that the uninducible genes are transcribed at a constitutive high level, rather than simply uninducible and off, is consistent with a model in which steroid inducibility is the result of removal of a repression, i.e., the DNA sequences that modulate inducibility keep the gene off in the absence of hormones. One need not necessarily postulate a repressor protein that interacts with the DNA. The sequence itself may keep the chromatin in a transcriptionally inactive configuration until acted upon by the hormone-receptor complex. It may be of note that the sequences from −88 to −77 that are missing in the constitutive variant are part of a 9-bp direct repeat TGAGAAG$^{G}_{A}$A which recurs 23 bases downstream. A 10-base direct repeat occurs in approximately the same position in the promoter region of MMTV, another well-characterized dexamethasone-responsive gene. Site-specific mutagenesis in the 5′ promoter region will ultimately delineate which sequences are responsible for hormonal induction, and hopefully will provide information as to the nature of the induction.

Induction of α_{2u} by Other Hormones

Glucocorticoid hormones are only one of many inducers of α_{2u} globulin in vivo. Androgens, thyroid hormones, growth hormone, and insulin all increase α_{2u} levels in rat liver, and estrogens strongly repress α_{2u} synthesis. However, L cells, and most tissue culture cell lines used for gene-transfer experiments, lack receptors for these other hormones. Several cell lines have been described with appropriate receptors (MCF-7 cells have estrogen receptors, CHO cells have androgen receptors, and 10T½ cells have thyroid hormone receptors). A dominant-acting vector for gene-transfer experiments in tissue culture cells has been developed. The vector consists of the prokaryotic neomycin-resistance gene from Tn5, linked to the SV40 T antigen promoter, and followed by the SV40 small t splice region, and the large T poly(A) addition site. This vector has proven to be very successful in transforming several tissue culture cell lines (including those mentioned above) to resistance to the neomycin analog G418. Various α_{2u} genes have been inserted into these cell lines, and the effects of estrogen, thyroxine, and androgens are currently being assessed.

Publications

Kurtz, D.T., D.K. Bishop, and M.M. Manos. 1983. Hormonal control of rat α_{2u} globulin gene expression. *ICN-UCLA Symposium on the Evolution of Hormone Receptor Systems*. (In press.)

Kurtz, D.T., L. McCullough, D.K. Bishop, and M.M. Manos. 1983. DNA sequences required for hormonal induction of rat α_{2u} globulin genes. *Cold Spring Harbor Symp. Quant. Biol. 47*: 985.

GENES FOR THE MAJOR STRUCTURAL PROTEINS: ORGANIZATION AND CONTROL OF EXPRESSION

S.H. Hughes, J. Feramisco, *D. Helfman, E. Kosik, T. Kost, J. Sorge, G.P. Thomas*

Immunological Screening of cDNA Expression Libraries

Relatively straightforward methods have been established for the construction of essentially complete cDNA or genomic DNA libraries from many sources. Although such libraries usually contain many thousands of members, the major difficulty is the identification of clones of particular interest. Final identifications are usually made by sequencing the cDNA insert, by using the cDNA to select a specific mRNA for in vitro translation, or both. Such assays are not ordinarily suited to screening large libraries for individual clones, and steps are usually taken to enrich for the clones of interest. The most commonly used procedures rely on cDNA hybridization probes made from an mRNA preparation that contains high levels of the sequence of interest. Variations of this general approach include physical fractionation of the mRNA by sedimentation, gel electrophoresis, or immunoprecipitation of polysomes. Alternatively, differential hybridization can be used if two mRNA preparations are available that differ principally in their content of the mRNA of interest. These approaches are generally limited to the identification of genes that are expressed at moderate to high levels.

At least two new techniques have been developed that permit the identification of clones for genes that are expressed at low levels. If part or all of the amino acid sequence of a protein is known, it is possible to synthesize specific oligonucleotides for use either as hybridization probes themselves or as primers for the enzymatic synthesis of specific cDNA probes. This procedure is limited by the degeneracy of the genetic code. Other techniques require that the cDNA preparation, ideally made from total poly(A)$^+$ mRNA, be cloned into plasmids that promote expression of the cDNA in *Escherichia coli*. The resultant "expression libraries" are then screened for appropriate translation products using either a functional or an immunological assay. These protocols bypass the need for enrichment of the mRNA and provide promising approaches for the cloning of genes expressed at low levels.

We have developed procedures for constructing cDNA expression libraries from total poly(A)$^+$ mRNA, and the immunological screening of such libraries with specific antibodies to identify the gene products. These techniques improve upon previously described immunological screening methods in that the expression library is made directly from unfractionated poly(A)$^+$ mRNA and that the antibody-antigen complexes are detected on the same nitrocellulose filters used to grow the colonies. A cDNA expression library of about 100,000 total members has been constructed using chick smooth muscle poly(A)$^+$ RNA as a template. After screening with anti-tropomyosin antibodies, we isolated about a dozen independent bacterial clones that express proteins antigenically related to tropomyosin. DNA sequence analysis demonstrated that these cDNAs encode tropomyosin. One of the clones contains the entire coding region of tropomyosin and expresses this protein in *E.coli*.

The same library has been screened for α-actinin; colonies have been identified that (1) react specifically with two independent actinin antisera and (2) produce a novel protein that can be immunoprecipitated with actinin antisera. Since the α-actinin protein has not yet been sequenced, DNA sequence data is not helpful in the identification of the clones; selection of mRNA and translation into protein will be necessary.

Construction of a cDNA Expression Library

Kurtz and Nicodemus have described a method for cDNA cloning in which two different oligonucleotide linkers, *Eco*RI and *Sal*I, are simultaneously ligated to the blunt-ended cDNA. After the linkers are digested with appropriate restriction enzymes, the cDNA can be ligated to an *Eco*RI–*Sal*I-cut plasmid vector which, if the insert is physically removed, cannot ligate to itself. This highly efficient method permits the use of an excess of vector and yields libraries in which greater than 95% of the bacterial clones contain cDNA inserts. However, with the simultaneous ligation of both linkers, one expects that at least 50% of the cDNA molecules will have the same linker at each end and therefore cannot be cloned in a double-cut vector. In practice, we find that the *Sal*I linkers ligate more rapidly than the *Eco*RI linkers so that greater than 50% of the cDNAs have homogeneous ends. John Fiddes and Doug Hanahan (Structure and Expression of Eukaryotic Genes Section) have modified the procedure to overcome this problem. One set of linkers is ligated to the end of cDNA corresponding to the 3′ end of the poly(A)$^+$ RNA before cleavage of the hairpin loop with S1 nuclease (see Fig. 1). Following the S1 reaction, the second set of linkers is ligated to the end of the cDNA that previously was protected by the hairpin loop. This greatly increases the percentage of cDNA molecules with different linkers at each end.

If the linkers are chosen appropriately, the cDNA can then be ligated to a plasmid expression vector that contains both a transcriptional and a translational start site. The sequential addition of linkers permits the insertion of the cDNA in a defined

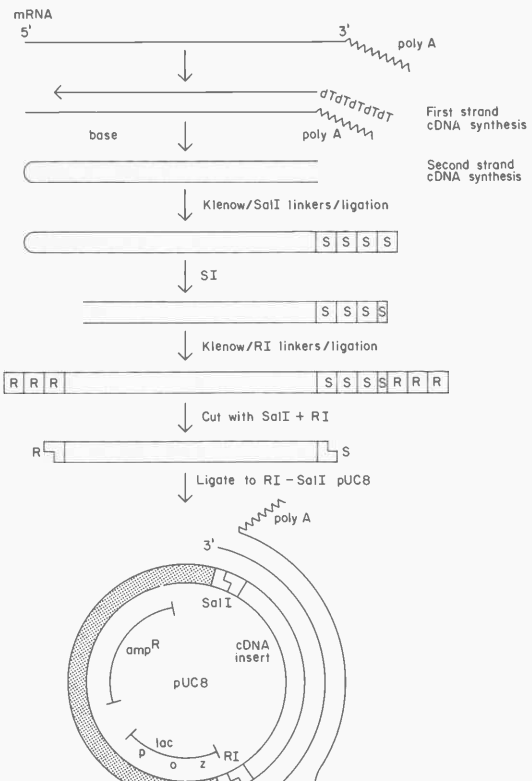


Figure 1

Construction of a cDNA expression library. At the top of the panel is shown the poly(A)$^+$ RNA, which is copied into single-stranded cDNA by reverse transcriptase, employing an oligo(dT) primer. The template RNA is degraded with base, and the second strand synthesized, generating a double-stranded DNA hairpin. The open end of the hairpin is filled in with the Klenow fragment of *E. coli* DNA polymerase I and ligated to *Sal*I linkers (S). The hairpin loop is destroyed with S1, and the ends of the resulting double-stranded cDNA are again filled in with the Klenow fragment of *E. coli* DNA polymerase I and ligated to *Eco*RI linkers (R). After digestion with *Eco*RI and *Sal*I, the double-stranded cDNA has a *Sal*I site at the end that corresponds to the 3′ end of the template RNA and an *Eco*RI site at the end that corresponds to the 5′ end of the template RNA. This cDNA is ligated to the larger *Sal*I–*Eco*RI fragment of pUC8. In such constructions, the transcript made in *E. coli* has the same polarity as the template mRNA.

orientation (Fig. 1). Under these conditions, approximately one bacterial clone in three should have a cDNA insert capable of expressing at least a portion of the protein the template mRNA normally translates.

This protocol has one drawback. Some cDNAs

will contain restriction sites that are the same as those in the linkers. This will give either an unclonable DNA insert or an insert that is always cloned in the same, possibly incorrect, translational frame. The pUC8/pUC9 plasmid expression vector system has been used in an attempt to overcome this problem. Both of these vectors have a polylinker with multiple cloning sites inserted in the aminoterminal region of the *E. coli lacZ* gene but differ in the orientation of the polylinker. It is therefore possible to take half of a cDNA preparation and add linkers in one temporal order and clone in the appropriate orientation in pUC8, then take the other half of the same cDNA preparation, add the same set of linkers in the opposite order, and clone in the correct orientation in pUC9. This will overcome most of the problems caused by internal restriction sites in the cDNA. We can also block cleavage of the cDNA with the appropriate methylases or use two nonoverlapping sites of linkers.

Figure 2 shows, in schematic form, the steps in antibody screening. The transformed *E.coli* are plated on detergent-free nitrocellulose sheets laid onto ampicillin plates. After the colonies reach 1–2 mm in diameter, replicas are made and the clones regrown either on selective media for immunological and cDNA screening or on glycerol plates for

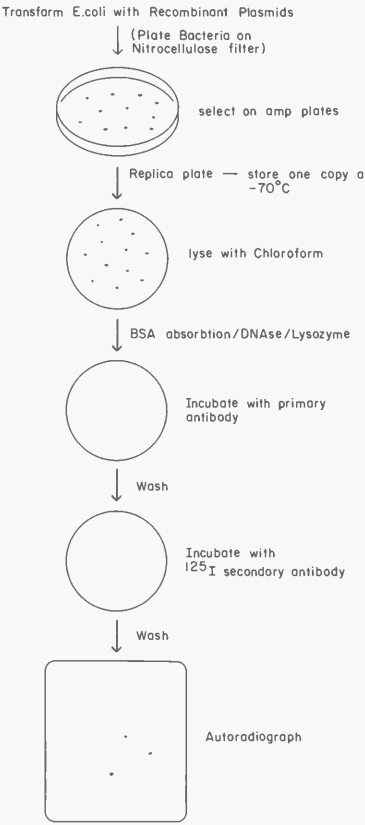


Figure 2
Immunological screening of a cDNA expression library. The transformed *E. coli* are plated on detergent-free nitrocellulose sheets on ampicillin plates. After the colonies grow up, replicas of the original filter are made and the clones grown up either on selective media for immunological and cDNA screening or grown up on glycerol plates for long-term storage at −70°. For the immunological screening, one of the replica filters is suspended in chloroform vapor to lyse the cells; then the filter is treated with a mixture of BSA, DNase, and lysozyme. The filter is then incubated with a specific antiserum, in our case rabbit anti-tropomyosin serum, then washed and incubated with an ^{125}I-labeled second antibody (goat anti-rabbit IgG). After washing, the appropriate clones are identified by autoradiography.

long-term storage at − 70°C. For the immunological screening, one of the replica filters is suspended in chloroform vapor to lyse the cells, then the filter is treated with a mixture of bovine serum albumin, DNase, and lysozyme. The filter is then incubated with a specific antiserum (we used rabbit anti-tropomyosin serum to isolate the tropomyosin clones), then washed and incubated with an ^{125}I-labeled second antibody (goat anti-rabbit IgG). After washing, the appropriate clones are identified by autoradiography.

One potential problem is the synthesis of a protein product lethal to *E. coli*. Fortunately, all that is required in the immunological screening procedure is that an antigenic fragment be produced. In the tropomyosin screening experiments, we successfully detected a recombinant making a protein that is less than 50% of the full-sized tropomyosin protein (containing only 133 of the 284 tropomyosin amino acids). It seems likely that in many cases where a complete or nearly complete protein would be lethal to *E. coli*, an antigenically active fragment would not. For example, Mary Jane Gething and her collaborators demonstrated that synthesis of a complete copy of influenza hemagglutinin is apparently lethal to *E. coli*. However, if the DNA sequences coding for the aminoterminal hydrophobic signal sequence are deleted, and the hemagglutinin DNA is inserted in the correct translational reading frame into a plasmid expression vector, these *E. coli* synthesize a protein antigenically related to flu hemagglutinin. Mary Jane Gething supplied us with such clones, and we have used our immunological screening technique to detect specifically the *E. coli* clones making the hemagglutinin-related protein. This argues for the generality of our protocol in two ways. Because the flu hemagglutinin is unrelated to tropomyosin, it is likely that the protocol will work with many different genes. In addition, we are able to detect clones that synthesize nonlethal proteins antigenically related to flu hemagglutinin. Even if there are problems with lethal synthesis of proteins in some cases, this problem can be overcome using inducible expression vectors. The pUC8/pUC9 vectors contain the *lacZ* operator/promoter region; expression of inserts introduced into these plasmids is inducible in an *E. coli* I^{q} strain.

We do not have a precise assessment of the sensitivity of the protocol. Clearly, the bacterial clones we have successfully detected make large amounts of the novel protein. It is likely, however, that the antisera will be the most variable (and crucial) reagent. From tests with purified proteins spotted onto nitrocellulose, the sensitivity depends critically on the quality of the antisera used. Polyclonal sera have been used in the tropomyosin α-actinin, and flu hemagglutinin experiments. A single monoclonal antibody might specifically detect a site near the amino terminus of the protein, which would mean only a small fraction of the appropriate cDNA clones would make an antigen that a particular monoclonal antibody would detect. However a mixture of several monoclonal antibodies recognizing antigenic determinants throughout the length of the protein could be substituted for polyclonal sera.

Avian Retrovirus Vectors

We have been modifying cloned copies of retroviral DNA with two specific goals: (1) to try and take advantage of the unique properties of retrovirus in the construction of vectors and (2) to learn more about the way retroviruses work. Of the known viruses that infect higher eukaryotes, only retroviruses act as vectors in nature. Retroviruses are nonlytic and when not carrying an oncogene have little or no effect on the growth or behavior of cells in culture. The viral genome associates stably with the host cell; as a requisite part of the virus life cycle, one or a few copies of the viral genome are integrated into host DNA. The infection is self-limiting; the production of viral proteins by the infected cell blocks viral receptors on the cell surface—infected cells are resistant to reinfection. Replication-competent retroviruses are relatively small. The prototype viruses are about 7.5 kb long, making the cloning and manipulation of the viral genomes reasonably straightforward. The efficiency of infection is high; an infection of cultured cells can be initiated by transfecting cloned viral DNA into susceptible cells, and in a week or two essentially every cell in the culture will be infected. On rare occasions, retroviruses acquire cellular oncogenes and cause them to be expressed at moderate to high levels. In most cases, the acquisition of the oncogene occurs concomitantly with the loss of one or more viral genes. There is one exception: Rous sarcoma virus (RSV) has aquired an oncogene, *src*, without losing any viral information.

RSV is therefore a natural helper-independent retrovirus vector, and it serves as the basis for our vector constructions. RSV was chosen because we could, by suitable manipulations, remove the *src* gene and replace it with any sequence we chose. The resulting constructions would be helper independent; there would be no requirement for selectable markers, and growth of the virus would be sufficient to ensure the propagation of any sequence inserted into the vector.

To exploit the virus as a vector successfully, it is necessary to understand the relationship between viral sequence and function. Over the past 2 years, we have made a variety of defined deletion mutants by manipulation of a cloned copy of the RSV genome (see Fig. 3). The RSV genome contains no site for the restriction enzyme *Cla*I, and the end points of the deletions were all created by ligation to synthetic *Cla*I linkers. In all cases, the exact end points of the deletions have been determined by DNA sequencing. In addition to facilitating the construction of a series of defined deletion mutants, the *Cla*I site can be used as the point of insertion of a variety of foreign DNAs.

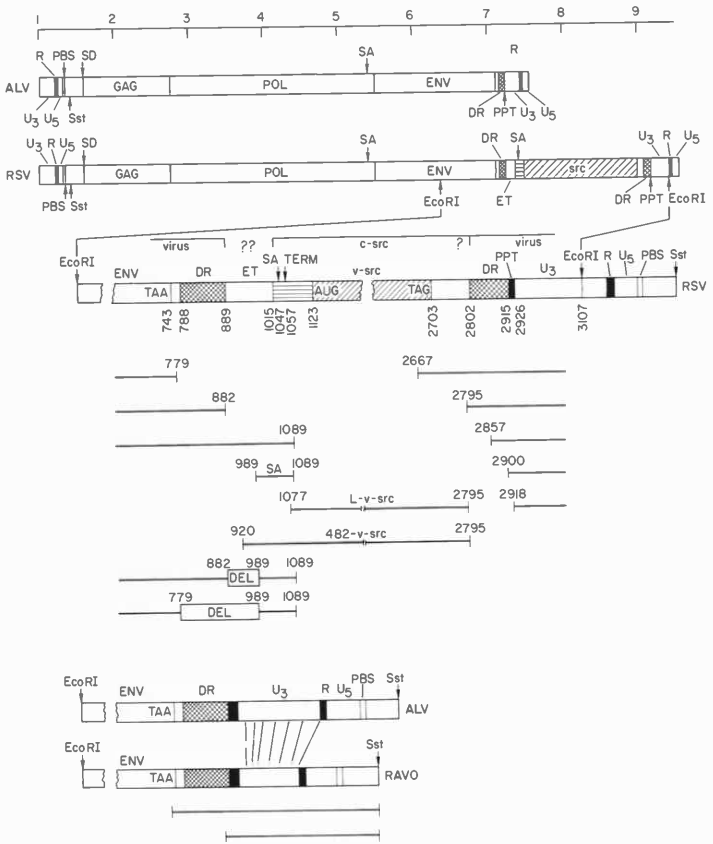


Figure 3

Helper-independent avian retrovirus vector constructions. At the top of the drawing is a scale in kilobases. Below this scale are drawings of an avian leukosis virus (ALV) and Rous sarcoma virus (RSV). The four genes (*gag*, *pol*, *env*, and *src*) are labeled on the drawings. Important regions and sites are also given: U_3, R, and U_5 in the LTRs; the primer binding site (PBS); the splice donor (SD) just inside gag; the two splice acceptors (SA) for *env* and *src* messages; the direct repeat (DR) present once in ALV and twice in RSV; the sequences of unknown origin near *src* (E.T.); and the polypurine trace adjacent to the LTR (PPT). The *Sst*I site just past the left-hand LTR and two of the four *Eco*RI sites, those flanking *src*, are also marked. The region between the *Eco*RI sites is shown on an expanded scale under the drawing of RSV. The numbers denote the distance from the *Eco*RI site in *env*. The position of a series of defined deletion mutants, generated by the addition of *Cla*I linkers is given below. Two *src*-containing fragments flanked by synthetic *Cla*I linkers; L-v-*src* (which lacks the splice acceptor) and 482-v-*src* (which contains the SA), have also been generated. Similarly, a small fragment (SA) has been made with an *Xho*I linker at 1089. This fragment has been used to generate double-deletion mutants. At the bottom of the figure is a schematic representation of the relationship of the LTRs and adjacent regions of an ALV virus and the endogenous virus RAV-0. Two fragments containing the RAV-0 LTR were created that extend from the natural *Sst* site just past the LTR (these constructions are done with the circular form of viral DNA) to a synthetic *Cla*I linker lying just past the DR or just past the PPT. These fragments were used, together with the 779 and the 882 deletions, to replace the ALV-RSV LTR region with a RAV-0 LTR.

Functions of the Noncoding Regions between *env* and the Long Terminal Repeat of RSV

The noncoding sequences adjacent to the *src* coding region are derived from at least three sources: (1) the avian leukosis virus (ALV) that acquired *src*, (2) the region of the host (probably chicken) genome that contains c-*src*, and (3) one segment (E.T.) of unknown origin. Figure 3 shows the arrangement of these segments in the RSV strain that we use, the Schmidt-Ruppin A (SRA) strain. The same sequences are present, although in a different arrangement, in the Prague C strain. Our analysis has demonstrated that at least two of the noncoding segments between *env* and the long terminal repeat (LTR) are required for viral replication, and that a third segment, unnecessary for replication, is required for expression of *src*, and presumably for other inserts as well.

Obviously only those sequences present in the parental ALV virus are needed for replication (see Fig. 3). There are two functionally separate segments we can identify: (1) the polypurine tract (PPT) that lies immediately adjacent to the LTR and (2) the direct repeat (DR) present in two copies flanking *src* in RSV and present only once in ALVs (see Fig. 3). The PPT is absolutely required for replication; viral genomes lacking this segment do not replicate and cannot be rescued by a helper virus (Sorge and Hughes, *J. Virol. 43*: 482 [1982a]). It is likely that these mutants cannot properly initiate plus-strand DNA synthesis; this would explain the *cis*-dominant phenotype of the mutants. The boundary is both sharp and absolute. Mutants deleted from position 884 or 1089 to position 2900 replicate normally; if the deletion is extended to position 2918, replication is completely abolished.

At least one copy of the DR is required as well. Virus variants that contain the PPT but no DR (deletions extending from 779 to 2900) do not replicate, even though the PPT is present. Such variants are not viable; even though RNA is synthesized and spliced, RNA from these variants does not appear in virions, even in the presence of a helper. Although the two direct repeats differ slightly in sequence, both functional deletion variants containing either the upstream copy (884–2900 and 1090–2900) or the downstream copy (779–2667 and 779–2795) replicate normally.

Although the noncoding sequences near *src* that are not of viral origin are not involved in replication, at least one plays a crucial role in *src* expression. *src* mRNA is created from a full-length transcript by splicing. Sequences just beyond the *gag* ATG are joined to sequences just upstream of the *src* coding region (see Fig. 3). Deletion of sequences just upstream of *src* (884–1077) allows normal viral replication; *src* expression is abolished. It is unlikely that the manipulations alone have a deleterious effect; a similar construction (1090–1077) that causes a small duplication expresses *src* normally. What function, if any, do the mysterious E.T. sequences have? We are currently testing a series of new deletions (779–989 and 882–989) in hopes of answering this question.

We also hope to be able to answer another interesting question. All the known RSV messages are apparently derived from a single full-length precursor. The subgenomic messages are derived from this precursor by splicing; however, even the subgenomic *env* and *src* messages contain the AUG at which *gag* translation initates, and the splice donor site lies just past the sixth *gag* codon. Both *env* and *gag-pol* messages appear to initiate translation at the *gag* AUG (see Fig. 3). By contrast, the *src* translation begins with a *src*-encoded AUG about 80 bp past the splice acceptor (SA) site in *src*. How is this possible? It is likely that the termination codon, TAG, located 4 codons past the *src* SA, plays a key role in allowing translation from the *src* AUG. We have subcloned the splice acceptor on a 100-bp fragment, and will soon modify the TAG termination codon by in vitro mutagenesis. This experiment will not only answer an important question in viral function; it may also permit us to express gene fragments cloned into vectors deleted for *src* using the *gag* AUG as the point of translation initiation.

Introduction of Foreign DNA into Retrovirus Vectors

One of our major goals is to use retroviruses to control the expression of a variety of genes, especially those of the cytoskeleton. To this end, we have introduced several new genes into the *src* deletion mutants described in the previous section.

The first major problem we encountered was stability. This is hardly surprising since the parent virus, RSV, is itself unstable. The major source of instability appears to be the DR sequences that flank *src*. At high frequency, there is recombination between these repeats; the recombination event eliminates the *src* sequences and gives rise to an ALV virus (see Fig. 3). Our initial constructions, which had inserts introduced into the 884–2667 or into the 1089–2667 deletions were, like RSV, unstable. To enhance stability, either the upstream or the downstream direct repeats could be eliminated. However the position of the SA (at 1057) makes elimination of the downstream direct repeat easier. Constructions with inserts into the 1089–2900 deletion are much more stable; however a small repeat (11 bp) is retained. For this reason, we have subcloned the SA site (989–1089) and are now constructing double-deletion mutants (779–989 plus 1089–2795, see Fig. 3) that retain the SA but completely eliminate the upstream DR. Other constructions of this type (884–989) are being used to probe for the possibility that the E.T. sequences play a role in *src* expression. We would like to eliminate all unnecessary sequences because the total size of the virus is strongly constrained. Constructions much larger than 10 kb do not grow, and if growth is forced,

deleted derivatives are obtained.

There are several potential uses of retrovirus vectors. One is as a tool for controlling the expression of genes; genes can be introduced into a retrovirus in such a fashion that their expression is dependent on the retroviral promoter. Another potential use would take advantage of the unique ability of a retrovirus, through its alternation of life cycles between DNA and RNA, to remove precisely the intervening sequences from genomic DNA, creating cDNA in vivo.

The most direct test of expression with the helper-independent retrovirus vectors has been the ability of constructions carrying the *E. coli* neomycin resistance gene to make chicken cells resistant to the drug G418. A construction was made in which the *neo* gene was inserted, in the proper orientation, into the deletion mutant 1089–2900. Chicken cells were infected under nonselective conditions and the virus allowed to spread through the culture. The cells were completely resistant to G418 challenge; uninfected cells were completely susceptible. We have also modified the *src* gene itself as a test insert (see Fig. 3). Tests for expression of these two inserts, *src* and *neo*, are trivial; vector constructions can be tested rapidly and easily. Once appropriate vector constructions are made, we will attempt to insert and express cytoskeletal genes.

The second set of experiments involves making a vector for the efficient conversion of genomic clones to cDNA clones both for use in the helper-independent retrovirus vector and for expression in *E. coli*. It would be helpful if the retrovirus were also a shuttle vector and carried plasmid sequences that would render the vector capable of growth in both *E. coli* and chicken cells. Our initial attempts with such vectors—vectors carrying a small plasmid inserted into the 889–2667 deletion—were successful; that is, virus was rescued into *E. coli*, albeit at very low frequency (D. Hanahan and S. Hughes, unpubl.). However, such constructions are quite unstable in chicken cells. Furthermore, since RSV is strongly size constrained, there is no room for additional sequences in these constructions. We are therefore attempting to build helper-dependent vectors, basing the selection for the defective vector virus on transformation by *src*. To leave as much room as possible, we are trying to determine how much of the 5′ region of the viral genome must be retained to have the virus replicate in the presence of a helper.

In Vivo Vectors and Activation of Endogeneous Oncogenes

RAV-0 is an endogeneous virus of chickens. By several criteria, including sequence (Hughes, *J. Virol. 43*: 191 [1982]), it is closely related to the ALV viruses. However, RAV-0 causes no apparent disease; by contrast, the related ALV viruses cause lymphoid leukosis. Two simple and several more complex explanations are possible. The ALV viruses activate the endogenous oncogene *c-myc* when they integrate nearby; either RAV-0 does not integrate next to *c-myc* or, alternatively, if it does integrate next to *c-myc*, it does not activate *c-myc*. Logic dictates that some difference in the RAV-0 and ALV viral genomes makes ALV oncogenic and RAV-0 not. If the ALV and RAV-0 genomes are compared, the major differences in sequence reside in the U_3 region of the LTR. It appears as if the RAV-0 U_3 was derived from the ALV U_3 by a series of small deletions (or, alternatively, as if the ALV U_3 was derived from the RAV-0 U_3 by a series of small insertions). Since the U_3 region is intimately involved in integration and transcription, these changes could explain the differences in oncogenicity between RAV-0 and ALV. To demonstrate the involvement of the LTR region, we have substituted the LTR of some of the *src* deletion mutants (which cause lymphoid leukosis) with the RAV-0 LTR.

A series of such recombinants have been made based on the 779 and 889 deletions. Such deletions were linked to RAV-0 fragments that do and do not contain the direct repeat region from RAV-0 (see Fig. 3). These recombinant viruses, which do not contain any coding region from RAV-0, are being tested for oncogenicity in vivo by Lyman Crittenden. If, as we expect, the ALV viruses that contain the RAV-0 LTR are not oncogenic in vivo, they will serve as the prototype in vivo vectors. It is possible that such vectors could be used to introduce sequences into the germ line as well as into somatic tissue.

The Organization of the Chicken β-Actin Gene

There is a single β-actin gene in chickens; this gene encodes a major cytoskeletal protein in nonmuscle cells. We have isolated two genomic clones that encode this entire gene by a direct screen of a chicken genomic library (Hughes et al., *Cold Spring Harbor Symp. Quant. Biol. 46*: 507 [1982]). Both of these clones, originally isolated as bacteriophage λ recombinants, have been subcloned into pBR322; both appear to contain a complete copy of chicken β-actin and adjacent sequences. The coding region of chicken β-actin is interrupted by four intervening sequences. A precise map of this region is given in Figure 4. This entire region has been sequenced, and the 3′ untranslated region as well. A comparison of the sequence of the genomic clone, with the 3′ untranslated region of two β-actin cDNA clones derived by D. Cleveland locates the poly(A) addition site. There is a very slight heterogenicity in poly(A) addition; one of the two cDNA clones has two additional base pairs before the poly(A) stretch.

The start of transcription has been determined by primer extension on β-actin mRNA, and lies about 1000 bp upstream of the AUG. Electron microscopy (Hughes et al., *Cold Spring Harbor Symp. Quant. Biol. 46*: 507 [1982]) does not reveal additional in-

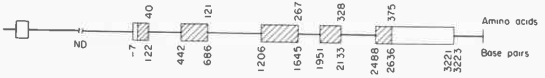


Figure 4
Organization of the chicken β-actin gene. The drawing depicts the single genomic copy of chicken β-actin. Exons are shown as boxes, introns as thin lines; the coding region is cross-hatched. Base pair coordinates are given from the AUG codon used to initiate translation. There is a 3′ untranslated region of about 600 bp. The site of polv(A) addition is heterogeneous; two sites (3221, 3223) 2 bp apart are used. Transcription begins about 1 kb upstream of the AUG. The region that includes sites at which translation is initiated has been sequenced; part of the first intervening sequence has not (marked ND).

tervening sequences. However a comparison of the sequences in the corresponding regions upstream of the AUG in the genomic clone and in a full-length β-actin cDNA clone (provided by D. Cleveland) suggests that there is a fifth intervening sequence in the 5′ untranslated region. Hybridization of the 5′ untranslated region of the cDNA clone to the genomic clones places the start of transcription within 1.2 kb of the AUG. The transcriptional start site has been located about 1000 bp upstream of the β-actin AUG; there is a fifth intervening sequence of about 900 bp in the 5′ untranslated region of the chicken β-actin gene. This large intervening sequence will permit the easy separation of the coding region and the promoter for β-actin, making it relatively easy to manipulate both the gene and its promoter.

Publications

Helfman, D.M., J.R. Feramisco, J.C. Fiddes, G.P. Thomas, and S. Hughes. 1983. Identification of clones that encode chicken tropomyosin by direct immunological screening of a cDNA expression library. *Proc. Natl. Acad. Sci. 80:* 31.

Hughes, S.H. 1982. Sequence of the LTR and adjacent regions of the endogenous avian virus RAV-0. *J. Virol. 43:* 191.

——. 1982. Synthesis, integration and transcription of the retrovirus provirus (Review). *Curr. Top. Microbiol. Immunol. 103:* (in press).

Hughes, S.H., J. Sorge, J.P. Thomas, J. Feramisco, L. Chow, and J. Garrels. 1982. Approaches for isolating genes encoding the cytoskeletal proteins and for reintroducing the genes into cultured cells. *Cold Spring Harbor Symp. Quant. Biol. 46:* 507.

Sorge, J. and S.H. Hughes. 1982. The polypurine tract adjacent to the U_3 region of the Rous sarcoma virus genome provides a cis-acting function. *J. Virol. 43:* 482.

——. 1982. The splicing of intervening sequences introduced into an infectious retrovirus vector. *J. Mol. Appl. Genet. 1:* 547.

STRUCTURE AND EXPRESSION OF EUKARYOTIC GENES

J.C. Fiddes, *W.R. Boorstein, M. DeLuca, D. Hanahan, W. Huse, K.D. Talmadge, and N.C. Vamvakopoulos*

Glycoprotein Hormone Genes

J.C. Fiddes, W.R. Boorstein, M. DeLuca, W. Huse, K.D. Talmadge, and N.C. Vamvakopoulos

The four glycoprotein hormones—chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid stimulating hormone (TSH)—play a major role in regulating reproduction (LH, FSH, and CG) and thyroid function (TSH). Three of the hormones, LH, FSH, and TSH, are synthesized in the anterior pituitary whereas the fourth, CG, is made in the placenta. In the female, a surge of pituitary LH stimulates the ovary to produce the steroids necessary for ovulation. During pregnancy, CG functions in a very similar way to LH in that it stimulates the corpus luteum of the ovary to produce the steroids necessary for the maintenance of pregnancy. This functional similarity between CG and LH is reflected in their close structural resemblance.

All four glycoprotein hormones are dimeric, consisting of dissimilar α and β subunits that are associated noncovalently. The α subunit is common to all four hormones whereas the β subunits are unique and give biological specificity to the hormone. The β subunits show sequence homologies to each other, indicating that they form a multigene family. The highest homology (82%) is observed between the β subunits of CG and LH. All other β subunits, when compared in pairs, have amino acid sequence homologies ranging from about 25% to 40%.

Previously, we have isolated and characterized cDNA clones for both the α and β subunits of CG. The α subunit was then used as a probe to isolate the α-subunit genes from a bacteriophage λ library. Comparison of the structure of the cloned α-subunit gene with the hybridizing fragments detected in total human DNA showed that there is a single copy of the α-subunit gene. This is expressed in the pituitary to make the α subunit for LH, FSH, and TSH and in the placenta to make the α subunit for CG.

More recently, we have been examining the structure of the β-subunit genes for two of the hormones, CG and LH. The βCG cDNA was used to isolate genomic recombinants from two independent human chromosomal libraries, one in bacteriophage Charon 4A and one in bacteriophage λ Charon 28. We purified a total of 16 hybridizing phage and determined restriction enzyme maps. We thus identified three different groups of overlapping recombinants, as shown in Figure 1. Eight separate regions that hybridize to the βCG cDNA probe were identified in these groups of phage and are shown in Figure 1. Detailed restriction enzyme mapping and DNA sequence analysis showed that seven of the genes, labeled 1–3 and 5–8 on Figure 1, are βCG genes or pseudogenes, whereas one of the genes, labeled 4 on Figure 1, codes for βLH.

The genes show an unusual structural arrangement. Four of the genes, β1 and β2 and β5 and β6, are found in inverted pairs with the 3′ ends separated by about 2.2 kb. In contrast to these, β3 and β4 and β7 and β8 form tandem gene pairs.

To determine whether these seven βCG genes and the single βLH gene represent all the CG and LH genes present in the human genome, we analyzed several different digests of human placental DNA by filter hybridization, using both βCG and βLH fragments as probes. Fragments corresponding to all eight genes were identified in this way and no additional fragments were detected. Thus, it appears that the human genome has a total of seven genes, or pseudogenes, coding for the β subunit of CG and a single gene coding for the β subunit of LH.

The significance of the complex organization of the β-subunit gene is not obvious. Examination of the distribution of restriction enzyme sites around the genes reveals extensive regions of repeats. For example, the positions of the *Kpn*I, *Xba*I, and *Xho*I sites preceding β3 and β8 are identical (Fig. 1). The *Kpn*I site is almost 5 kb upstream from the proposed transcriptional start. The inverted and tandem pairs presumably arose from a complicated series of duplications and inversions encompassing an area considerably larger than the coding regions themselves. It is interesting to note that βLH, which appears to be coded for by a single gene, is a much older gene in evolutionary terms than βCG, which is coded for by a multigene family. Presumably βCG evolved from βLH prior to the gene duplication and rearrangement.

Although it is not known to which chromosome the β subunit of CG maps, we have some evidence for the linkage of all the βCG hybridizing fragments. A single hybridizing band of 50–60 kb is observed in filter hybridizations of human DNA digested with either *Eco*RI or *Sal*I. However, despite this suggestive evidence that the genes are all linked, extensive screening of two distinct libraries failed to produce an overlapping series of sequences linking the seven βCG genes and the one βLH gene.

DNA sequencing and restriction enzyme mapping reveal that all of the genes have the same basic structure. There are two introns that separate codon -16 of the signal peptide from codon -15, and codon 41 of the mature protein from codon 42. The two introns, A and B, are ~ 350 and ~ 230 nucleotides long, respectively. We have almost established the complete nucleotide sequence of two of the βCG genes, 5 and 6, and the single βLH gene, 4. The two βCG genes are very similar, with an overall nucleotide sequence homology of greater than 95%. Most of the differences are in the introns and

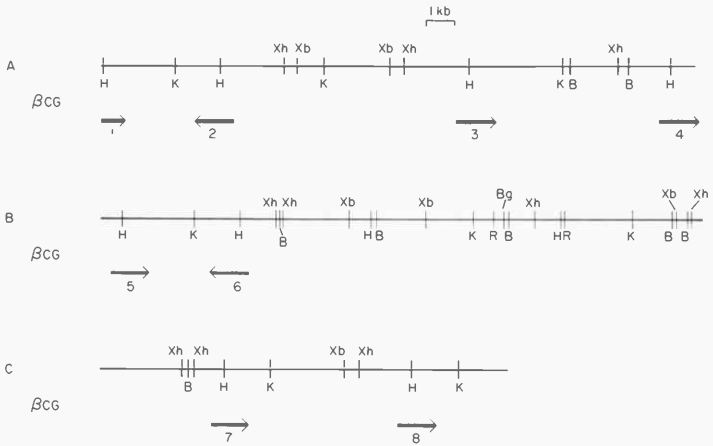


Figure 1

Restriction enzyme maps of the three groups of genomic isolates (*A*, *B*, C). The positions of the eight genes (1–8) are indicated by arrows that point in the proposed direction of transcription. Restriction enzyme analysis and DNA sequencing has shown that genes 1–3 and 5–8 code for βCG whereas gene 4 codes for βLH. Each gene spans 1.45 kb from the cap site to the poly(A) site.

in the 350-nucleotide-long 5′ untranslated region. The βLH gene is also very closely related to the βCG genes. The nucleotide sequence homology is about 95% whereas the amino acid sequence homology is about 80%. Very few silent third-position changes are observed.

Detailed comparison of the restriction enzyme maps of all eight genes shows that they are very similar, although no two genes are identical. We have used the slight differences between the genes to study their expression. One of the genes, β3, is identified by a single silent third-position change that generates a new restriction enzyme site. Analysis of placental cDNA clones with this restriction enzyme showed that two out of 15 cDNAs contained the new site, showing that β3 was expressed.

Another difference, this time between genes 5 and 6, was detected by sequencing. In this case, the single nucleotide change alters the coding potential of the gene. Gene β5 has a codon for Asp at position 126, whereas in gene β6 the corresponding codon is for Ala. The sequence containing Asp corresponds to the published amino acid sequence and to the previously determined cDNA sequence. Analysis of all seven βCG genes shows that genes 1, 3, and 5 appear to have Asp at this position whereas genes 2, 6, 7, and 8 have Ala. None of the cDNA clones that we have analyzed has Ala. This evidence suggests that the actively expressed βCG genes are numbers 1, 3, and 5.

We have also made constructions of genes βCG 2, 3, and 5–8 to examine their transient expression in COS cells (the 5′ end of βCG 1 has not been cloned). RNA was isolated from the transfected cells and analyzed with S1 nuclease to establish correct initiation of the βCG genes directed by their own promoters. In these experiments, we have detected correct initiation of genes 3 and 5 but can detect no initiation from genes 2, 6, 7, and 8. The βLH gene (4) has not been studied in this way. The transient expression results are therefore consistent with the analysis of the cDNA clones and suggest that not all of the βCG genes are expressible despite their strong structural similarity.

We are now attempting to use oligonucleotide probes to isolate the β-subunit genes for both TSH and FSH to establish whether they are linked to the β subunits of CG and LH and to see if they are coded for by single or multiple genes.

Isolation of cDNA and Genomic Clones for the Hypothalamic Releasing Factor, gnRH

J.C. Fiddes, W.R. Boorstein, and M. DeLuca

The release, and probably the synthesis, of the pituitary glycoprotein hormones LH and FSH is controlled by the hypothalamic peptide, gonadotropin-releasing hormone (gnRH). The sequence of this 10-amino-acid-long peptide has been established previously. In collaboration with James Roberts and

Marion Evinger (Columbia University) and Alex Markham (ICI, England), we have been trying to use oligonucleotide probes synthesized to correspond to the amino acid sequence to isolate the gene coding for gnRH. We are interested in isolating this gene to establish whether gnRH, in common with other small polypeptide hormones, is made initially in a larger precursor form, and to provide a probe for studying its synthesis in the brain.

Two approaches have been taken in our attempt to isolate sequences coding for gnRH. In one, we have made cDNA libraries from both rat hypothalamic and human placental mRNA and screened them using oligonucleotide hybridization probes. This has not resulted in the identification of any recombinants that positively hybridize to the oligonucleotides, possibly because of the very low abundance of gnRH mRNA. In the second approach, we have used the oligonucleotide probes to screen directly both rat and human genomic libraries in bacteriophage λ vectors. This approach avoids the need to make very large cDNA libraries. Approximately 50 positively hybridizing bacteriophage λ recombinants have been isolated from the two libraries by this approach. We are now in the process of subcloning these phage λ recombinants into M13 vectors to identify by DNA sequencing which, if any, of them contain sequences coding for gnRH.

cDNA Cloning Methods

J.C. Fiddes and D. Hanahan

We have developed a directional cDNA cloning method that is very efficient and is well suited to the construction of cDNA expression libraries. This procedure is a modification of the double-linker method of Kurtz and Nicodemus (*Gene 13:* 145 [1981]). Instead of ligating cDNA to a single set of oligonucleotide linkers, as had been done previously, they used a mixture of two different sets of linkers. Double-stranded cDNA, following digestion of the hairpin loop with S1 nuclease, was ligated to a mixture of *Eco*I and *Sal*I linkers, and the resulting products were ligated to a *Sal*I–*Eco*RI vector prepared from pBR322 DNA. This considerably increased the efficiency of cDNA cloning because the problems of self-ligation of the vector were largely eliminated.

In theory, this approach should result in 50% of the cDNAs with nonhomologous ends and the other 50% of the cDNAs with homologous ends that therefore cannot be cloned into a double-cut vector. In practice, we find that considerably less than 50% of the cDNA molecules have heterogeneous ends, due to appreciable differences in the rates of ligation of the *Eco*RI and *Sal*I linkers. To overcome this problem, we have modified the procedure to ensure that the cDNA molecules all have mixed linker ends. Double-stranded cDNA is synthesized by standard methods, and the hairpin loop at the end corresponding to the 5′ end of the mRNA is left intact. One set of oligonucleotide linkers is then ligated to this cDNA. These linkers will ligate only to the end of the cDNA corresponding to the poly(A) tail because the hairpin loop will block ligation to the other end. The hairpin loop is then cleaved with nuclease S1 and a second set of linkers is ligated to the cDNA. The cDNA is then digested with the restriction enzymes corresponding to both sets of linkers, which results in cDNA molecules with mixed linker ends of defined orientation. These molecules can then be cloned with very high efficiency into double-cut plasmid vectors.

One advantage of adding the linkers sequentially is that the orientation of the cDNA within the vector is known. As well as being very useful in the analysis of the cDNA clones, this advantage also permits the method to be used for the construction of expression libraries that can be screened immunologically. In collaboration with D. Helfman, J. Feramisco, P. Thomas, and S. Hughes, we have used the sequential linker method to make cDNA libraries from chicken smooth muscle in the expression vectors pUC8 and pUC9. These have been screened with antibodies against tropomyosin, and several tropomyosin cDNA clones have been isolated and characterized.

Isolation of cDNA Clones for the Sodium Channel

J.C. Fiddes and W. Huse

The sodium channel is a transmembrane protein essential for nerve and muscle excitation. This channel has been isolated from the electric organ of the eel, *Electrophorus electrocus*, and partially characterized. The protein consists of a large polypeptide of molecular weight <250 kD that probably forms the sodium-selective pore, and possibly smaller subunits. Using polyadenylated mRNA isolated from the electric organ, we have made cDNA expression libraries in the vectors pUC8 and pUC9. These have been screened immunologically with antibodies, provided by William Agnew, directed against the sodium channel large polypeptide. Ten recombinants that reacted positively with the antibodies have been identified and purified. We are now in the process of trying to establish that these recombinants contain sequences that code for the sodium channel. As no amino acid sequence information is known for the sodium channel, this identification is being made by mRNA selection and by characterization of the protein products made by the recombinants in *Escherichia coli*.

Publications

Boorstein, W.R., N.C. Vamvakopoulos, and J.C. Fiddes. 1982. Human chorionic gonadotropin β-subunit is encoded by at least eight genes arranged in tandem and inverted pairs. *Nature 300:* 419–422.

Fiddes, J.C. and D. Hanahan. 1983. A directional cDNA cloning procedure using sequential addition of oligonucleotide linkers. (Submitted.)

Fasano, I., J.C. Fiddes, M. Wigler, and M. Goldfarb. 1983. Nucleotide sequence of the RNA transcript from the T24 bladder carcinoma transforming gene. (Submitted.)

Helfman, D.M, J.R. Feramisco, J.C. Fiddes, G.P. Thomas, and S.H. Hughes. 1983. Identification of clones that encode chicken tropomyosin by direct immunological screening of a cDNA expression library. *Proc. Natl. Acad. Sci. 80:* 31–35.

Talmadge, K., M.-J. Gething, and J.C. Fiddes. 1983. Expression of the multiple genes for the β-subunit of human chorionic gonadotropin. (In preparation.)

Talmadge, K., N.C. Vamvakopoulos, W.R. Boorstein, and J.C. Fiddes. 1983. Comparison of the seven genes for human chorionic gonadotropin β-subunit with the single gene for human luteinizing hormone β-subunit. (In preparation.)

MOLECULAR GENETICS OF THE MOUSE

L.M. Silver, *L. Cisek, J. Danska, H. Fox, M. Harper, C. Jackson, M. Krangel, D. Lukralle, C. Parra, N. Sarvetnick, J. Uman, P. Weinstein*

Our laboratory combines classical and molecular approaches to obtain an understanding of the organization and expression of the mouse genome. Most of our efforts are directed toward a small region of the mouse genome known as the *t* complex. The *t* complex is located along the proximal half of chromosome 17 and includes the entire major histocompatibility complex (MHC or *H-2*). Variant forms of the *t* complex are known as *t* haplotypes and exhibit a series of effects on embryogenesis, spermatogenesis, and meiotic recombination along the *t* complex region. Partial *t* haplotypes obtained as the products of rare recombinant events carry a subset of the genetic factors responsible for the various *t* haplotype effects. This indicates the presence within complete *t* haplotypes of multiple and interacting mutant genes. Most of the studies described below represent our effort to identify and characterize the DNA sequences, genes, and gene products that mediate the *t* haplotype effects.

t Complex Proteins Expressed during Spermatogenesis

L. Silver, L. Cisek, J. Uman, J. Garrels, J. Danska [in collaboration with Norman Hecht, Tufts University]

We have used a system of high-resolution two-dimensional gel electrophoresis (see Quest 2-D Gel Laboratory Section) to identify and characterize testicular cell proteins specified by *t* haplotypes. So far, a total of nine independent *t* complex proteins (TCPs) have been identified. Mutant forms of eight of these proteins are expressed in common by all complete *t* haplotypes studied. Alternate wild-type forms of six of these proteins have been identified, and evidence from cell-free translation experiments suggests that all of these proteins are encoded directly by genes within the *t* complex. Partial *t* haplotypes have been used to map the genes specifying each TCP to a subregion of the *t* complex, and correlations have been made between particular *Tcp* genes and the *t* haplotype genes involved in sperm differentiation. A quantitative analysis was performed on TCP expression during particular stages of spermatogenesis. The expression of three of the TCP proteins was of particular interest. Both p63/6.9 (TCP-1) and TCP-3 peak in expression during the round spermatid stage of haploid germ cell differentiation. The *Tcp-2* gene only begins to express in round spermatids and is highly active during the last stages of spermiogenesis. Genetic data suggest that all three of these genes could be involved in *t* haplotype effects on sperm differentiation.

t Complex Proteins Expressed during Embryogenesis

M. Krangel, J. Garrels

Embryos homozygous for complete *t* haplotypes die at specific stages during development and display distinct morphologic abnormalities. As a first step toward an understanding of the effects of *t* haplotypes on embryonic development, we are embarking on a project to identify and characterize *t* haplotype-encoded proteins that are expressed during embryogenesis. Strain-129 embryos and 129 embryos congenic for different *t* haplotypes will be metabolically labeled with [^{35}S]methionine and analyzed by high-resolution two-dimensional gel electrophoresis. By analyzing embryos at different stages of pre- and early postimplantation development, we should be able to follow the expression of a large number of proteins through early development, and identify polymorphic proteins that are encoded by *t* haplotypes. Once this set of proteins is defined, a variety of criteria, including genetic mapping, time course of expression, adult tissue distribution, and patterns of expression in embryos carrying different *t* haplotypes, will be used to identify proteins most likely to be mediating the effects of *t* haplotypes on embryonic development. Detailed characterization will likely require the identification of cell types that express these products in quantities sufficient for biochemical analysis. If such sources can be obtained, we should be in a position to begin to ask questions as to the roles of these proteins in embryonic development.

Cloning Sequences Coding for TCP Proteins

N. Sarvetnick, C. Muckeman, G.P. Thomas

The TCP proteins described above are specified by the *t* complex region of chromosome 17. We are identifying clones that encode two of these proteins. Toward this end, we have constructed a testis cDNA library of 20,000 members using the double-linker method developed at Cold Spring Harbor Laboratory. This library has been subjected to several different screens to help in choosing clones that are possible TCP-1 candidates. Total poly(A)$^+$ RNA was size-fractionated on two successive sucrose gradients and translated, and the fraction most enriched in TCP-1 mRNA was chosen. Two "differential" screens were used; one is with similarly fractionated $T^{hp}/^+$ liver RNA. Liver contains approximately 5% of the TCP-1 in the testis; T^{hp} is a variant form of chromosome 17 with a deletion covering TCP-1, and should maximize the differential. The second differential screen is a slightly smaller-size fraction of message than the testis fraction chosen to

be enriched for TCP-1. As estimated from two-dimensional gels, this fraction contains very little TCP-1 message but is enriched for several other proteins in common with the enriched fraction. Following screening, about 70 clones fit the criteria of strong hybridization to the testis fraction and weak hybridization to liver and the smaller testis fraction. These clones are being subjected to further analysis. After eliminating a group of cross-hybridizing clones, the members were subjected to a different level of analysis. Each clone was used to probe a blot of restriction enzyme-digested genomic DNA to identify restriction fragment-length polymorphisms. Any polymorphisms that correlate with the presence of *t* chromatin will be mapped by using numerous partial *t* haplotypes available in our colony. All clones will need to be subjected to hybrid selection for definitive identification. Analysis on Northern blots has satisfactorily double-checked the differential expression between testes and liver of chosen clones. From in vivo labeling experiments, the TCP-2 protein has been shown to become prominent in the two-dimensional gel pattern only in the final stage of spermatogenesis. In fact, this protein is made in *vast* quantities at this stage, exceeding levels of actin and tubulin. By exploiting this knowledge, we can create a probe from RNA of this late fraction of spermatogenesis and screen the library. After eliminating actin and tubulin, the only other prominent hybridization should be from TCP-2 clones. Once the clones specifying these proteins have been obtained, we can isolate genomic clones from a 129 and a *t* homozygous library. These genomic clones will enable us to compare and map genes and/or gene clusters within the *t* complex. We will also define the pathway and extent of expression of *Tcp-1* and *Tcp-2* during spermatogenesis. Finally, we can define whether or not each gene is identical to a *t* factor involved in transmission ratio distortion.

Molecular Dissection of *t* Complex DNA

H. Fox [in collaboration with G. Martin, University of California, San Francisco, and A. Frishauf and H. Lehrach, European Molecular Biology Laboratory, Heidelberg]

We would like to isolate the DNA sequences responsible for the varied properties of the *t* complex. These will include both coding sequences, in the case of genes involved in such processes as segregation distortion, sterility, and lethality, and noncoding sequences responsible for recombination suppression. To accomplish this goal, we plan to obtain molecular markers dispersed throughout the *t* complex. By classical mapping of a gene between two markers, it could be isolated through chromosome-walking techniques. In parallel, by comparing the ordering of these markers within the *t* chromosome and wild-type chromosome 17, as well as comparing detailed restriction maps and sequences of genomic *t* and wild-type clones obtained with these probes, the general organization of *t* complex DNA may be discerned.

We have constructed a genomic library from a homozygous t^{w5} embryonic stem cell line. Two approaches were used to isolate *t* complex-specific clones from this library. The first of these was to use an *H-2* cDNA probe, provided by Lee Hood (California Institute of Technology), to isolate the t^{w5} *H-2* genes. Since the *H-2* region is an integral component of the *t* complex, all of the clones obtained will be from the *t* complex. By isolation of unique subclones from these genomic clones, we can obtain molecular markers for a 3-cM region of the *t* complex. These markers will be especially useful for the isolation of the lethal genes, since many of these map near the *H-2* complex. Twenty-four unique *H-2* clones have been isolated so far. One of the clones, λt^{w5}P3, contains a 10-kb *Eco*RI fragment, identical to the genomic restriction fragment found on Southern blots to be specific for the t^{w5} haplotype. We isolated a subclone 8 kb downstream from the gene, which appeared to hybridize exclusively to t^{w5} haplotype genomic DNA on Southern blots. It was then found that the same hybridization was obtained with mice of the t^{lub3} and t^{lub7} haplotypes, members of the t^{lub1} lethal group, which is distinct from the t^{w5} lethal group. Other members of the t^{lub1} complementation group did not show this hybridization. Examination of the *Eco*RI genomic *H-2* restriction pattern of these mice showed the t^{lub3} and t^{lub7} pattern to be a hybrid between those of t^{w5} and t^{lub1}, containing the t^{w5}-specific 10-kb band, while having other bands present in t^{lub1} and not in t^{w5}. The t^{lub3} and t^{lub7} haplotypes most likely arose by recombination events within the *H-2* region of feral female mice doubly heterozygous for t^{w5} and t^{lub1}. Work is in progress on the analysis of the other *H-2* clones.

We are using a novel, second method to isolate DNA distributed throughout the *t* complex. Jon-Erik Edstrom (EMBL, Heidelberg) has used a micromanipulator to dissect out the proximal half of chromosome 17, corresponding to the *t* region. DNA was purified in a nanoliter droplet, a complete *Eco*RI digestion was performed, and the fragments were cloned into a λ vector. Repetitive clones were screened out and the remaining clones were subcloned into pUC8. To prove their localization to the *t* complex, we looked for polymorphisms on genomic blots between congenic inbred mice containing *t* haplotypes, which are thus identical at all DNA sequences except those mapping to *t*. Clones with polymorphic restriction fragments could then be further localized within the *t* region by using partial *t* haplotypes. We have so far obtained seven *t*-specific probes this way, three of which have been mapped using the partial *t* haplotypes. Clone Tu66e identifies two *t*-specific bands, one of which maps to the proximal region and one of which maps to the middle area; clone Tu122s identifies a single *t*-specific band which maps to the middle area; and clone Tu108 identifies three polymorphic bands, all

of which map to the distal region. Many more clones will be examined, and a more detailed analysis will be made using other partial *t* haplotypes. Large genomic clones are also being isolated, using these smaller clones as probes.

t^{Inl} Is a Novel, Variant Form of Mouse Chromosome 17 with a Deletion in a Partial *t* Haplotype

L. Silver, D. Lukralle, J. Garrels

Moutier discovered, in a mouse from a noninbred Swiss/Orleans laboratory stock, a spontaneous dominant mutation that mapped to the *T* locus and was named T^{Orl}. Genetic analyses indicated that T^{Orl} was not a simple mutation at one locus, but rather a deletion over a 3-cM region of chromosome 17 that included both *T* and *quaking* (*qk*). Further experiments have demonstrated that T^{Orl} is associated with recessive genetic properties affecting sperm function, characteristic of the proximal region of complete *t* haplotypes. These results were interpreted as evidence for the location of proximal *t* haplotype sperm factors within the region deleted by T^{Orl}.

To investigate the possibility that the T^{Orl} deletion is associated with a region of mutant *t* chromatin, a high-resolution, two-dimensional gel analysis was performed on [^{35}S]methionine-labeled protein from animals heterozygous for the T^{Orl} mutation. The results of this experiment demonstrate that all five of the proximally located *t* haplotype-specific proteins are expressed by animals carrying T^{Orl}. This result clearly demonstrates the association of the T^{Orl} mutation with an extended segment of *t* haplotype DNA. At least three separable factors (one proximal, one central, and one distal) interact to distort male transmission ratio. Only a complete *t* haplotype can be transmitted consistently at a very high ratio—loss of either the proximal end or the distal end causes a loss of the high transmission. To determine whether the Tt^{Orl} haplotype carries a proximal *t* haplotype distortion factor, we set up a series of crosses to obtain two parallel genotypes in which Tt^{Orl} was present in *trans* to a partial, distal *t* haplotype. Tt^{Orl}, t^{h17}, and t^{Lub2} each carry only a subset of the eight variant *Tcp* genes associated with all complete *t* haplotypes. However, a genotype with Tt^{Orl} in combination with either t^{h17} or t^{Lub2} reconstitutes the complete set of eight variant *Tcp* genes. If the three *t* haplotype distortion factors can interact in *trans* and if the Tt^{Orl} haplotype carries the proximal factor *Tcd-1*, then Tt^{Orl}/t^{h17} and Tt^{Orl}/t^{Lub2} males should exhibit severe transmission ratio distortion. The results from this experiment dramatically demonstrate that the Tt^{Orl} haplotype can function in *trans* to promote a high transmission frequency for each of the partial, distal *t* haplotypes studied. The cumulative transmission ratio obtained for the 169 offspring born to seven males was 95%. As neither Tt^{Orl} nor the distal *t* haplotypes are transmitted at a high frequency when isolated in separate genotypes, it seems likely that the synergistic effect reported here for *trans* heterozygotes is a consequence of a positive interaction among gene products from Tt^{Orl} and a distal *t* haplotype. This implies that it is the *t* chromatin associated with the Tt^{Orl} haplotype and not the deletion that promotes the high transmission frequency.

Polymorphism of Ribosomal DNA Patterns within an Inbred Strain

M. Harper, L. Chow

The 18S and 28S ribosomal genes of the mouse are present in tandem repeats of approximately 40 kb, each containing three *Eco*RI sites. One of these *Eco*RI fragments contains the origin of the 45S transcript, most of the 18S gene, and a region of nontranscribed spacer DNA. The size of this *Eco*RI fragment varies in a strain-specific manner. Particular fragments are characteristic of different inbred strains of mice. Using a probe to the 18S gene, we have also found a polymorphism within the highly inbred strain 129/SvJ. In some 129/SvJ mice, the probe hybridizes to a single RI fragment of 13 kb, in others it hybridizes to the 13-kb fragment and an additional 16-kb fragment.

We are investigating this unusual phenomenon in two ways. We are studying the genetics of the system to understand the inheritance of the two types of patterns in the 129/SvJ strain. The second approach is to compare the structure of the two RI fragments at the molecular level. The 13-kb and 16-kb RI fragments have been cloned into Charon 4A, and these fragments and their internal *Sal*I fragments have been subcloned into pBR322. The region that is transcribed into the 45S transcript is conserved between the two forms, whereas the nontranscribed region diverges. 5′ to the site of origin of the 45S transcript is a *Sal*I fragment that contains multiple *Pvu*II sites. The size of this fragment varies between the two forms and is probably analogous to the "variable" region in the BALB/c strain described by Arnheim (SUNY, Stony Brook). 5′ to this *Sal*I–*Sal*II fragment is an *Eco*RI–*Sal*I fragment that also contains variable regions. From mapping studies and electron microscopy studies performed by Louise Chow, it appears that this region also contains a number of direct as well as indirect repeats. Despite the potentially destabilizing features of this genomic region, it remains very stable in the mouse genome, showing that there are constraints on homologous recombination in vivo.

Publications

Biddison, W.E., D.D. Kostyu, J.L. Strominger, and M.S. Krangel. 1982. Delineation of immunologically and biochemically distinct HLA-A2 antigens. *J. Immunol. 129:* 730.

Krangel, M.S., W.E. Biddison, and J.L. Strominger. 1983. Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes. II. Variant

DK1: Evidence for a discrete CTL recognition region. *J. Immunol.* (in press).

Krangel, M.S., D. Pious, and J.L. Strominger. 1982. Human histocompatibility antigen mutants immunoselected in vitro: Biochemical analysis of a mutant which synthesizes an altered HLA-A2 heavy chain. *J. Biol. Chem. 257:* 5296.

Krangel, M.S., S. Taketani, D. Pious, and J.L. Strominger. 1983. HLA-A2 mutants immunoselected in vitro. Definition of residues contributing to an HLA-A2 specific serological determinant. *J. Exp. Med. 157:* 750.

Krangel, M.S., S. Taketani, W.E. Biddison, D.M. Strong, and J.L. Strominger. 1982. Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes. I. Variants M7 and DK1. *Biochemistry 21:* 6313.

Magnuson, T., C. J. Epstein, L.M. Silver, and G.R. Martin. 1982. Pluripotent embryonic stem cell lines can be derived from t^{w5}/t^{w5} blastocysts. *Nature 298:* 750.

Magnuson, T., G.R. Martin, L.M. Silver, and C.J. Epstein. 1983. Studies on the viability of $t^{w5}t^{w5}$ embryonic cells in vitro and in vivo. *Cold Spring Harbor Conf. Cell Proliferation 10:* (in press).

Pious, D., M.S. Krangel, L.L. Dixon, P. Parham, and J.L. Strominger. 1982. HLA antigen structural gene mutants selected with an allospecific monoclonal antibody. *Proc. Natl. Acad. Sci. 79:* 7832.

Silver, L.M. 1982. Genomic analysis of the H-2 complex region associated with mouse *t* haplotypes. *Cell 29:* 961.

——.1983. Reevaluation of the evidence for the generation of new lethal *t* haplotypes by mutation. *Immunogenetics* (in press).

Silver, L.M. and M. White. 1982. A gene product of the mouse *t* complex with chemical properties of a cell surface-associated component of the extracellular matrix. *Dev. Biol. 91:*423.

Silver, L.M., D. Lukralle, and J.I. Garrels. 1983. T^{Orl} is a novel, variant form of mouse chromosome 17 with a deletion in a partial *t* haplotype. *Nature 301:* 422.

Silver, L.M., G.R. Martin, and S. Strickland., eds. 1983. "Teratocarcinoma stem cells." *Cold Spring Harbor Conf. Cell Proliferation 10* (in press).

Silver, L.M., J. Uman, J. Danska, and J.I. Garrels. A diversified set of testicular cell proteins specified by genes within the mouse *t* complex. *Cell* (in preparation).

Taketani, S., M.S. Krangel, D. Pious, and J.L. Strominger. Structure of a mutant HLA-A2 antigen obtained by selection with an allospecific monoclonal antibody. (In preparation.)

CELL CYCLE-MODULATED GENE EXPRESSION

D. Zipser, *J. Lewis, L. Rodgers, D. Matkovich*

We have recently succeeded in isolating the thymidine kinase (*tk*) gene from the A-29 line of Chinese hamster cells, and in doing so, have accomplished the first major technical objective in our intended study of the genetic determinants that govern the S-phase-specific cell cycle expression of mammalian *tk* genes. This clonal isolation from a recombinant phage library was facilitated in large measure by the technique of tRNA suppressor rescue developed here at Cold Spring Harbor Laboratory by Kenji Shimizu and Mike Wigler. The hamster *tk* gene is contained with 13.3 kb of genomic DNA in a λL47 recombinant phage designated λ34S4 (Fig. 1). Based on the following lines of preliminary evidence, we believe the hamster *tk* gene may extend over 8.5 kb. (1) We have isolated a recombinant phage designated λ52 which contained the genomic DNA in the 2.6-kb, 4.2-kb, and 5.5-kb *Eco*RI fragments. This λ52 was, however, biologically inactive in transformation assay on mouse Ltk⁻ cells. The hamster *tk* gene in λ34S4 may, therefore, contain DNA sequences within the 1.8-kb and 3.2-kb *Eco*RI fragments. (2) The digestion of λ34S4 with *Sal*I and *Sma*I abolishes λ34S4 *tk* transformation activity, and, therefore, sequences within the 2.6-kb and 4.2-kb *Eco*RI fragments must be part of the hamster *tk* gene. (3) The λ34S4 clone has no detectable nucleotide sequence homology to the cloned herpes simplex virus I *tk* gene, but it does share limited homology with the previously cloned chicken *tk* gene. Using high-stringency Southern blot as a criterion, the 3.2-kb *Eco*RI and 4.2-kb *Eco*RI fragments of λ34S4 react with a nick-translated chicken *tk* gene probe. This data suggests that the hamster *tk* gene in λ34S4 extends from the *Sma*I site within the 4.2-kb *Eco*RI into the 3.2-kb *Eco*RI fragment, a distance of approximately 8.5 kb.

Based on our preliminary experiments, we believe the hamster *tk* mRNA is a 1350-nucleotide polyadenylated RNA. We detect this RNA in Northern blots probed with the 3.2-kb and 4.2-kb *Eco*RI fragment but not with the 2.6-kb and 1.8-kb *Eco*RI fragments of λ34S4. Since both the 3.2-kb and 4.2-kb *Eco*RI fragments show homology with the chicken *tk* gene, we expect that the 1350-nucleotide RNA these probes detect is in fact the hamster *tk* mRNA. Using the 3.2-kb *Eco*RI fragment of λ34S4 as a nick-translated probe, we have analyzed the concentration of the 1350-nucleotide RNA in poly(A)⁺ RNA preparations obtained from A-29 under various growth conditions. This 1350-nucleotide mRNA is readily detected in RNA prepared from asynchronous midlog cultures of A-29 cells, but is virtually undetectable in RNA prepared from G_0-phase populations of A-29 cells, synchronized by serum starvation methods. This result is compatible with a body of indirect experimental evidence that suggests that the cell-cycle-dependent S-phase-specific expression of hamster *tk* activity is regulated most proximally by the S-phase appearance of hamster *tk* mRNA.

We are presently defining the extent to which the expression of the hamster *tk* gene in λ34S4 is cell-cycle dependent when transfected into a variety of mammalian tk⁻ host cells. Our preliminary evidence suggests that the expression of the cloned hamster *tk* gene in mouse Ltk⁻ cells shows a moderate degree of S-phase specificity. We are analyzing the pattern of *tk* gene expression in BHK tk⁻ ts13, rat tk⁻, and CHO tk⁻ cells transfected with λ34S4 as well.

The ultimate objective of this work is, of course, to define the mechanisms operating to restrict the appearance of the hamster *tk* mRNA to the S phase of the eukaryotic cell cycle. Because the hamster *tk* mRNA is represented among the least abundant class of cytoplasmic poly(A)⁺ RNAs, we are prevented from directly assaying cell-cycle phase-specific rates of *tk* gene transcription, modes of RNA processing, and *tk* mRNA stability from which we might discern the physiological processes governing hamster *tk* regulation. As an alternative approach to this problem, we intend to construct chimeric hamster *tk* genes, combining specific fragments of the hamster *tk* gene with fragments of the herpes *tk* gene, which is expressed constitutively through all phases of the cell cycle in mouse cells transformed with the gene. These gene constructions will be transfected into appropriate tk⁻ host cells, and tk⁺ transformants will then be analyzed for their cell-cycle pattern of *tk* activity. These constructs will be designed to assay 5′ and 3′ flanking sequence, hamster *tk* coding, and

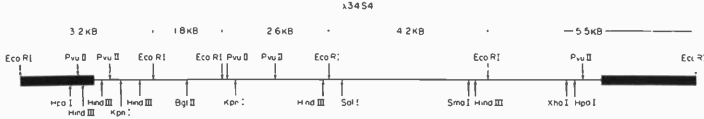


Figure 1
λL47 recombinant phage λ34S4 containing hamster *tk* gene.

intron sequences for genetic determinants that regulate S-phase-specific *tk* gene expression. When such determinants are identified in chimeric *tk* genes, we intend to analyze their mutagenesis techniques, and when indicated, analyze their orientation and positioning requirements within the chimeric genes as well. This knowledge of the nature and position of the genetic determinants of the cell cycle regulation within the *tk* gene may permit us to propose with confidence the physiological processes governed by these determinants.

Publications

Lewis, J.A., J.L. Biedler, and P.W. Melera. 1982. Gene amplification accompanies low level increases in the activity of DHFRase in antifolate resistant Chinese hamster lung cells containing abnormally banding chromosomes. *J. Cell Biol.* 94: 418.

Lewis, J.A., J.P. Davide, and P.W. Melera. 1982. Selective amplification of polymorphic DHRF genes in Chinese hamster cells. *Proc. Natl. Acad. Sci.* 79: 6961.

Lewis, J.A., K. Shimizu, and D. Zipser. 1983. Isolation and preliminary characterization of the thymidine kinase gene from Chinese hamster fibroblast cells. (Submitted.)

Shimizu, K., J.A. Lewis, M. Goldfarb, and M.H. Wigler. 1983. A suppressor rescue method for the isolation of genes by DNA-mediated transformation. (Submitted).

Cell Biology

The Cell Biology Group has had for the past several years a keen interest in the organization of the cytoplasm and in cell motility and the underlying molecular mechanisms governing these processes. In the last year or two, this work has led us into two related areas of research: the cell biology of transformation and of the heat-shock or stress response. It is becoming increasingly clear that a full understanding of these biological phenomena will require the analysis of the functions of several key protein components of these systems.

With a variety of biochemical and cellular experimental techniques now available, such as immunology, two-dimensional gel electrophoresis, microinjection of tissue culture cells, protein chemistry, and DNA cloning, we have been probing the roles of the cytoskeletal, transforming, and heat-shock proteins as indicated below.

The makeup of the group has changed over the past year, most notably with the departure of our dear colleague, Guenter Albrecht-Buehler. He has assumed the role of Professor of Cell Biology at Northwestern University School of Medicine in Chicago. The loss of Guenter will be felt for years to come. Kent Matlack, who is a graduate student from Berkeley, has joined us to work on the biochemistry of the cytoskeleton, and Tohru Kamata, a postdoctoral fellow from Tokyo, has begun working on the biochemistry of the human oncogene product, p21. Robert Franza joined our group to study transformation by two-dimensional gel electrophoresis after serving as a Resident at Dartmouth Medical School. Additions to our technical staff were Georgia Binns, Cecilia Fraser, Gladys Blose, Patricia Smith, and Joseph Suhan.

CELL BIOCHEMISTRY

J. Feramisco, J. Lin, *G. Binns, A. Calasso, B. Daniels, C. Fraser, N. Haffner, D. Helfman, T. Kamata, K. Matlack, F. Matsumura, S. Matsumura, M. Szadkowski, W.J. Welch*

The Mammalian Stress Response

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

Mammalian cells incubated under adverse conditions undergo what has been termed the stress response. As was described in last year's report, such a response is most notably characterized by changes at the protein synthetic level. Briefly, in the stressed cell, the rate of synthesis of a small number of proteins (the stress proteins) is increased along with a concomitant decrease in the production of most other cellular polypeptides. Although their synthesis is increased during the stress response, almost all of the stress proteins (except for one or two) appear to be expressed at lower levels in cells grown under "normal" tissue culture conditions. The stress response, in general, appears to be a defensive one in nature since a brief induction of the stress proteins appears to confer a degree of protection to the cell upon subsequent stress situations. Whereas it is clear which proteins are induced following the onset of the stress response, their function in both the normal and stressed cell and the manner by which they afford the cell protection are not known and constitute the focus of our research.

To gain a better understanding of the stress response and the role the stress proteins serve, we have been examining the response in mammalian cells both at the cellular and protein levels. With regard to changes in the cell, we have found that cells grown under stress appear to: (1) show a more flattened and spread morphology; (2) have an increased number of intracellular actin bundles; and (3) display a rearrangement in their intermediate filament network, with the filaments redistributing from their normal splayed array throughout the cytoplasm into a tight cap near the nucleus (Thomas et al., *Cold Spring Harbor Symp. Quant. Biol. 46:* 985 [1982]). At the protein level, mammalian cells grown under stress and labeled with [^{35}S]methionine synthesize at high levels proteins with apparent molecular masses of 72, 73, 80, 90, 100, and 110 kD. In addition, we recently have found, as have others, a smaller mammalian stress protein with an apparent molecular mass of approximately 28, kD that contains little or no methionine residues. The increases in the amounts of the stress proteins in the stressed cell appear to result from changes at both the transcriptional and translational levels. Specifically, mRNAs coding for the stress proteins appear elevated and these messages are preferentially translated while transcription and translation of other cellular mRNAs appear reduced. The mechanisms by which such controls are exerted in the stressed cell are not understood and constitute one aspect of our research (see also Thomas, Protein Synthesis Section).

Examination of the stress proteins induced in a variety of different mammalian cell types by two-dimensional gel electrophoresis indicates that the molecular mass and isoelectric charge of the individual stress proteins are well conserved amongst different species. The only major differences amongst the various cell types are the number and multiple charge variants of the 72-kD stress protein. For example, in gerbil fibroma and Chinese hamster ovary cells, only one to two isoelectric variants of 72 kD are found, while in BHK cells approximately four and in HeLa cells as many as seven to eight 72-kD charge variants are observed. Interestingly, we have found that all of the HeLa 72-kD (and 73-kD) isoforms appear related, as determined by one-dimensional peptide mapping. In addition, antibodies raised against the HeLa 72-kD stress protein also cross-react with the 72-kD isoforms from other mammalian cell types as well as with the avian 70-kD heat-shock protein. Hence, although there appear to be differences between the number of 72-kD isoforms induced in each cell type, the protein in general appears to be well conserved across evolutionary lines.

In addition to heat-shock treatment, exposure of cells to a variety of biological agents gives rise to the stress response (Thomas et al., *Cold Spring Harbor Symp. Quant. Biol. 46:* 985 [1982]). Such agents include amino acid analogs, certain drugs, transition series metals, and inhibitors of oxidative phosphorylation to name a few. Although it is clear that all of these diverse treatments in some way adversely affect cellular metabolism, no common cellular target appears obvious. Furthermore, it is now apparent that induction of all six stress proteins is not a coordinate event, since certain treatment of cells results in the enhanced synthesis of only a subset of the stress proteins. For example, we have found that two of the stress proteins, the 80-kD and 100-kD species, are also induced in cells grown in the absence of glucose (i.e., the "glucose-regulated proteins"). Similarly, the same two proteins also are induced in cells treated with calcium ionophores (Welch et al., *J. Biol. Chem.* [1983, in press]). Whereas the synthesis of the 72-kD and 73-kD stress proteins is unaffected, these same two treatments also result in the decreased production of the 90-kD stress protein. Thus, it appears that the rates of synthesis of the 80-, 90-, and 100-kD proteins are all affected to different extents by calcium ionophores, glucose deprivation, and by stress. The question then arises as to whether there is any connection between these various effectors. While we are continuing to investigate this question, it is

worth noting that others have reported a possible relationship between intracellular calcium concentrations and the activation of glucose transport. It will be interesting to determine whether the onset of stress in the cell also affects glucose uptake and/or metabolism.

One approach to dissect the biological role of the stress response and the function of the stress proteins is to learn more about the nature of the proteins themselves and to determine their location in the cell. We have found through metabolic labeling studies (Welch et al., *J. Biol. Chem.* [1983, in press]) that the 100-kD stress protein is a glycoprotein and that the 80-kD and 90-kD stress proteins are both phosphoproteins, with there being as many as six to eight phosphorylated variants of 90-kD. In addition, the 100-kD protein was found to incorporate phosphate in some but not all cell types examined. Our cell fractionation studies have shown that the 90-kD protein is located almost exclusively in the cytoplasm, whereas the 80-kD and 100-kD stress proteins appear associated with intracellular mem-

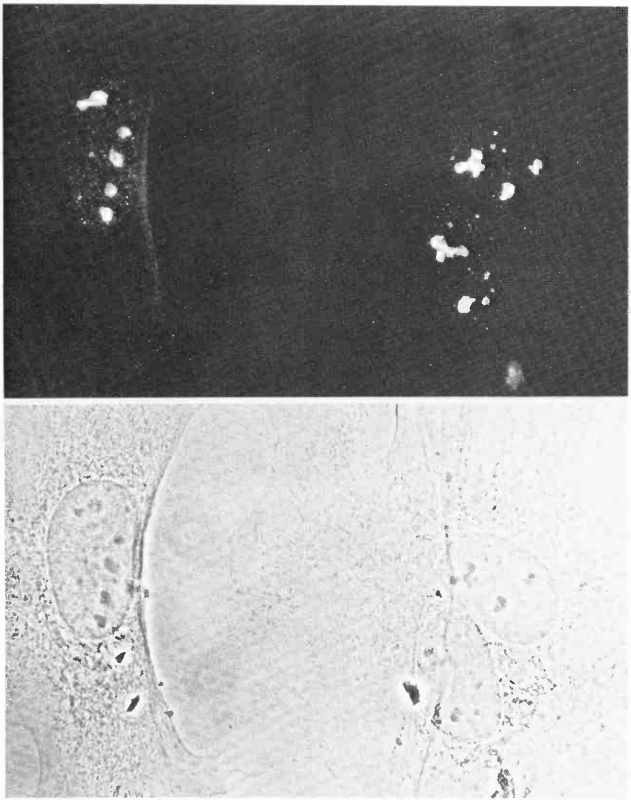


Figure 1
Immunofluorescent localization of the 72 kD heat-shock protein in stressed human cells. Antibodies specific to the 72 kD protein indicate that this induced, heat-shock protein is localized primarily in the nucleoli, sites of ribosome assembly, and RNPs. (*Top*) Immunofluorescence; (*bottom*) phase contrast.

branes. The 72-kD and 73-kD proteins, related proteins as described earlier, fractionate equally between the nucleus and the cytoplasm following heat-shock treatment of cells. As was described in last year's report, we have successfully purified the 72-kD and 73-kD proteins as well as the 90-kD protein. A number of physical properties of the purified proteins were determined, including their Stokes' radii, $S_{20,w}$ value, isoelectric point, and native molecular weight (Welch and Feramisco, *J. Biol. Chem. 257:* 14949 [1982]). More recently, we have succeeded in purifying both the 80-kD and 100-kD proteins from Triton-extracted, stressed HeLa cells (W.J. Welch and J. Feramisco, in prep.).

Using the purified proteins as antigens, we have prepared and characterized polyclonal antibodies against the 72-kD, 80-kD, 90-kD, and 100-kD stress proteins (Welch et al., in *Heat shock: From bacteria to man*, p. 257, Cold Spring Harbor Laboratory [1982]). In addition, Jim Lin has prepared a monoclonal antibody against the 100-kD stress protein. Production of more monoclonal antibodies against all of the stress proteins currently is in progress. The specificity of all our antibodies has been confirmed by immunoprecipitation and analysis of the immunoprecipitates by two-dimensional gel electrophoresis as well as by preabsorption of each individual antibody with the purified antigen. Indirect immunofluorescence studies using our 80-kD antibody showed vesicularlike staining of the antigen, a result consistent with our cell fractionation data showing 80-kD to be present within intracellular membranes. A similar analysis using the 90-kD serum revealed the antigen to be present with discrete particles throughout the cytoplasm. The identity of these particles is presently under examinaton. Staining with both the monoclonal and polyclonal antibodies against the 100-kD protein showed the antigen to be present primarily in or on the Golgi apparatus and less prominently within the nucleus and on the plasma membrane. Upon heat-shock treatment, the number of cells displaying nuclear staining increased while the Golgi staining became less prominent (Lin et al., in *Heat shock: From bacteria to man*, p. 267, Cold Spring Harbor Laboratory [1982]). Finally, the location in the cell of the 72-kD stress protein has generated much excitement. While little or no staining is observed in cells grown at 37°C, heat-shock-treated cells display intense nuclear fluorescence. Such nuclear staining appears to be specific for the nucleoli. Following reversal of the heat-shock treatment, the nucleolar staining disappears (W.J. Welch and J. Feramisco, in prep.). Since the nucleolus is at least in part associated with ribonucleoprotein (RNP) formation, we are examining the possibility that the 72-kD stress protein is in fact part of a RNP complex. Preliminary studies show this to be the case.

Associations of Vinculin In Vivo

K. Matlack, J. Feramisco

Physical contact between cells within a tissue and between a cell in culture and its substrate occurs largely at small areas comprising only a small fraction of the cell's surface (Izzard and Lochner, *J. Cell Sci. 21:* 129 [1976]). These specialized areas, known as adhesion plaques, are also the sites at which microfilaments terminate on the inner surface of the cell membrane (Heath and Dunn, *J. Cell Sci. 29:* 197 [1978]). Vinculin, a protein first isolated from chicken smooth muscle, localizes to these sites in cultured cells, as well as colocalizing with fibronectin on the upper surface of the cell (Geiger, *Cell 18:* 193 [1979]; Feramisco and Burridge, *J. Biol. Chem. 255:* 1194 [1980]; Burridge and Feramisco, *Cell 19:* 587 [1980]). Vinculin from at least one nonmuscle source is a calcium-dependent actin-binding protein that binds to actin filaments at their ends (Burridge and Feramisco, *Cold Spring Harbor Symp. Quant. Biol. 46:* 507 [1982]). Its localization within cells to the sites at which microfilaments terminate, then, raises the possibility that vinculin may be involved not only in stabilizing the ends of microfilament bundles but in establishing their positions as well. Vinculin's colocalization with fibronectin in cultured cells also suggests that it may be involved in interactions between the cell's cytoskeleton and the extracellular matrix.

Little is known, however, about the structures in which vinculin is found. Vinculin is likely to be only one of a number of proteins involved in an adhesion plaque, and the way in which the adhesion plaque functions in the cytoskeleton will require kr.owledge of the interactions among these proteins and their interactions with actin. We are trying to identify components involved in adhesion plaques or in the interaction of the cytoskeleton with the extracellular matrix by identifying proteins that are associated with vinculin. Presently, we are trying to use immunoprecipitation with antisera against chicken gizzard vinculin to recover complexes including vinculin that are formed when living chicken embryo fibroblasts are exposed to a variety of cross-linking agents. We hope to purify components that we find associated with vinculin, to localize them using immunological methods both in cells with relatively stable cytoskeletons and in cells in which the cytoskeleton is being assembled, and to study in vitro the interactions between these proteins and vinculin.

Molecular Cloning of Cytoskeleton Protein: Immunological Screening of Expression Libraries

D. Helfman, J. Feramisco, J. Fiddes, P. Thomas, S. Hughes

(For more information, see Genes for the Major Structural Proteins: Organization and Control of Expression Section.)

Relatively straightforward methods have been established for the construction of essentially complete cDNA or genomic DNA libraries from many

sources. Such libraries usually contain many thousands of members; the major difficulty is the identification of clones of particular interest. Final identifications are usually made by sequence analysis of the DNA insert, by using the DNA to select a specific mRNA for in vitro translation, or both. Such assays are not usually suited to screening large libraries for individual clones, and steps are usually taken to enrich for the clones of interest. The most commonly used procedures rely on cDNA hybridization probes made from a mRNA preparation that contains high levels of the sequence of interest. Variations of this general approach include the physical fractionation of the mRNA by sedimentation, gel electrophoresis, or immunoprecipitation of polysomes. Alternatively, differential hybridization can be used if two mRNA preparations are available that differ principally in their content of the mRNA of interest. These approaches are generally limited to the identification of genes that are expressed at moderate to high levels.

At least two techniques have been developed that permit the identification of clones for genes that are expressed at low levels. If part or all of the amino acid sequence of a protein is known, it is possible to synthesize specific oligonucleotides for use either as hybridization probes themselves or as primers for the enzymatic synthesis of cDNA probes. This procedure is limited by the degeneracy of the genetic code. Other techniques require that the cDNA preparation, ideally made from total poly(A)$^+$ mRNA, be cloned into plasmids that promote expression of the cDNA in *Escherichia coli*. The resultant expression libraries then are screened for appropriate translation products by using either a functional or an immunological assay. These protocols bypass the need for enrichment of the mRNA and provide promising approaches for the cloning of genes expressed at low levels.

A cDNA library of ~9,000 members has been prepared from chicken smooth muscle mRNA by using the plasmid expression vector pUC8. Addition of *Sal*I and *Eco*RI linkers at different stages during the preparation of the cDNA resulted in a population of molecules, most of which had *Eco*RI linkers at the end of the cDNA that corresponded to the 5′ end of the template mRNA and *Sal*I linkers at the end that corresponded to the 3′ end of the mRNA. The cDNA molecules then were inserted into a *Eco*RI-*Sal*I-cut plasmid vector, pUC8, that contains the transcriptional and translational start sequences from the *lac*Z gene upstream of the *Eco*RI site. The sequential addition of the linkers to the cDNA ensured that most of the cDNAs were inserted into pUC8 in the proper orientation for expression. The colonies were replica-plated onto nitrocellulose filters and lysed in situ with chloroform vapor. The library was screened for colonies producing products immunologically related to chicken tropomyosin by incubating the filters first with a rabbit antitropomyosin antibody and second with a ^{125}I-labeled goat anti-rabbit IgG. Two colonies were detected that reacted specifically with the antisera. Plasmids from both clones have been partially subjected to sequence analysis; both plasmids contain cDNAs that encode tropomyosin. These protocols are potentially useful for the identification of cDNA clones for genes expressed at low levels from large cDNA expression libraries.

Microinjection of Monoclonal Antibodies against Tubulin into Living Fibroblasts

S. Blose, J. Feramisco

(For more information, see Biology of the Cytoskeleton Section.)

Four monoclonal antibodies have been generated against either α- or β-tubulin subunits and one that has activity against both subunits. Cells were microinjected with these antibodies and the effects on cytoplasmic microtubules and 10-nm filaments were assessed. Two monoclonals, DM1A and DM3B3, an anti-α- and anti-β-tubulin, respectively, caused the 10-nm filaments to collapse into tight juxtanuclear caps; the other monoclonal antibodies had no effect. The filament capping was observed to be complete at 1.5–2 hours after injection. During the first 30 minutes after injection, a few cytoplasmic microtubules near the cell periphery could be observed. By 1.5 hours, when most of the 10-nm filaments were capped, the complete cytoplasmic array of microtubules was observed. Cells injected in prophase were able to assemble a mitotic spindle. These results suggest that though a direct interaction of antibodies with either α- or β-tubulin subunits, 10-nm filaments were dissociated from an interaction with microtubules.

Monoclonal Antibodies Directed against the Human Oncogene Protein, p21

T. Kamata, Y. Suarde, G. Binns, C. Fraser, M. Wigler, J. Feramisco

Recently, there have been important leads into the control of the transformation process through the identification of human genes that are capable of converting normal cells into transformed cells (see Mammalian Cell Genetics Section). In general, these newly described oncogenes show homology to previously described genes of Harvey or Kirsten sarcoma viruses. The genes encode a protein of ~21 kD that is thought to be able to alter the normal growth phenotype to that of a transformed phenotype. To unravel the molecular mechanisms of the transformation process in terms of the cell, it will be of utmost importance to characterize p21 biochemically. We have begun a program aimed at the understanding of the transformation process some of which will be described here.

To have specific monoclonal antibodies to p21, four synthetic peptides were made, coupled to KLH, and injected into mice (and rabbits). The four peptides correspond to: (1) the aminoterminal region where a single amino acid substitution is thought to

alter the "normal" version of p21 to the "transformation" version, (2) the middle of the protein where a site common to all p21s is present, and (3) the carboxyterminal region where divergence occurs between the various p21s. We have produced 10 stable hybridomas secreting antibodies to the peptide from the region in (2) above and are in the process of getting similar antibodies specific to the other two regions.

Multiple Forms of Tropomyosin Associated with Microfilaments from a Variety of Cell Types

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

It is known that skeletal muscle tropomyosin, in association with troponin complex, regulates actomyosin interaction in a calcium-dependent manner. Because no troponin complex was found in smooth muscle or nonmuscle cells, the biological functions of tropomyosin in these cells are not yet determined. Using the microfilament isolation method that we have developed (Matsumura et al., *J. Biol. Chem.* [1983, in press]), we have found multiple forms of tropomyosin associated with our microfilament preparation from a variety of cell types, including chicken embryo fibroblasts and myoblasts, rat REF-52 cells, rat L6 myoblasts, "normal" rat kidney (NRK) cells, mouse NIH-3T3 cells, mouse myeloma (SP-2) cells, mouse neuroblastoma cells, gerbil fibroma (CCL-146) cells, baby hamster kidney (BHK-21) cells, and human fibroblast (KD) cells. Five forms of tropomyosin with apparent molecular weights of 40K, 36.5K, 35K, 32.4K, and 32K and six forms of tropomyosin with apparent molecular weights of 46K, 44K, 38K, 36.5K, 32.8K, and 32.6K can be identified from rat cell lines and chicken embryo fibroblasts, respectively, by two-dimensional gel analysis. These protein spots are: (1) immunoprecipitable with rabbit polyclonal antibody against smooth muscle tropomyosin from total cell extracts; (2) lacking tryptophan and proline; (3) heat stable; and (4) able to bind to skeletal muscle F actin. Expression of these multiple forms of tropomyosin was found to be different between normal REF-52 and its viral transformed cells (see details in next section). Because microfilaments in transformed cells appear to be less ordered, it is interesting to know whether different forms of tropomyosin may be responsible for this. It may further suggest different functions in the stabilizing and/or bundling of microfilaments for these tropomyosin isoforms in nonmuscle cells. Purification and characterization of individual forms of tropomyosin are currently in progress.

Use of Monoclonal Antibodies to Isolate and Characterize Tropomyosin-enriched and α-Actinin-enriched Microfilaments from Chicken Embryo Fibroblasts

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

We have used monoclonal antibodies against tropomyosin and against α-actinin to isolate two classes of microfilaments, i.e., tropomyosin-enriched and α-actinin-enriched microfilaments, respectively, from cultured chicken embryo fibroblasts. As described in last year's report, the microfilament isolation method consists of three simple steps, i.e., Triton/glycerol extraction to stabilize microfilaments, homogenization in Mg^{++}-ATP to disperse microfilaments, and immunoprecipitation of microfilaments by either anti-tropomyosin or anti-α-actinin monoclonal antibody in a native condition. Electron microscopic studies of the isolated tropomyosin-enriched microfilaments showed periodic localization of tropomyosin along the microfilaments with a 35-nm repeat (Fig. 2A). On the contrary, the isolated α-actinin-enriched microfilaments showed no obvious periodicity. About 10–30 individual α-actinin-enriched microfilaments with average lengths varying from 1 to 5 μm were associated at certain parts of microfilaments by anti-α-actinin monoclonal antibodies (Fig. 2, B and C). This area may represent the α-actinin-enriched region along microfilaments. These two classes of microfilaments also differ in their protein composition. Molar ratios of major identifiable proteins in the isolated microfilaments are α-actinin (dimer):actin (monomer):tropomyosin (dimer)-trace (< 0.03):8.06:1.00 for tropomyosin-enriched microfilaments and 1.00:17.61:0.43 for α-actinin-enriched microfilaments. Both the isolated microfilaments retained their abilities to activate myosin ATPase activity.

Differential Expression of Tropomyosin Forms in the Microfilaments Isolated from "Normal" and Transformed Rat Cultured Cells

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

Microfilaments were isolated from "normal" and simian virus 40 (SV40)- or adenovirus type 5 (Ad5)-transformed REF-52 cells by a newly developed method (Matsumura et al., *J. Biol. Chem.* [1983, in press]). Electron microscopy shows that microfilaments isolated from transformed cells are much shorter in length than those from "normal" cells. By one- and two-dimensional gel analysis of such isolated microfilaments, we have found changes in the pattern of the five identifiable species of tropomyosin (*M*, 40,000, 36,500, 35,000, 32,400, and 32,000) upon transformation (Fig.3). A decrease in the level of one or both of major tropomyosins (*M*, 40,000, 36,500) of normal cells and an increase of one or both of minor tropomyosins (*M*, 35,000, 32,000) are common to both types of viral transformants. REF-52 cells transformed by a defective SV40 (*dl884*) incapable of expressing the viral small t protein do not show these changes, suggesting the involvement of this protein in the altered pattern of tropomyosin gene expression. The degree of alteration in the patterns of tropomyosin correlates well with the degree of morphological transformation. Furthermore, the changes in tropomyosin patterns

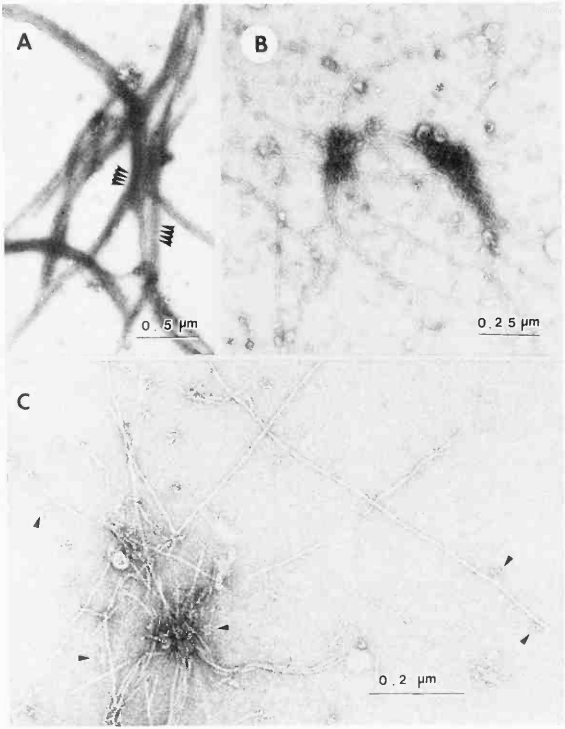


Figure 2
Tropomyosin-enriched (*A*) and alpha-actinin-enriched (*B, C*) microfilaments isolated from chicken embryo fibroblasts by anti-tropomyosin and anti-α-actinin monoclonal antibodies, respectively.

are to be specific to the morphological changes induced by transformation, because "rounding-up" after trypsin or cytochalasin B treatment does not cause these changes in tropomyosin patterns. Similar changes in tropomyosin patterns are also observed both in total cell lysates and in cell-free translation products directed with total mRNA. These results would suggest that at least five mRNAs are present for these multiple forms of tropomyosin and that the changes in tropomyosin patterns are regulated at the level of RNA rather than by post-translational modification.

The differential expression of different forms of tropomyosin appears to be a general phenomenon. In addition to DNA viral transformation described above, we have found similar changes in tropomyosin patterns in various types of transformants, including RNA virus-transformed rat kidney cells, chemically transformed BALB/c mouse 3T3 cells, and NIH-3T3 cells transformed by tumor cell DNA.

Tropomyosin is one of the major components of microfilaments of cultured cells. In fibroblasts, tropomyosin appears to be localized in stress fibers or actin cables but to be absent in the movable portions of cells, such as ruffling membranes. This observation, coupled with the ability of this protein to stabilize actin filaments, suggests that tropomyosin may regulate the organization of microfilaments into bundles as stress fibers. Therefore, the altered patterns of tropomyosin expression may be one of the causes for morphological transformation. To explore this hypothesis, purification and charac-

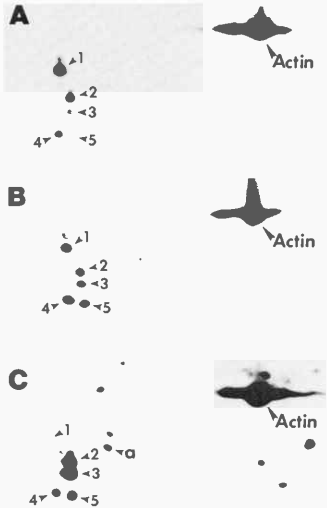


Figure 3
Two-dimensional gel analysis of the microfilament fractions from "normal" and transformed cells (pH 5–7 ampholytes for the first dimension, 12.5% polyacrylamide for the second dimension). After electrophoresis, gels were processed for fluorography. Only those parts of the two-dimensional gels containing tropomyosin and actin are shown here. (*A*) Microfilaments from normal cells, REF-52; (*B*) microfilaments from SV40-transformed cells, REF-WT4A; (C) microfilaments from adenovirus-transformed cells, Ad5D.1A. Numbers indicate multiple forms of tropomyosin (TM-1 to TM-5). Note the decrease in the level of both TM-1 and TM-2 and the increase of both TM-3 and TM-5 in the microfilaments from SV40 transformants (*B*). TM-1 is entirely missing and TM-3 is greatly increased in the microfilaments from adenoviral transformants (C). A protein found in the microfilaments from adenovirus-transformed cells (a in C) is heat stable but does not appear to be a tropomyosin.

terization of each form of tropomyosin are currently in progress.

A New Actin-binding 83K Protein Found in L6 Myoblasts

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

Our new method for microfilament isolation has allowed us to detect a new actin-binding protein with a molecular weight of 83,000 in the microfilaments of L6 myoblasts. This protein is missing in the microfilaments isolated from myotubes, suggesting a role of this protein in the formation of thin filaments during myogenesis. We have found that the 83K protein, like tropomyosin, is a heat-stable protein. Using this property, we have purified the protein from REF-4A cells because the same protein is also present in this cell line. Purified protein shows binding to skeletal muscle F actin and is focused around pI 7–8 as four spots. Furthermore, 83K protein appears not to have villinlike or gelsolinlike activities, i.e., severing of F actin, although its *M*, is similar to these proteins. None of the previously reported actin-binding proteins, except tropomyosin, is known to be heat-stable. Therefore, we believe that 83K protein is a new actin-binding protein. Immunofluorescent localization with polyclonal rabbit antiserum raised against this 83K protein has revealed that the protein is localized in stress fibers of myoblasts but disappears after fusion into myotubes. This is consistent with the finding that the 83K protein is missing in the microfilaments of myotubes. Further characterization of 83K protein as well as detailed intracellular localization during myogenesis is currently in progress.

Production of Monoclonal Antibodies to 83K and Multiple Forms of Tropomyosin

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

As described in previous sections, we have found a new microfilament-associated protein (83K), which may play some roles in myogenesis, and a multiple form of tropomyosin, which may have different functions in stabilizing and/or bundling of microfilaments. We have begun to prepare monoclonal antibodies to these proteins by immunizing mice with gel-purified 83K and with individual forms of tropomyosin. These antibodies will be used in conjunction with microinjection techniques and immunofluorescence and immunoelectron microscopy to probe the in vivo function and intracellular localization of these proteins.

In a preliminary fusion experiment with microfilament fractions from L6 myoblasts as antigens to immunize mice, we have isolated two interesting hybridoma clones, NHY3 and NHY6. Clone NHY3 secreted antibody that gave the immunofluorescence staining pattern on L6 myoblasts similar to that obtained by rabbit antiserum against purified 83K protein. The other clone, NHY6, producing antibody gave the α-actininlike staining pattern. Experiments identifying the specific antigens recognized by these antibodies are currently in progress.

Publications

Blose, S.H., D.I. Meltzer, and J.R. Feramisco. 1982. 10nm-Filaments induced to collapse in cells microinjected with antibodies against tubulin. *J. Cell Biol.* 95: 229a.

Feramisco, J.R. and K. Burridge. Purification and characterization of alpha-actinin from non-muscle cells. (In preparation.)

Feramisco, J.R., J.E. Smart, K. Burridge, D. Helfman, and G.P. Thomas. 1982. Co-existence of vinculin and a

vinculin-like protein of higher molecular weight in smooth muscle. *J. Biol. Chem. 257:* 11024.

Helfman, D.M., J.R. Feramisco, J.C. Fiddes, G.P. Thomas, and S.H. Hughes. 1983. Identification of clones that encode chicken tropomyosin by direct immunological screening of a cDNA expression library. *Proc. Natl. Acad. Sci. 80:* 31.

Lin, J.J.-C., F. Matsumura, and S.H. Blose. 1983. A monoclonal antibody that cross-reacts with tropomyosin and vimentin. (Abstract submitted to ASBC meeting.)

Lin, J.J.-C., F. Matsumura, and S. Yamashiro-Matsumura. Tropomyosin- and alpha-actinin-containing microfilaments isolated from chicken embryo fibroblasts by monoclonal antibodies. (In preparation.)

Lin, J.J.C., J.R. Feramisco, S.H. Blose, and F. Matsumura. 1983. Monoclonal antibodies to cytoskeletal proteins. In *Monoclonal antibodies and hybridomas: Progress and applications* (ed. R.H. Kennett et al.). Plenum Press, New York. (In press.)

Lin, J.J.-C., W.J. Welch, J.I. Garrels, and J.R. Feramisco. 1982. The association of the 100,000 dalton heat-shock protein with the Golgi apparatus. *In Heat shock: From bacteria to man* (ed. M.J. Schlesinger, M. Ashburner, and A. Tissières), p. 267. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Matsumura, F. and J.J.-C. Lin. 1982. Visualization of monoclonal antibody binding to tropomyosin on native smooth muscle thin filaments by electron microscopy. *J. Mol. Biol. 157:* 163.

Matsumura, F., J.J.-C. Lin, and S. Yamashiro-Matsumura. 1983. Use of monoclonal antibodies to isolate and characterize tropomyosin-enriched and alpha-actinin-enriched microfilaments from chicken embryo fibroblasts. (Abstract submitted to ASBC meeting.)

Matsumura, F., S. Yamashiro-Matsumura, and J.J.-C. Lin. 1983. Isolation and characterization of tropomyosin-containing microfilaments from cultured cells. *J. Biol. Chem.* (in press).

Matsumura, F., J.J.-C. Lin, S. Yamashiro-Matsumura, M. Hightower, and W. Topp. 1982. Changes in the species of tropomyosin localized in microfilaments upon viral transformation. *J. Cell Biol. 95:* 284a.

Matsumura, F., J.J.-C. Lin, S. Yamashiro-Matsumura, P.G. Thomas, and W.C. Topp. Differential expression of tropomyosin forms in the microfilaments isolated from normal and transformed REF-52 rat embryo cell lines. (Submitted.)

Sugino, H. and F. Matsumura. 1983. Fragmin induces tension reduction of actomyosin threads in the presence of micromolar levels of Ca^{2+}. *J. Cell Biol. 96:* 199.

Thomas, G.P., W.J. Welch, M.B. Mathews, and J.R. Feramisco. 1982. Molecular and cellular effects of heat shock and related treatments of mammalian tissue-culture cells. *Cold Spring Harbor Symp. Quant. Biol. 46:* 985.

Wang, K., J.R. Feramisco, and J.F. Ash. 1982. Fluorescent localization of contractile proteins in tissue culture cells. *Methods Enzymol. 85:* 514.

Welch, W.J. and J.R. Feramisco. 1982. Purification of the major mammalian stress proteins. *J. Biol. Chem. 257:* 14949.

——.1983. Intracellular localization of the 72K stress protein in normal and heat-shocked cells. (In preparation.)

Welch, W.J., J.I. Garrels, and J.R. Feramisco. 1982. The mammalian stress proteins. In *Heat shock: From bacteria to man* (ed. M.J. Schlesinger, M. Ashburner, and A. Tissières), p. 257. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Welch, W.J., J.I. Garrels, G.P. Thomas, J.J.-C. Lin, and J.R. Feramisco. 1983. Biochemical characterization of the mammalian stress proteins and identification of two stress proteins as glucose- and Ca^{++}-ionophore-regulated proteins. *J. Biol. Chem.* (in press).

Whitehouse, S., J.R. Feramisco, J.E. Casnellie, E.G. Krebs, and D.A. Walsh. Studies on the kinetic mechanism of the catalytic subunit of the cAMP-dependent protein kinase. (Submitted.)

Yamashiro-Matsumura, S. and M. Kawata. 1981. Methionyl tRNA synthetase induced conformational change of *E. coli* $tRNA^{fmet}$. *J. Biol. Chem. 256:* 9308.

——. Purification of methionyl and glutaminyl tRNA synthetase by affinity elution. (Submitted.)

S. Blose, *G. Blose, A. Bushnell, G.-Y. Cai, D. Meltzer, P. Renna, J. Suhan*

10-nm Filaments Induced to Collapse in Cells Microinjected with Monoclonal Antibodies against Tubulin

S. Blose, D. Meltzer, J. Feramisco

Numerous studies have proposed that the spatial array of 10-nm filaments in a cell is dependent on interactions with an intact cytoplasmic network of microtubules. To probe this possible interaction in vivo, CCL 146 cells were microinjected with several monoclonal anti-tubulins: DM1A and DM3A1 were mouse monoclonal IgG_1 anti-α-tubulins; DM1B and DM3B3 were mouse monoclonal IgG_1 anti-β-tubulins; and mouse monoclonal (IgM) DM3B2 was an anti-α/β-tubulin (Fig. 1). After microinjection, cells were fixed and stained for double-label immunofluorescence at various times to assess any changes in distribution of the 10-nm filaments or microtubules. Within 2–3 hours after injection of DM1A or DM3B3, all of the 10-nm filaments were collapsed into tight aggregates (Fig. 2). If these antibodies were first preabsorbed with assembled microtubules and then injected, the filament collapsing was abolished, while preabsorption with CCL 146 vimentin had no effect on collapsing. Microinjection of DM1B, DM3B2, or DM3A1 had no effect. In the microinjected cells, tubulin staining was observed to be diffuse in the first 30–60 minutes after injection, followed by a period in which some microtubules were then observed (Fig. 3). At 1.5–3 hours after injection, when the 10-nm filaments were maximally collapsed, the complete normal cytoplasmic array of microtubules was observed (Fig. 3). Several cells injected in the early prophase also appeared to assemble a normal mitotic spindle surrounded by a 10-nm filament cage (Fig. 4). This suggests that the injected antibodies did not inhibit microtubule assembly into a spindle. Although the mechanism of 10-nm filament collapse in response to microinjected anti-tubulin is not yet understood, it is likely that interference with cytoplasmic microtubules (through α- and/or β-tubulin) may be responsible. The interference may be through: (1) a transient depolymerization of the microtubules and/or (2) the coating of the microtubule surface with antibody that sterically blocks microtubule-10-nm filament interactions.

Molecular Basis of the Monoclonal Antibody (LCK-16) Cross-reaction with Tropomyosin and 10-nm Filament Proteins: Vimentin and Desmin

S. Blose, F. Matsumura, J. Lin [in collaboration with K. Weber and N. Geisler, Max Planck Institute for Biophysical Chemistry, Goettingen,

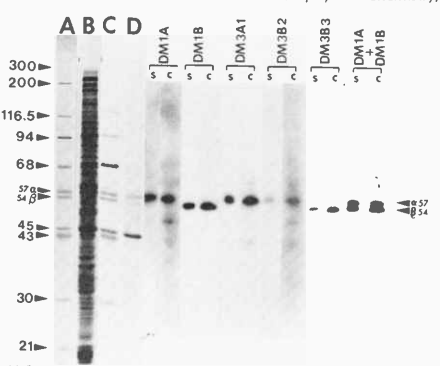


Figure 1

Western blot analysis of the monoclonal anti-tubulin binding to a total cell lysate of the CCL-146 cells. Lane A, M_r standards $\times 10^{-3}$; 57 and 54, correspond to α- and β-tubulin, respectively. Lane B, the total cell lysate. Both of these lanes were 12%-SDS gels stained with Coomassie blue. Lanes C and D, blots of A and B, respectively, stained with amido black. Monoclonal antibody blots of these lanes (s, M_r standards corresponding to lane A; and c, cell lysate corresponding to lane B) revealed that DM1A and DM3A1 stained α-tubulin; DM1B and DM3B3 stained β-tubulin; DM3B2 stained predominantly α-tubulin. The last two lanes (DM1A + DM1B) show that the mixing of DM1A and DM1B stained both tubulin subunits.

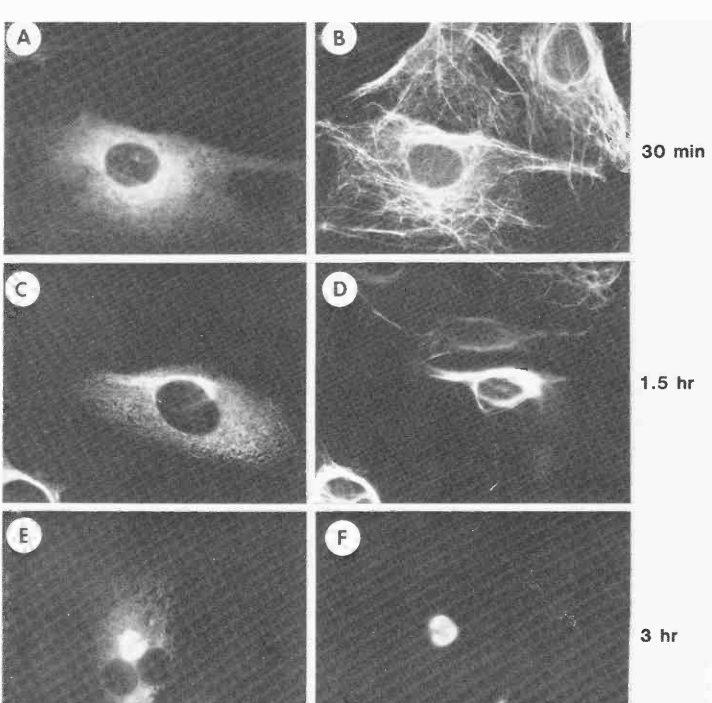


Figure 2

Fluorescent micrographs of cells microinjected with DM3B3, monoclonal anti-β-tubulin, and stained to observe the vimentin filament distribution at 30 min (*A* and *B*), 1.5 hr (C and *D*), and 3 hr (*E* and *F*) after injection. Each pair, *A-B*, *C-D*, and *E-F*, represents the same cell stained for the distribution of DM3B3 with TMRITC goat anti-mouse (*A,C,E*) and for the distribution of the vimentin filaments with FITC-labeled #240 anti-vimentin (*B,D,F*). At each time point, the injected DM3B3 (*A,C,E*) was found to be diffusely distributed in the cytoplasm. Within the first 30 min after injection, the vimentin filaments (*B*) appeared to have the same distribution as an uninjected cell. By 1.5 hr after injection (*D*), the vimentin filaments had collapsed next to the nucleus. At 3 hr after injection (*F*), the vimentin filaments had completely moved into a tight cap next to the nucleus.

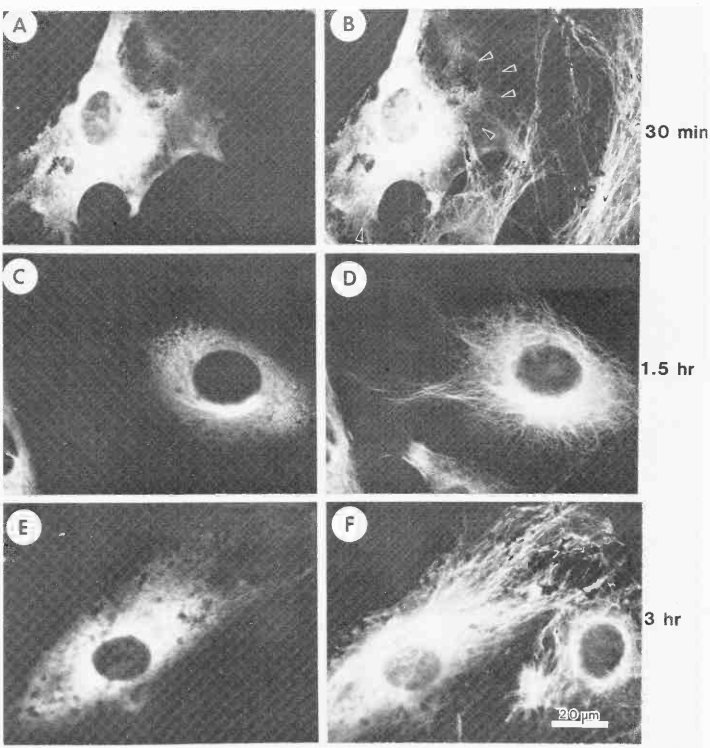


Figure 3

Fluorescence micrographs of cells microinjected with DM3B3 (monoclonal anti-β-tubulin) and stained to observe the microtubule distribution at 30 min (*A,B*), 1.5 hr (*C,D*), and 3 hr (*E,F*) after injection. Each pair, *A-B*, *C-D*, and *E-F*, represents the same cell stained for the distribution of DM3B3 with TMRITC-labeled goat anti-mouse (*A,C,E*) and for the distribution of microtubules probed with FITC-labeled guinea pig anti-tubulin (GP1T4) (*B,D,F*). At each time point, the injected DM3B3 (*A,C,E*) was found diffusely distributed in the cytoplasm. Within the first 30 min after injection, the microtubules (*B*) were hard to visualize with GP1T4,except in the cell periphery. At 1.5 hr postinjection (*D*), microtubules were observed in their normal radial array, and, by 3 hr postinjection, the microtubules maintained their normal cytoplasmic distribution (*F*) at a time when the vimentin filaments were maximally collapsed into a cap (see Fig.2F).

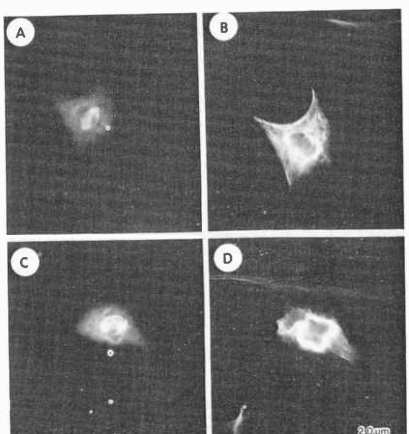


Figure 4
Fluorescence micrographs of two metaphase cells 30 min after microinjection with DM3B3. Each pair, *A-B* and *C-D*, represents the same cell stained for the distribution of DM3B3 with TMRITC goat anti-mouse (*A,C*) and for vimentin filaments with FITC-labeled #240 anti-vimentin (*B,D*). DM3B3 was visualized on the metaphase spindle microtubules (*A,C*); the antibody did not block assembly of the spindle to this stage. The distribution of vimentin filaments formed a cage around the spindle and was not affected by the injected anti-tubulin.

West Germany, and J. Heuser, Department of Physiology and Biophysics, Washington University, St. Louis, Missouri]

We have recently described a mouse monoclonal antibody, LCK-16 (IgM), that cross-reacts with two unrelated cytoskeletal proteins—tropomyosin and vimentin (Blose et al., *Cold Spring Harbor Symp. Quant. Biol. 46:* 455 [1982]). LCK-16 decorates 10-nm filaments in a periodic fashion with a repeat of decoration at ~31–38 nm (Fig. 5A). In collaboration with Dr. John Heuser, we have confirmed that the antibody decoration of the 10-nm filament appears to be helical as determined by stereo-electron micrographs of deep-etched, processed specimens decorated with antibody. The LCK-16 also decorates tropomyosin-containing microfilaments with a 33-nm repeat (Fig. 5B). The antibody causes the microfilaments to aggregate into large macromolecular structures and has been used to isolate native microfilaments from a variety of cell types (Matsumura et al., *J. Biol. Chem.* [1983, in press]). In our attempt to determine where LCK-16 binds to the tropomyosin molecule, paracrystals of skeletal muscle tropomyosin were generated (Matsumura and Lin, *J. Mol. Biol. 157:* 163 [1982]) and then decorated with LCK-16. Electron micrographs made of these negatively stained specimens revealed that (1) the antibody bound with a 38.3 ± 1.8-nm periodicity and (2) that the antibody bound over the aminoterminal overlap region of the paracrystal (Fig. 5C). This region represents approximately one-third of the N-terminal end of tropomyosin. Future studies will focus on the location that LCK-16 binds to within the aminoterminal region.

In collaboration with Dr. K. Weber and N. Geisler, we are also determining where LCK-16 binds to desmin molecule (a 10-nm-filament protein). Desmin was fragmented by enzyme proteolysis and chemical cleavage according to published schemes (Geisler et al., *Cell 30:* 277 [1982]; Geisler et al., *Nature 296:* 448 [1982]). The various fragments were then probed by solid-phase radioimmunoassay using LCK-16. Our preliminary results indicate that LCK-16 binds desmin in the region spanning from the aminoterminus to the sole tryptophan. Work in progress seeks to determine with greater resolution the site to which LCK-16 binds. From these studies, we hope to determine if the tropomyosin and desmin molecules have common functional and/or structural domains.

Publications

Blose, S.H. and A. Bushnell. 1982. Observations on the vimentin-10nm filaments during mitosis in BHK-21 cells. *Exp. Cell Res. 142:* 57.

Blose, S.H., F. Matsumura, and J.J.-C. Lin. 1982. Structure of vimentin 10nm-filaments probed with a monoclonal

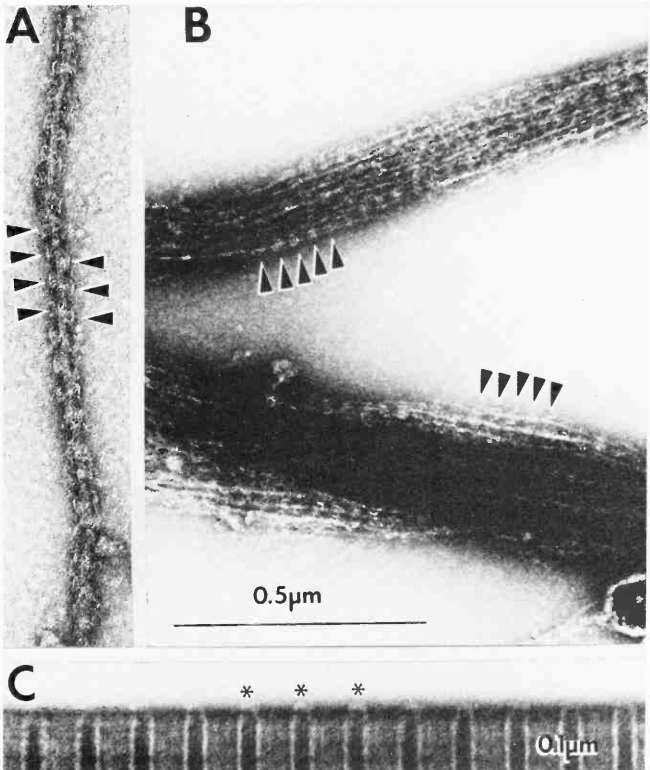


Figure 5
Electron micrographs of negatively stained (uranyl acetate) specimens decorated with a mouse monoclonal LCK-16 (IgM) that cross-reacts with tropomyosin and 10-nm filaments (desmin and vimentin). (*A*) 10-nm Filament decorated with LCK-16. The antibody bound in a helical fashion (arrowheads) with a repeat period of approximately 38 nm. (*B*) Tropomyosin-containing microfilaments isolated from tissue culture cells. LCK-16 caused the lateral aggregation of the filaments and decorated (arrowheads) them with ~33-nm period repeat. (C) Skeletal muscle tropomyosin Mg^{++}-paracrystal decorated with LCK-16. The antibody (*) bound in the amino-overlap region of the molecule; this domain represents a region spanning from the amino terminus one-third into the molecule.

antibody that recognizes a common antigenic determinant on vimentin and tropomyosin. *Cold Spring Harbor Symp. Quant. Biol. 46:* 455.

Blose, S.H., D.I. Meltzer, and J.R. Feramisco. 1982. 10nm-filaments induced to collapse in cells microinjected with antibodies against tubulin. *J. Cell Biol. 95:* 229a.

Lin, J.J.-C., F. Matsumura, and S.H. Blose. 1983. A monoclonal antibody that cross-reacts with tropomyosin and vimentin. *Fed. Proc.* (in press).

Lin, J.J.-C., J.R. Feramisco, S.H. Blose, and F. Matsumura. 1982. Monoclonal antibodies to cytoskeletal proteins. In *Monoclonal antibodies, hybridomas: A new dimension in biological analyses* (ed. R.H. Kennett et al.). Plenum Press, New York. (In press.)

Lin, J.J.-C., K. Burridge, S.H. Blose, A. Bushnell, S.A. Queally, and J.R. Feramisco. 1982. Use of monoclonal antibodies to study the cytoskeleton. In *Cell and muscle motility* (ed. R.M. Dowben and J.W. Shay), vol. 2, p. 63. Plenum Press, New York.

QUEST-2D GEL LABORATORY

J.I. Garrels, *R. Franza, J. Emanuele, L. Cascio, P. Smith*

Our goal for several years has been the establishment of protein data bases derived from quantitative analysis of two-dimensional gels. We have previously established techniques and equipment for routine production of standardized two-dimensional gels, and we have been developing a software package for complete two-dimensional gel analysis. In previous reports, we have described our data management system, our programs for spot detection and integration, and our programs for pattern matching. During the past year, we have greatly enhanced the matching programs, we have developed programs to create and manage our standard patterns (representing spots seen on many gels), we have developed programs to reduce data from multiple films and multiple gels into a single data base file for each sample, and we have developed numerous programs for analysis of data after entry into the data base.

The QUEST Software System

The QUEST software package is large. It contains over 150 separate programs and about 5 million characters of Fortran code and documentation. The programs are all written in a consistent style and use similar naming conventions and data structures throughout. The system is highly integrated in the sense that programs share many subroutines for access of the data-base files and programs communicate with one another by sending packets of data through common memory. The QUEST system has proven easy to use, especially since all user interaction takes place at formatted screens. A thorough description of the QUEST system from the point of view of the user is now in press (Garrels et al., in *Two-dimensional gel electrophoresis of proteins: Methods and applications*, Academic Press, New York [1983]).

The QUEST system is large because it supports all aspects of quantitative two-dimensional gel electrophoresis including the experimental protocols, the record-keeping for the gel laboratory, the film scanning and data reduction, and the analysis of data contained in the data-base files. In this report, we will describe more fully the programs for data reduction and data analysis. The process of data reduction is summarized in Figure 1. A typical sample might be run on several different types of two-dimensional gel (say three different pH ranges) and each gel is usually exposed to film several times (say three times). The task of the data reduction system is to quantitate and combine all of this data into a single data file that best represents the intensity of each protein in the original sample.

The data reduction begins by scanning the film (SCAN) and the calibration film (CALSCAN). A series of programs then detects and integrates the spots resulting in a FILMSPOTS file (FSP). The FSP file is next matched to an appropriate STANDARD file (STD), which represents all spots previously seen on this type of gel. The matching procedure produces a file called MATCHLIST (MTL). Next the data from each FSP file derived from the same gel is combined, using the MATCHLIST information, into a GELSPOTS file (GSP). This file contains the best quantitation of each spot on the gel, considering all available film exposures. Finally, the data from all three gels is combined into a single file called SMPSPOTS (SSP). In the latter process, the computer must know where each protein of the sample appears on each of the separate types of gels. This information is provided to the system by matching the standard patterns to one another.

The final SMPSPOTS files are important components of the protein data base. However, the data base must be flexible. What happens if yet another gel is run, if longer exposures later become available, or if details of the initial match are found to be incorrect? Additions and corrections to the data will frequently occur and our data-base structure must allow the data to be constantly updated and improved. For this reason, we consider the FILMSPOTS and MATCHLIST files to be more permanent than the SMPSPOTS file. With these files available, we can always redisplay and reedit the match of any film to its standard. An automatic series of programs will generate a new SMPSPOTS file from the modified MATCHLIST and FILMSPOTS files. If additional films representing the given sample have been analyzed and matched in the meantime, their data will also be utilized in the generation of the updated SMPSPOTS file. The system presently allows the combining of data from as many as 10 gels per sample with up to five exposures of each gel to film. By this strategy, each protein is quantitated from the gel or gels where it best resolved using the level of exposure to film that best represents its intensity without saturation.

For analysis of reduced (SMPSPOTS) data, we aim to make the data as visible to the user as possible. From any image displayed on the color TV screen, a user can indicate any spot of interest. A simple command brings up the standard spot number, the name of the spot (if known), and the intensity of the protein in any preselected set of samples. If desired, the intensity of the spot throughout a series of samples will be plotted in the corner of the screen. To read other information that might have been previously entered about the spot, the user can bring up forms on the terminal screen containing entries relating to transformation, posttranslational modification, mutations, cell-type specificity, subcellular localization, etc.

To ask which proteins are increased or decreased

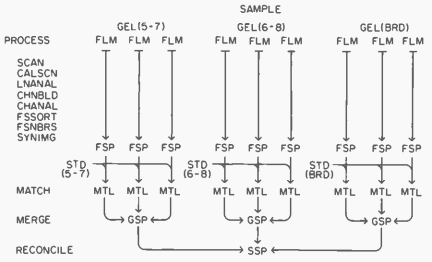


Figure 1

Flow chart for processing of two-dimensional gel data. A radiolabeled sample (*top*) is prepared for two-dimensional gel electrophoresis and the proteins are resolved on several types of gels (for example, three different pH ranges). Each gel is exposed to film for several different lengths of time (three times shown here) to detect both the very intense and the very faint proteins. The task of the data reduction programs is to quantitate and combine the data from these nine films into a single file (the SMPSPOTS or SSP file) in which the best quantitative determination for each protein is recorded. The programs following the SCAN and CALSCAN automatically detect and integrate the spots, creating a FILMSPOTS file (FSP) for each film. The FILMSPOTS files are matched to STANDARD files (STD) to create MATCHLIST (MTL) files. Following the matching process, which is partly manual and partly automatic, the final two stages of data reduction proceed automatically. The MERGE process combines data from all films representing the same gel (yielding a GELSPOTS or GSP file), and the RECONCILE process examines the data from each gel to determine the best overall value for each spot. If a spot is detected without overlap on several gels, its intensity from each gel will be averaged. If a spot is overlapped, or badly shaped on one gel, that data will be ignored and only data from gels that properly resolved the spot will be used. At the end of the data-reduction process, all available information about the original sample has been reduced to a single SMPSPOTS (SSP) file.

under the conditions of an experiment, the user can specify his criteria on the MAKESETS form. The members of a spot set can be selected by degree of quantitative change between specified samples, by range of intensity, by range of coordinates, and by spot quality (excluding overlapped or poorly shaped spots if desired). Using any displayed image, the members of any set can be indicated by a change in color. The computer can be asked to combine spot sets to give, for example, proteins that both increase in transformed cells and that respond to a growth factor. Many spot sets can be selected and stored permanently.

An overall measure of difference between any two samples can be obtained by the COMPARE form which can be used to plot either a scatter plot (a log-log plot in which each protein is plotted as its intensity in the first sample versus its intensity in the second sample), or as a histogram of protein intensity ratios. These programs report satistically the standard deviation of the distribution of intensity ratios, which is a measure of the overall difference.

Until the QUEST system was complete, including these data reduction and data analysis programs, data could not be entered into the permanent data base and little information could be extracted from the system. Now data are flowing into the system at a steady rate and our data on normal and transformed cells are being analyzed. Further improvement to the throughput of the system will require a faster computer supporting two or more graphics work stations, allowing several users to enter and analyze data simultaneously.

Studies of Human Disease

During 1981 we concluded our electrophoresis work on human fibroblasts from individuals with cystic fibrosis (CF). In the final round of experiments, we prepared 75 samples from 21 cell lines representing both normal and CF genotypes. Each of these samples has been run on two-dimensional gels optimized for different pH and molecular-weight ranges. A total of 484 gels and nearly 1000 film exposures have been completed as part of these experiments.

The complete computer analysis of this much data is still beyond the capacity of our computer system, but we have done a computer-assisted analysis of approximately 1100 proteins from 13 cell lines. The computer was used to generate a large map of the human patterns and to number each of the proteins. The films were compared visually for major differences and the observed differences were recorded by spot number in computer files. The computer was then used to determine whether any of the observed differences correlated with the presence of the genes for cystic fibrosis. Among the 13 cell lines (4 normals, 2 heterozygotes, and 7 CF homozygotes), 20 proteins were found to be missing or greatly reduced in one or more cell lines. These appear to be natural polymorphisms in the population; none of the

observed differences correlated with the presence of the cystic fibrosis genes.

Our data on human fibroblasts from the cystic fibrosis study and the Huntington's disease study (see Annual Report 1981) constitute an important resource that we intend to analyze more fully to form a human fibroblast data base. This is now being done gradually, but since our computing resources are still limited, the task will require some months. We hope within the next 12–18 months to upgrade to a system with much more speed and storage capacity, and we expect the upgraded system to support two or more graphic work stations so that matching and analysis can be done by several users at once.

Studies of Transformed Cells

Our main application of the quantitative two-dimensional gel technology is the thorough characterization of a single cell line and its transformed derivatives. Our criteria for choosing a line are that it be stable in culture, that its origin and history are known, that it can be grown in defined media, and that it can be transformed by various agents including SV40 and adenovirus. The line chosen is the REF52 rat embryo line recently described by W. Topp and M. Hightower (see Annual Report 1981). A defined medium for REF52 cells has been reported by Topp and Hightower, in collaboration with D. McClure of the University of California, San Diego. These workers have isolated a series of SV40 transformants of REF52, and some of these transformed lines have been further selected for growth in soft agar and for tumorigenesis in nude mice. The nutritional requirements and growth characteristics of each transformed line are already known. Other derivatives of REF52, including adenovirus and Kirsten murine sarcoma virus transformants, are being characterized by Topp and colleagues. With this series of cell lines and the helpful cooperation of Bill Topp, we have begun an extensive series of studies to determine how protein changes are related to growth control and transformation.

Since his arrival to the laboratory, Robert Franza has cultured all of the normal and transformed REF52 lines and prepared the samples that we consider necessary to establish the core of our REF52 data base. Each line has been labeled with [^{35}S]methionine at a variety of densities and growth rates. The normal REF52 line has been subcloned to measure its clonal heterogeneity, and the stability of the parent line has been determined by labelings done over a period of months in continuous culture. The labeling protocols have included short pulses, long steady-state labelings, and pulse-chase experiments. In addition to normal [^{35}S]methionine labeling, many lines have also been labeled with [^{33}P]phosphate and with [^{3}H]mannose.

Each line has been grown in both regular (serum-containing) medium and in defined medium. Single components have been selectively deleted from the defined medium to reveal the response to EGF, vasopression, HDL, insulin, and hydrocortisone. Other growth factors, such as IGF2, and other treatments, such as tumor promoters, tunicamycin, calcium ionophores, and heat shock, have been tested for their effects on normal REF52 and some of its transformants.

For each of these samples, gels of several pH and acrylamide concentrations have been run and both long and short exposures of the gels to film have been prepared. A large number of reproducible, high-quality films are now being quantitatively processed by the system. Experiments with labeling times, defined versus regular medium, and some heat-shock experiments have already been partially analyzed for both normal and SV40-transformed cells. As this baseline data on normal and transformed REF52 cells is entered into our computer, we can define sets of proteins that are altered in rate of synthesis, turnover, or phosphorylation with each alteration of the medium or at each stage of transformation. For any protein of particular interest, we can ask the computer to create a table and a graph immediately that will report the amount of the protein in each sample of any experiment that has been analyzed. This core of data will be informative in terms of overall relatedness of responses and in terms of defining proteins of particular interest. Moreover, it will serve as background information against which to compare the results of our next line of experimentation.

We have begun, in collaboration with Jim Feramisco, a series of experiments based on microinjection of cloned genes into the nucleus of REF52 cells. Initial experiments have shown successful transformation of REF52 cells by microinjection of the SV40 *A* gene contained on the pSV7 plasmid kindly provided by Dr. T. Shenk of the State University of New York, Stony Brook. Approximately 30 cells growing on a small square of glass were injected with the plasmid. After the injected cells had proliferated to approximately 300 cells, they were labeled with [^{35}S]methionine and run on a two-dimensional gel. Figure 2 shows the gel obtained. It is clearly possible to detect thousands of proteins from the small numbers of cell involved in microinjection experiments. This demonstration opens the way for the study of early events that occur in cells after injection of a transforming gene, a mutated gene, or a purified protein or antibody. It is possible to label cultures in which every cell is known to be injected and in which the exact cell numbers and doubling times are known by direct observation. Using the knowledge of protein changes in REF52 cells that is becoming available in our data base, we can use the microinjection techniques to dissect further the responses by direct manipulation of gene expression and functional protein content.

Collaborative Experiments

We have continued fruitful collaborative experiments with several groups at Cold Spring Har-

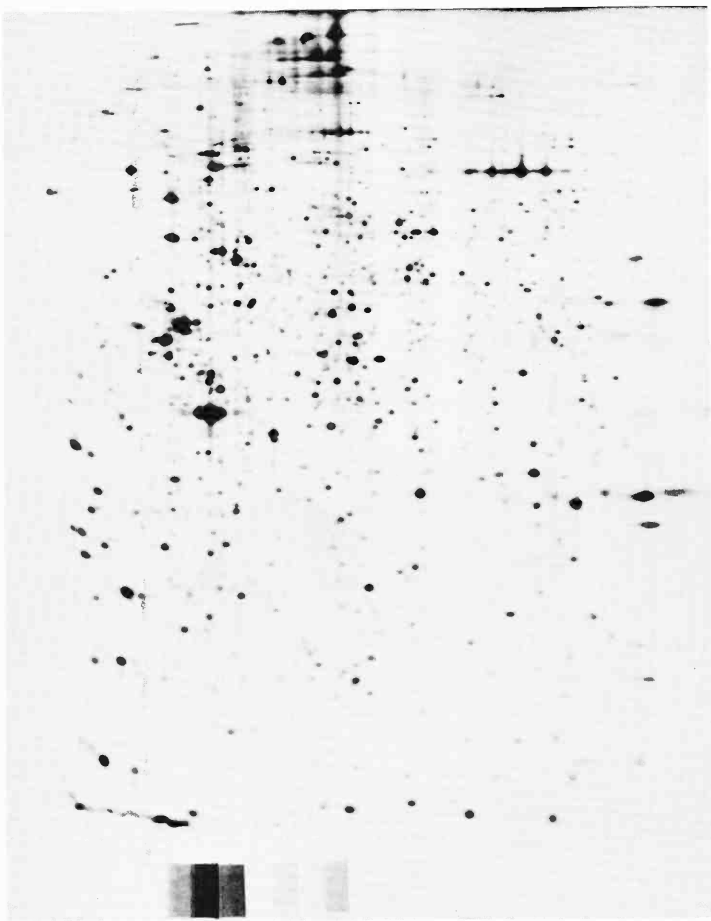


Figure 2
Proteins from REF52 cells transformed by microinjection. Normal REF52 cells on a glass chip were microinjected with a plasmid containing the SV40 early region. DNA was placed directly into the nucleus by pressure injection from a micropipette. Nine days later, the chip covered with about 300 morphologically transformed, rapidly dividing cells was labeled with 250 μCi of [^{35}S]methionine for 12 hr and the proteins were resolved by two-dimensional gel electrophoresis. The pattern shown represents the broad-range pH gradient resolving most proteins from pH 4 to 8. About 4.5 million dpm were applied to this gel and it was exposed to film for only 19 hr. This result demonstrates that we can now study transformation in REF52 cells at many time points beginning immediately after microinjection of transforming DNA. The experiment was done in collaboration with Jim Feramisco using the pSV7 plasmid kindly provided by Dr. T. Shenk.

bor Laboratory. The heat-shock work has continued in conjunction with Bill Welch, Jim Feramisco, and Paul Thomas. The major heat-shock proteins of HeLa and rat cells have been detected and quantitated on our system, and their modifications by phosphate and carbohydrate have been examined. (See Cell Biochemistry Section for details.)

We have continued to collaborate with Lee Silver to examine the protein differences in mice carrying various *t*-locus haplotypes. A set of nine *t*-locus-specific proteins has been identified, and these have been further mapped into specific regions of the *t* locus on chromosome 17. (See Molecular Genetics of the Mouse Section and the references below for more details.)

In collaboration with Michele Franceour and Mike Mathews, we have analyzed proteins immunoprecipitated by antisera from patients with lupus erythematosis. Most of these recognize proteins of ribonucleoprotein complexes and the same proteins have been identified in whole-cell protein patterns for HeLa cells. A recent antisera has been shown to recognize a different nuclear protein which is more predominant in dividing than in quiescent cells, and this protein is especially high in SV40 or adenovirus-transformed cells. This protein will be further characterized in collaboration with Mike Mathews.

Publications

Francoeur, A.M., J.I. Garrels, and M.B. Mathews. Purification and characterization of the lupus antigen, LA. (In preparation.)

Garrels, J.I. 1983. Quantitative two-dimensional gel electrophoresis of proteins. *Methods Enzymol. 100:* 411.

——. 1983. Major phosphoproteins of cultured muscle cells: Kinetics of phosphorylation and induction by heat shock. In *Muscle development: Molecular and cellular control* (ed. M.L. Pearson and H.F. Epstein), p. 43. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Garrels, J.I., J.T. Farrar, and C.B. Burwell IV. 1983. The QUEST system for computer-analyzed two-dimensional gel electrophoresis of proteins. In *Two-dimensional gel electrophoresis of proteins: Methods and applications* (ed. J.E. Čelis and R. Bravo). Academic Press, New York. (In press)

Lane, E.B., M. Kurkinen, B.L.M. Hogan, and J.I. Garrels. 1983. Coexpression of vimentin and cytokeratins in cells of the early mouse embryo. *Nature 303:* 701.

Lin, J.-C., W.J. Welch, J.I. Garrels, and J.R. Feramisco. 1982. The association of the 100-KD heat-shock protein with the Golgi apparatus. In *Heat shock: From bacteria to man* (ed. M.J. Schlesinger, M. Ashburner, and A. Tissières), p. 267. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Silver, L.M., D. Lukralle, and J.I. Garrels. 1983. T Orl is a novel, variant form of mouse chromosome 17 with a deletion in a partial t haplotype. *Nature 301:* 422.

Silver, L.M., J. Uman, J. Danska, and J.I. Garrels. 1983. Polymorphic t complex proteins and the origin of mouse t haplotypes. *Cell* (in press).

Thomas, G.P., J.I. Garrels, and M.B. Mathews. Differential induction of the stress response proteins in HeLa cells and chick fibroblasts. (In preparation.)

Welch, W.J., J.I. Garrels, and J.R. Feramisco. 1982. The mammalian stress proteins. In *Heat shock: From bacteria to man* (ed. M.J. Schlesinger, M. Ashburner, and A. Tissières), p. 257. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Welch, W.J., J.I. Garrels, G.P. Thomas, J.-C. Lin, and J.R. Feramisco. 1983. Biochemical characterization of the mammalian stress proteins and identification of two stress proteins as glucose- and Ca^{2+} - ionophore-regulated proteins. *J. Biol. Chem. 258:* 7102.

Neurobiology

Year-round neurobiology has been growing by leaps and bounds, and in the last year, the two neurobiology groups have easily doubled in size. Although much of the emphasis remains focused on the leech, an exquisite model system introduced to modern neuroscience by John Nicholls, work is also being carried out on the far more comlex mammalian brain. The attraction of leech neurobiology continues to be the possibility of a multifaceted approach combining antibody technology with electrophysiology, biochemistry, histology, and developmental studies. Interestingly, what is still missing is precisely what Cold Spring Harbor Laboratory is famous for—no one has yet peeked at leech DNA. The Neurobiology summer teaching program was updated again with the introduction of new courses emphasizing the latest technologies in the field.

LEECH NEUROBIOLOGY

B. Zipser, *T. Flanagan, M. Flaster, C. Schley*

In the past few years, monoclonal antibodies against single types of neurons or small sets of neurons have taught us a great deal about the molecular neuroanatomy of the adult leech nervous system. While new antibodies are still being generated, we are using our existing antibodies to analyze in detail the temporal and spatial pattern of antigen expression during neuronal development, the biochemical nature of the neural antigens, and the physiological function of neurons carrying these specific antigens. We are also happy to have succeeded in disseminating monoclonal antibody technology among our colleagues in the leech field. Other laboratories such as that of Bill Kristan (University of California, San Diego) and Gunter Stent (University of California, Berkeley) are tooling up to generate their own leech monoclonals, thus promising to make leech monoclonal antibodies a vibrant field.

Characterization of a Previously Unknown Type of Leech Neuron

T. Flanagan

While many of the monoclonal antibodies generated over the course of the last few years stain neurons that repeat from ganglion to ganglion—such as the N cells responding to noxious stimulation—some antibodies were made that label single types or small sets of neurons found only in one or two specialized ganglia. An example is Lan3-11, a monoclonal antibody that stains a pair of cell bodies only present in the second ganglion of the head and in the second ganglion of the midbody chain. To explore the specialized function of such a neuron previously largely ignored in leech neurobiology, the Lan3-11-labeled neurons were characterized with respect to their detailed morphological and electrophysiological signatures.

The pair of Lan3-11-labeled cells in segmental ganglion 2 have been identified through a double-labeling experiment combining intracellular Lucifer Yellow injection with antibody staining (Fig. 1). These neurons are readily identified in the live ganglia. Their large cell bodies are easily distinguished from neighboring pressure cells and Leydig cells because of their characteristic action potential, a 35-mV spike. Unlike the Leydig cells, the pair of Lan3-11-labeled cells is not electronically coupled to each other.

After intracellular recordings were taken, the Lan3-11 somata were filled with a fluorescent dye (Fig. 1b). Lan3-11 cells project into adjacent anterior and posterior ganglia, but do not project directly into segmental sensory fields through the ganglionic roots and they display only sparse neurite arborizations within their ganglion of origin. This may suggest that these cells have specialized to coordinate events anterior and posterior to the second segmental ganglion, rather than processing information primarily obtained within the second segmental ganglion. The first segmental ganglion contains neurons that process signals descending from cephalic photoreceptors (E. Peterson, pers. comm.). In subsequent studies, we plan to examine the Lan3-11 cells' response to photoreceptor stimulation, as Lan3-11 cells may be second-order photosensory interneurons.

We have not found any somata homologous to the Lan3-11 cells in other segmental ganglia. The results already available from this study indicate the utility of antigenic markers in locating unique cell types within neuronal systems, and further studies are intended to indicate the functional significance of such rare cells. In this application, the antigen serves as a marker, and while we have a genuine interest in the identity of this antigen (i.e., the Lan3-11 antigen may be a neuropeptide), the characterization of cells does not depend upon the characterization of antigens.

Characterization of Broadly Distributed Neuronal Antigens

M. Flaster, C. Schley, T. Flanagan, B. Zipser

In addition to neuron-specific monoclonal antibodies whose staining pattern includes a single neuron pair or only a handful of neurons in each segmental ganglion, we have generated monoclonal antibodies that stain large sets of neurons. One such antibody, Laz2-1, stains about 40 neurons in each segmental ganglion (Fig. 2A). This relatively large, but nevertheless restricted set of antigen-bearing cells, includes a diversity of cell types. To understand the significance of this staining pattern, a multifaceted investigation has been initiated. Intracellular recording and dye injection of cells in the Laz2-1 staining pattern has shown that some, but not all of the primary mechanosensory cells, are members of this set. Other identified neurons include the annulus erector motor neurons and the anterior pagoda cells (of yet unspecified function). Morphologically, all members of this set studied to date project peripherially via the roots of the ganglion in which they are situated. As additional members of the antigenically related set are identified, we hope to discern a relationship among the cells that may include either a common function or a common pattern of connections.

In addition to the physiological investigations, we have begun a developmental study using Laz2-1 with our collaborators, R. Stewart and E.R. Macagno at Columbia University. The Laz2-1 antigen appears in a few neurons quite early in embryogenesis, but does not appear in some cells until well after all

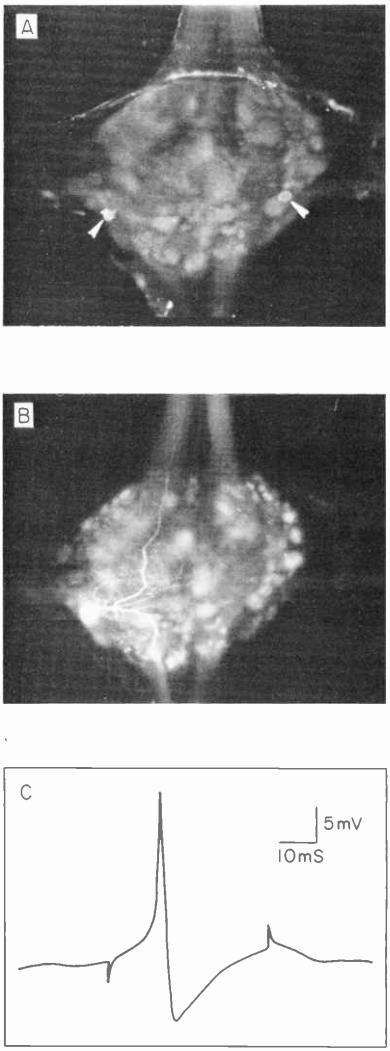


Figure 1
(*A*) Rhodamine-labeled Lan3-11 cells. (*B*) Lucifer Yellow-filled Lan3-11 cell in the same preparation as above. (C) Electrical recording of the Lan3-11 cell prior to Lucifer Yellow-injection.

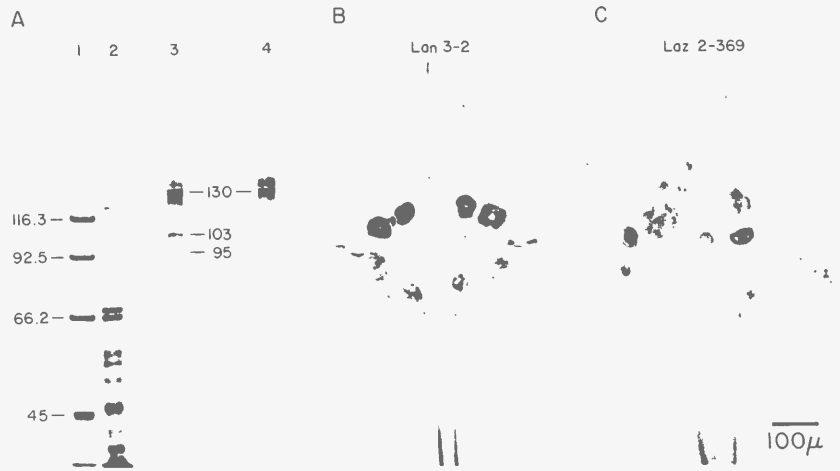


Figure 2
Immunoblot and histological data on Lan3-2 and Laz2-369. (*A*) On a 10% SDS-acrylamide gel, high-molecular-weight markers were run on lane 1 and extracted central nervous system proteins on lanes 2, 3, and 4. Proteins were transferred from the gel to nitrocellulose. The strip of nitrocellulose carrying lanes 1 and 2 was stained with amido black to visualize molecular-weight markers and leech central nervous system proteins. Lanes 3 and 4 were separately stained with monoclonal antibodies Lan3-2 and Laz2-369, respectively. Both monoclonal antibodies stain major broad bands centered at 130K. Lan3-2, in addition, stains two minor bands at 103K and 95K. (*B*) Lan3-2 brilliantly labels the two pairs of nociceptive cell bodies in a 4% paraformaldehyde-fixed leech ganglion in the presence of Triton X-100. Lan3-2-stained axons are visible in the fiber tracts above and below the ganglion. Lan3-2 (not shown here) will stain surface antigens in live ganglia. (*C*) Laz2-369 stains the pair of lateral nociceptive cell bodies in a live ganglion, suggesting that its antigen is localized on surface membrane.

cells in a typical midbody segmental ganglion are presumed to be postmitotic. Cellular developmental events concomitant with the appearance of the Laz2-1 antigen in identified cells may be experimentally accessible.

Neuron-specific Monoclonal Antibodies Cross-react with Other Select Tissues

N. Hogg, M. Flaster, B. Zipser

Three monoclonal antibodies, originally studied because of their neuron-specific staining in the leech central nervous system, have been characterized further, both immunocytochemically and biochemically (with Western blot staining) in the central nervous system and peripheral tissues. The three antibodies react both with neurons and select epithelial tissue in the central nervous system, gut, and penis. Antibody Lan3-8, reacts with neurons in the nerve cord and gut and with epithelial cells in the penis; it binds to a 65K molecule in all three tissues. Lan3-2 and Laz2-369 are considered as a related pair of antibodies; the former stains all (four) and the latter generally only half (two) of the neurons in a standard midbody ganglion responding to nociceptive stimulation (see Fig. 3). In the gut, both antibodies label patches of epithelial cells and Laz2-369 stains a previously unknown type of gut neuron. Whereas a given antibody stains different bands in gut and central nervous system immunoblots, comparing the bands of both antibodies for the same tissue extract makes it apparent that there are similarities in the molecular species that both antibodies recognize.

For each monoclonal antibody, the histologically identified tissue antigens need to be correlated with proteins identified on Western blots. Of particular interest are the broad 130K bands of central nervous system extract to which Lan3-2 and Laz2-369 bind (see Fig. 2A). The question is raised whether the molecular species in these bands represents a family of proteins that serve a specific nociceptive cell function.

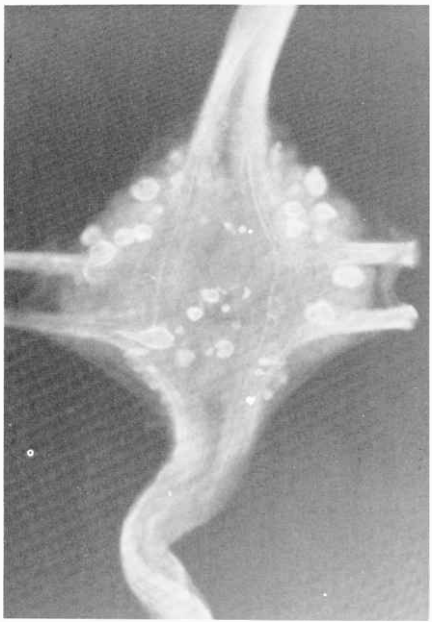


Figure 3
Histological data on Laz2-1. (A) Laz2-1 stains about 45 cell bodies in a leech ganglion. Laz2-1 labels axons in the fiber tracts below and right and left of the ganglion.

Gel Band Fusion

B. Zipser, M. Flaster, C. Schley

One of the major goals of our research is to detect neuron-specific molecules (proteins) using monoclonal antibody technology and then to further characterize them biochemically. A powerful first step in the biochemical characterization of leech nervous system antigens is their identification on nitrocellulose replicas of SDS-gels (immunoblots); however, the antigen(s) that binds a monoclonal antibody on immunoblots is not necessarily the same as the antigen(s) localized by the same monoclonal antibody in fixed tissue. We tested the strength of the correlation between histological and immunoblot data by generating monoclonal antibodies against SDS-denatured protein in a particular immunoblot band and successfully obtained new monoclonal antibodies with histological and biochemical binding properties similar to those of the original neuron-specific monoclonal antibodies. Two previously described monoclonal antibodies—Lan3-2, which stains both pairs of N cells in a typical leech segmental ganglion, and Laz2-369, which characteristically stains only the lateral N-cell pair—both elicit bands on SDS-gel immunoblots, including bands centered at 130 kD. We excised the 130-kD region from SDS-polyacrylamide gels and used it as immunogen in two spleen cell fusions. Hybridomas were screened histologically on whole mounts of leech ganglia in one fusion and were screened on immunoblots in a second fusion. Of the 350 clones screened histologically, 16% were positive. Of these, 60% were positive on immunoblots, with two monoclonal antibodies resembling Lan3-2 and one resembling Laz2-369. Of the 148 clones screened initially on immunoblots, 30% were positive of which three monoclonal antibodies resembled Lan3-2 while three others resembled Laz2-369.

The Lan3-2-like monoclonal antibodies share the original Lan3-2 properties: They stain all four N cells in a typical midbody segmental ganglion, they stain an array of axon bundles in the ventral quadrants of cryostat cross sections of the interganglionic connectives, and they elicit immunoblot bands at 130, 103, and 95 kD as does the original monoclonal antibody, Lan3-2. The Laz2-369-like monoclonal antibodies characteristically stain the lateral N-cell pair, stain an array of axon bundles in the ventral quadrants of cross sections of the interganglionic connectives, and elicit an immunoblot band centered at 130 kD, as well as apparently high-molecular-weight material just below the stacking gel/running gel boundary, as does Laz2-369. We did not succeed in obtaining monoclonal antibodies that separated any of the properties of the original N-cell monoclonal antibodies. Entirely novel monoclonal antibodies, as judged by their immunoblot staining patterns, were also isolated. They are conveniently grouped into two classes: monoclonal antibodies yielding multiple bands, some of which include the three Lan3-2 bands as well as several different bands of both higher and lower molecular weight, and monoclonal antibodies yielding single bands of varying width and mean molecular weight in the 130-kD region. Both of these classes are currently being analyzed in more detail.

Gel band fusions are an interesting experimental strategy from several points of view. They can demonstrate that determinants present in extracted protein and in fixed tissue are equivalent. Additionally, the frequency of histologically positive monoclonal antibodies whose binding is SDS-resistant is enhanced (roughly twofold in our experience), a useful property when one wishes to move from histological to biochemical analyses of neuronal antigens. Finally, should a family of CNS proteins of similar molecular weight exist, they would be demonstrable using this approach.

The Expression of Antigens by Embryonic Neurons and Glia in Segmental Ganglia of the Leech *Haemopis marmorata*

B. Zipser [in collaboration with E. Macagno and R. Stewart, Columbia University]

Monoclonal antibodies raised against adult leech nervous systems were screened on embryos of the leech *Haemopis marmorata* to determine when in development specific antigens are first expressed and the order in which they are expressed by different cells or tissues. Three of the monoclonal antibodies produced by Zipser and McKay (*Nature 289:* 549 [1981]) were screened: Lan3-1, Lan3-5, and Lan3-6. Each monoclonal antibody shows a different pattern of labeling in the adult leech nerve cord (Zipser, *J. Neuroscience 2:* 1453 [1982]). The embryonic stages studied were from 5 days after egg deposition to 30 days (hatching). The pattern of labeling was assayed in whole mounts using HRP-conjugated second antibodies. The principal results are: (1) Antigens recognized by Lan3-5 are first expressed by the glia of the roots of the anterior segmental ganglia at 6–7 days, several days later by the interganglionic connective glia, and near the end of embryonic development by ganglionic neurons. An anterior-to-posterior temporal gradient is observed in the expression of these antigens. In addition, Lan3-5 also labels the protonephridia and nephridia from early development onwards. (2) Antigens recognized by Lan3-6 are first expressed by a pair of neurons in each segmental ganglion and later in development by additional neurons. By the time of hatching, however, only about half of the neurons that label in the adult have done so, implying that some neurons express these antigens postembryonically. Labeling with Lan3-6 is first seen in neuronal somata, and only later in neuronal processes. (3) Antigens recognized by Lan3-1 and expressed by segmentally specific neurons in ganglia 5 and 6 are not detectable during embryonic development but are detected in early postembryonic stages. Thus, these three mono-

clonal antibodies provide an approach to the study of different aspects of the development of the leech nervous system, specifically the relation between glial and neuronal differentiation and the genesis of segmentally specific phenotypes.

A New Monoclonal Antibody Specifically Stains the Pressure Cells of the Leech

C.M. Loer, C. Schley

The success of Zipser and colleagues in producing monoclonal antibodies to the leech nervous system has brought other neurobiologists to Cold Spring Harbor to learn the techniques of hybridoma production and culture. Curtis Loer, a graduate student from Bill Kristan's lab at the University of California, San Diego, came to the Zipser lab to produce monoclonal antibodies to be used in studying the development of the nervous system of the glossophoniid leech, *Haementeria*. Under the expert guidance of Carol Schley and Birgit Zipser, 201 hybridoma clones were produced and screened for the production of antibodies to the *Haementeria* nervous system. Of 52 positive clones (an unusually high rate of positive clones), some 30 were chosen to be saved and studied. Antibodies from the clones bound to a large variety of components of the leech nervous system: to all or almost all neurons (5), axon hillocks (6), muscle, glial, and connective tissue (12), unidentified fibers (6), varicosities (4), small subsets of neurons (8), and others (11). Some of the antibodies gave staining patterns similar or identical to antibodies produced in the past to *Haemopis* nervous tissue, while others revealed patterns never before seen. One particularly impressive antibody specifically stains two pairs of well-characterized sensory neurons found in each ganglion, the pressure (P) cells (Fig. 4). This antibody is unusual in that not only is it quite specific for these cells, but it marks the neurites of the P cells as well as their cell bodies. This particular antibody should prove very useful in studying the development of these sensory neurons.

Publications

Hogg, N., M. Flaster, and B. Zipser. 1983. Cross-reactivities of monoclonal antibodies between select neuronal and epithelial tissues. *J. Neurosci. Res.* (in press).

Macagno, S., R. Stewart, and B. Zipser. Monoclonal antibodies serve as developmental markers for leech peripheral and central neurons. (Submitted.)

Zipser, B. 1982. Complete distribution patterns of neurons with characteristic antigens in the leech central nervous system. *J. Neurosci. 2:* 1453.

——.1982. Monoclonal antibodies against specific neurons. Second Embo Practical Course: Immunocyto-

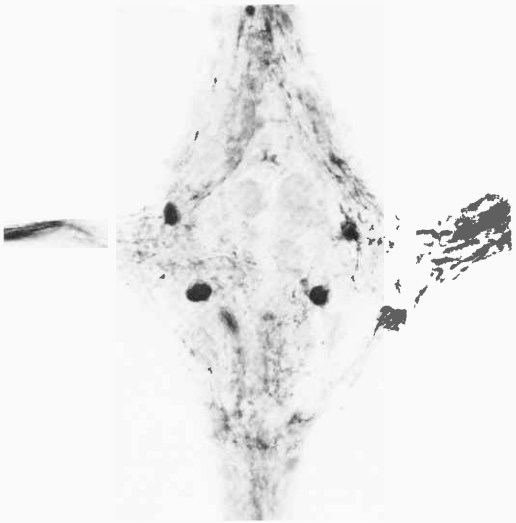


Figure 4
The Loer monoclonal antibody staining pattern in a *Haemopis* ganglion.

chemistry and its application in brain research. Amsterdam.
——.1982. Preparation of monoclonal antibodies and their advantage in identifying specific neurons. *Adv. Cell. Neurobiol.* (in press).
Zipser, B. and C. Schley. 1983. Two differently distributed central nervous system antigens to which a single monoclonal antibody binds can be differentiated through different fixations. *N.Y. Acad. Sci.* (in press).

ANATOMICAL STUDIES WITH MONOCLONAL ANTIBODIES

S. Hockfield, *E. Waldvogel, L. Kleina, S. Hendry*

Much of neurobiology over the last hundred years has involved the description of different neuron types. Anatomical, physiological, and pharmacological approaches have all been used to categorize neurons in an attempt to understand how the nervous system is organized. Until the advent of hybridoma technology, there was no means to look for molecules that correlated with or determined these characteristics. Our studies using monoclonal antibodies examine the molecular diversity of neurons in the central nervous system of vertebrates and invertebrates to determine relationships among neuron subclasses at the molecular level.

Antibodies to the Leech Nervous System

In the last year, we have further developed techniques for immuno-electron microscopy, allowing us to visualize antibody binding sites while preserving ultrastructural quality. This has allowed us to pursue our analysis of the subcellular organelles that carry specific antigens. The relative simplicity of the leech central nervous system is seen in the repeating organization of approximately 400 neurons in each segmental ganglion. The positions, sizes, and shapes of these neurons have been shown to be consistent by anatomical, physiological, and immunological techniques. In the leech, however, interactions between neurons do not occur on cell bodies but on fine processes of these cells (axons). The organization of axons in the leech is apparently more complex than that of cell bodies, as 5000 axons run in a bundle (called the connective) between each pair of ganglia. We have found that the antibodies first described to recognize subsets of neuron cell bodies in the leech by Zipser and McKay in 1981 also recognize subsets of axons (Hockfield and McKay 1983). Using this information, we have shown that antibody-identified axons occupy consistent positions in the connective. Although the general features of axon position and symmetry between the two halves of the connective are quite closely conserved, the fine details are not. For example, the number of axons in a particular fascicle may vary as may the position of the fascicle within an arc of approximately 30°. The determination of axon position must be a regulated event. In many cases where small subsets of axons are antibody positive, these axons travel together in bundles (fascicles) and are not distributed throughout the connective, even when the cell bodies recognized by that antibody are dispersed in the ganglia. This suggests that the presence of the antigen recognized by a particular antibody may be related to axon position. Many hypotheses for determination of axonal trajectory have postulated the presence of surface markers to serve as recognition cues. We have shown that in at least two cases when antibody-positive axons travel together in fascicles, the antibody recognizes an antigen present on the surface of the axons. This observation strengthened the possibility that these antigens might be involved in axon position finding. One requirement for such a molecule is that, if it does provide cues for the determination of specific routes, it be present from the earliest stage of axon outgrowth. We have shown, using immuno-electron microscopy, that during embryogenesis the antigens are indeed present from the earliest stages of axon outgrowth. Importantly, even in young embryos, the antibody-positive axons travel together in bundles and the antigen is located on the surface of these axons. Other details of the organization of specific identified axons are described in the Molecular Studies of the Nervous System Section.

What does the demonstrably great degree of antigenic diversity among leech neurons reflect in the functioning nervous system? Does this diversity reflect variations in a single characteristic among neurons, or are there many characteristics that vary to differentiate neurons from one another? Our electron microscopic studies of the organelles that carry the antigens recognized by monoclonal antibodies address these questions. It was possible that all the variability might be reflected in a single characteristic, such as different surface antigens as described above. We have found, however, that antibody-binding sites, while consistent among all elements recognized by each antibody, vary considerably between antibodies. For example, in contrast to the results described above, antibody Lan3-8 recognizes an internal antigen. Lan3-8 binds to an antigen present in all neuron cell bodies of the leech central nervous system but in only a subset of axons. At the ultrastructural level, using electron microscope-immunocytochemistry, this antibody appears to bind to cytoskeletal elements of axons. In 1-μm-thick plastic sections of leech ganglia, Lan3-8-stained cell bodies have an uneven distribution of antigen similar to a neurofilamentous network. The molecular weight of the Lan3-8-antigen (65,000 daltons) approximates that of one of the core neurofilament proteins. However, immunoblots run concurrently with Lan3-8 and an antibody that recognizes intermediate filament proteins shows a slight difference in molecular weight between the Lan3-8 antigen and a major band identified with the anti-neurofilament antibody.

Lan3-8-positive axons are located towards the perimeter of each of the large lateral connectives and in the medial connective. Unlike axons stained with antibodies that recognize surface antigens, Lan3-8-positive axons are not grouped together into

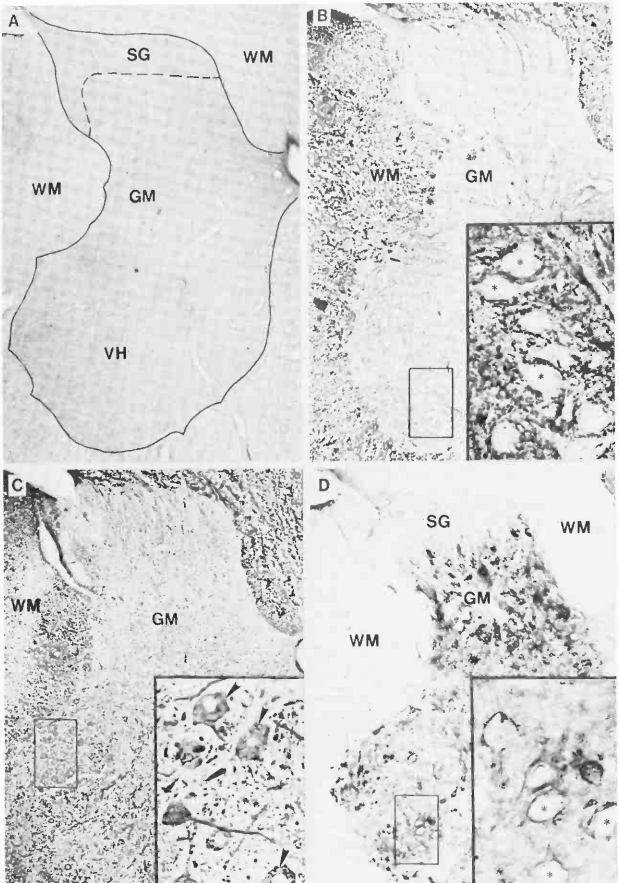


Figure 1

Transverse sections (50 μm thick) of the cervical spinal cord illustrate the staining patterns achieved with four different monoclonal antibodies (*A*). An antibody that did not bind to spinal cord sections left both the gray (GM) and white matter (WM) unstained. SG, substantia gelatinosa; VH, ventral horn. (*B* Cat-101 bound to axons in the white matter (WM) and gray matter (GM). In the ventral horn, antibody-labeled axons outline the unstained, negative images of cell bodies. The small rectangular area is shown at high magnification (*inset*) with unstained cell bodies (asterisks) outlined by stained processes. (*C*) Cat-201 bound to axons in the white (WM) and (GM) matter and to cell bodies. Prominently stained cell bodies are found in the ventral horn motor neuron pools. The *inset* shows antibody-staining localized to the cytoplasm of labeled cell bodies (arrowheads). (*D*) Cat-301 bound to neuron cell bodies and proximal dendrites in many areas of the spinal cord gray matteer (GM) but not to axons. The substantia gelatinosa (SG) contains no antibody-labeled cell bodies. The large ventral horn neurons (asterisks) shown enlarged in the *inset* demonstrated that Cat-301 binds to the surface of neurons. Magnification, 6 ×; *insets*, 25.6 ×.

fascicles and often travel among a number of unstained axons. Although it is tempting to correlate surface antigens with axons that form fascicles and internal antigens with axons dispersed in the connective, results with another antibody, Lan3-6, indicate that there is no simple correlation between the subcellular location of an antigen and axon fasciculation. Lan3-6 recognizes an internal antigen in axons that form a small number of axon fascicles. Further studies will be necessary to show what kinds of antigens actually participate in the determination of axon position. That different antibodies have different subcellular binding sites shows that there is more than a single characteristic that can vary to differentiate one neuron from another.

Antibodies to the Vertebrate Central Nervous System

Perhaps the most exciting result of the past year was the generation of antibodies that recognize subsets of neurons in the vertebrate central nervous system (McKay and Hockfield 1982). This showed that the hybridoma technique so successful in the leech could also be applied to the analysis of the vertebrate brain. The initial panel of approximately 50 antibodies (generated against the adult vertebrate spinal cord) included antibodies specific for cell bodies, for axons only, and for axons and cell bodies. Each antibody has a characteristic staining pattern in every area of the central nervous system we have examined. In the spinal cord (Fig. 1), for example, antibody Cat-201 binds to axons and to neuron cell bodies in the ventral horn whereas Cat-301 binds to cell bodies in both the ventral and dorsal horns. Each antibody binds to a different functional group of neurons, as ventral horn neurons are involved in generating movements and dorsal horn neurons are involved in processing sensory input from the skin.

Antibody Cat-301 Is Present on the Surface of Specific Neuronal Subsets

Cat-301 is an intriguing antibody in two respects. First, at the light microscopic level, it appears to bind to an antigen on the surface of neurons; and second, it binds to an unusual subset of neurons in several areas of the central nervous system.

Using electron microscopic immunocytochemistry, we have now shown that Cat-301 binds to an antigen on the external surface of neurons (Fig. 2). Binding sites are irregularly distributed over the surface of a neuron, dense on the membrane between synapses, and sparse or absent at the synaptic cleft. The antigen appears to extend around presynaptic profiles, similar to the distribution of extracellular matrix and basal lamina described at a peripheral synapse, the neuromuscular junction. At the neuromuscular junction, the extracellular matrix has been shown to be an important element of synaptic structure and can actually determine the location of the synapse on the muscle. Using standard electron microscopic techniques, extracellular matrix is absent from synapses in the central nervous system. In fact, neither surface molecules nor components of extracellular space have been described in the vertebrate central nervous system. Studies currently in progress will show if the Cat-301 antigen is a component of synaptic extracellular matrix in the central nervous system as the extracellular matrix at the neuromuscular junction is in the peripheral nervous system.

In the spinal cord, Cat-301 recognizes many kinds of neurons with the notable exception of the interneurons (local circuit neurons) in the substantia gelatinosa. In other areas of the central nervous system, more limited classes of neurons bind Cat-301. In the cerebellar cortex, only one cell type, the Lugaro cell, is recognized by Cat-301. This cell has not been integrated into descriptions of cerebellar circuitry because it was only rarely seen. Cat-301 may prove useful in identifying Lugaro cells so that they could be further studied.

Three areas of the brain involved in visual perception contain Cat-301-positive neurons. In the visual cortex, patches of Cat-301-positive neurons alternate with patches of Cat-301-negative neurons. The periodicity of these patches matches the dimensions of the ocular dominance columns described by Hubel and Weisel. The superior colliculus also receives visual input, and here, too, subsets of neurons are recognized by Cat-301. In the thalamus, the lateral geniculate nucleus receives visual input and layers of the lateral geniculate containing antibody-labeled cells are separated from layers containing unlabeled cells (Fig. 3). Further studies will show whether Cat-301-positive neurons in the lateral geniculate, superior colliculus, and visual cortex are physiologically related.

The thalamus is a major relay station in all sensory processes. Cat-301 also differentiates among cell types throughout the thalamus. In addition to the lateral geniculate nucleus, several, but not all, thalamic nuclei contain Cat-301-positive neurons. The distribution of antibody-identified neurons within a given nucleus reveals features of the nuclear organization, which, in some cases, was not apparent using previously described techniques. For example, in the ventro-basal nucleus, involved in skin and joint perceptions, patches of labeled neurons interdigitate with patches of unlabeled neurons. These patches may correspond to areas receiving a particular class of input. An intriguing possibility raised by these observations is that neurons serving similar functions for different modalities may be antigenically related. The neurons along a pathway for a single modality (vision, skin sense [somatosensory], or smell, for example) are physiologically related, that is, they have similar responses to particular stimuli. For each modality, the basic organization of relay stations is similar. In the visual system and in the somatosensory system, similar kinds of neurons are recognized

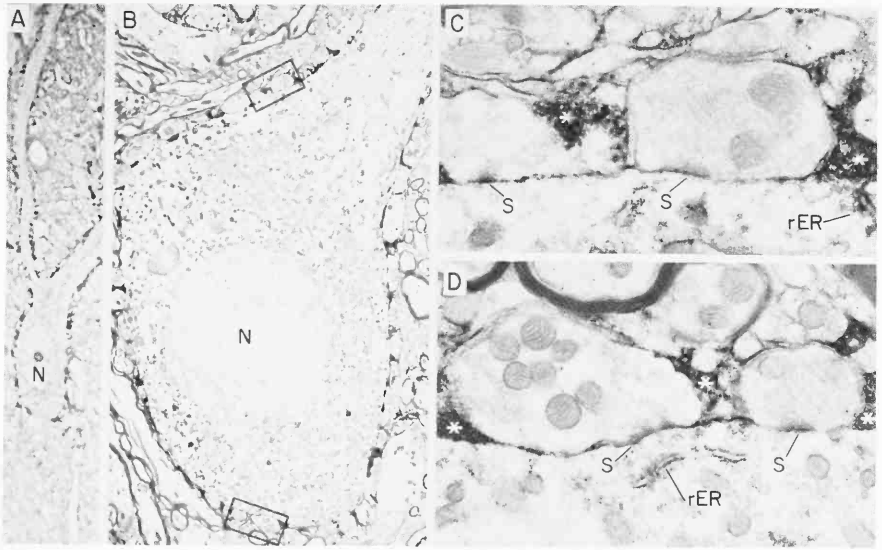


Figure 2

Light (*A*) and electron (*B—D*) micrographs of a layer-IV neuron from a 1% glutaraldehyde, 1% paraformaldehyde-fixed cat spinal cord. (*A*) Cat-301 staining is visible around the cell body and proximal dendrites of a layer-IV neuron whose nucleus (N) contains the nucleolus in this section (520×). (*B*) In a low-power electron micrograph of another section of the cell shown in *A*, Cat-301 staining along the surface of the cell body and a proximal dendrite appears interrupted by other profiles. Areas on boxes are shown enlarged in (C) and *D* (2800×). (C) Area in the upper box in *A* shows two axo-somatic synapses. Antibody staining (white asterisks) is present in the extracellular space along the surfaces of both the pre- and postsynaptic profiles but is excluded from the synapse (S) (23,000×). (*D*) Area in the lower box in *A* also shows antibody staining (white asterisks) around pre- and postsynaptic elements but excluded from synapses (s). A short stretch of rough endoplasmic recticulum (rER) in the postsynaptic cell stains with Cat-301 (23,000×).

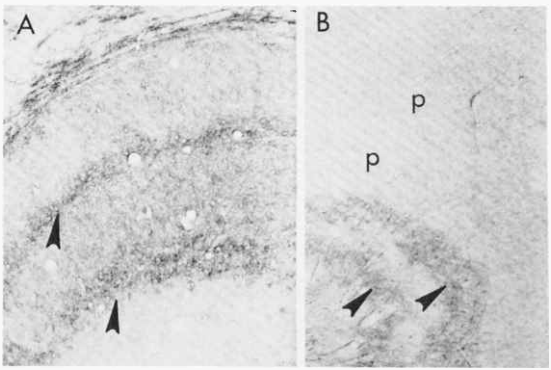


Figure 3
Antibody Cat-301 recognizes subsets of neurons in the lateral geniculate nucleus (LGN) of the thalamus in the cat (*A*) and monkey (*B*). (*A*) In the cat LGN, many neurons in the interlaminar zones (arrowheads) are heavily stained by Cat-301. Fewer neurons are stained in the intervening layers. (*B*) In the monkey LGN, many Cat-301-positive neurons are found in the magnocellular layers (arrowheads) while the parvicellular layers (p) are relatively free of labeled neurons. In both the cat and monkey, particular classes of physiologically identified neurons are found in layers that contain Cat-301-labeled cells.

by Cat-301 at each relay station. Using Cat-301 to analyze these structures may reveal similarities of organization conserved through sensory systems.

Studies on the Embryonic Vertebrate Nervous System

Many theories of neuronal development have postulated the presence of specific markers that guide neurons to make appropriate connections. As Cat-301 recognizes a surface antigen that might be involved in the formation or maintenance of specific connections, we have studied the developmental time course of the appearance of the Cat-301 antigen. In the spinal cord, the motor neurons in the ventral horn are generated before birth. During the first two postnatal weeks, the development of the adult pattern of synapses on motor neurons is completed. Very few motor neurons have Cat-301 surface staining at birth. The number of Cat-301 surface-labeled neurons increases during the first postnatal weeks, but even at 3 weeks, the adult pattern is not yet seen. This is consistent with the possibility that Cat-301 may be a component of synaptic extracellular material, as at the neuromuscular junction many extracellular components are seen only after synaptogenesis is complete. While the function of such molecules is not yet known in detail, the extracellular matrix has been shown to play an important role in maintaining the organization of receptors in the postsynaptic membrane.

To explore further molecules that might function in cell-cell interactions during development, we have now generated a panel of antibodies against early embryonic spinal cord. We have screened over 2000 cell lines and have obtained many antibodies that recognize neural elements in the embryonic spinal cord. These antibodies distinguish among cell types present early in development. Some antigens are transiently expressed whereas others are expressed in the developing and adult nervous system. Together with the antibodies raised against the adult nervous system, we can now trace the development of specific neuronal types.

Publications

Hockfield, S. and S. Gobel. 1982. An anatomical demonstration of projections to the medullary dorsal horn (trigeminal nucleus caudalis) from rostral trigeminal nuclei and the contralateral caudal medulla. *Brain Res. 252:* 203.

Hockfield, S. and R. McKay. 1983. Monoclonal antibodies demonstrate the organization of axons in the leech. *J. Neurosci. 3:* 369.

——. 1983. A surface antigen expressed by a subset of neurons in the vertebrate central nervous system. *Proc. Natl. Acad. Sci.* (in press).

Hockfield, S., R. McKay, S. R. Hendry, and E.G. Jones. 1983. Structural and organizational features of mammalian CNS demonstrated with monoclonal antibodies. *Cold Spring Harbor Symp. Quant. Biol. 48:* (in press).

McKay, R., S. Hockfield, and J. Johansen. 1983. The organization of molecularly distinct axons in the leech connective. *Cold Spring Harbor Symp. Quant. Biol. 48:* (in press).

McKay, R., J. Johansen, and S. Hockfield. Monoclonal antibody identifies a 63,000 dalton antigen found in all central neuronal cell bodies but in only a subset of axons in the leech. (Submitted.)

MOLECULAR STUDIES OF THE NERVOUS SYSTEM

R. McKay, S. Hockfield, *J. Johansen, L. Kleina, J. Monnier, O. Sundin, K. Fredrickson*

Our interest in using molecular techniques to study the nervous system continues. In the past year we have made progress in a number of areas. The most important changes are that we now devote a great deal of our time to studying the vertebrate nervous system and our studies on the leech have focused around a particular question, the organization of the leech connective.

The Leech Connective

R. McKay, S. Hockfield, J. Johansen, L. Kleina

The leech ganglion contains 400 neuronal cell bodies and these ganglia form a chain along the length of the animal. Many cell bodies send processes up and down this chain so that ganglia are hooked together by about 5000 axons that run in a structure called the connective. Although our monoclonal antibodies were initially screened for their ability to bind to specific cell bodies, it soon became clear that they also recognized subsets of axons in the connective. When we asked how these specific axons were arranged, we were surprised by the fact that the connective was stereotypically and symmetrically organized. Our surprise was derived from the fact that the leech connective had been studied ultrastructurally and this simplifying feature of its organization not understood. The reason for this is that the organization in the connective is not geometrically precise, but the results we obtain when we use antibodies to detect specific axons suggest that order exists.

Naturally, we wanted to confirm these interpretations with a different technique. Fortunately, in the leech, we can readily fill identified single cells with a tracer, horseradish peroxidase. This enzyme fills the processes of the cell and allows us to locate them in the connective at the electron microscopic level. The question we asked was, Do the processes of physiologically identified cells occupy stereotyped positions in the connective? We chose to study the processes of the mechanosensory cells first because we have several antibodies that bind to specific subsets of neurons that include these cells. The axons of the mechanosensory cells always run in an arc of about 30° of the lateral connectives; often the axons of physiologically similar cells run together. (Fig. 1, left, shows the positions of the seven main mechanosensory axons and Fig. 1, right, shows an electron micrograph of three filled touch cell axons.) We next filled other types of cells—the serotonergic Retzius cells and the Leydig cells; their axons also run in the same quadrant of the connective as the process of the mechanosensory cells. We hope to map the connective in this way and we are already studying the arrangement of these axons in the nerve bundles that run from the ganglion to the periphery.

The filling of identified cells confirms our immunohistochemical observation that some axons occupy stereotyped positions in the leech connective. The immunohistochemical evidence also suggests other important features of the organization of the connective. First, axons appear to be arranged in bundles or fascicles. Some features of this fasciculation are obviously conserved in a bilaterally symmetrical way in both halves of the connective. More importantly, we have shown that axons which run together in fascicles can share surface antigenic markers recognized by a monoclonal antibody, for example Lan3-2 (Hockfield and McKay 1983). This evidence suggests that markers of this kind might promote specific fasciculation. We know from studies on other organisms that axons choose specific growth routes in early development, so we have looked at the time of appearance of this surface antigen. We reported in the 1981 Annual Report that the antigens bound by Lan3-2 are on the surface of axons early in the development of the nervous system. This kind of data is intriguing and suggests that specific molecules, in addition to marking specific axons in the adult, may also play a direct role in regulating axon organization.

To pursue the biochemistry of this type of surface antigen further, we have followed two routes. We have continued to generate similar monoclonal antibodies using our initial shotgun approach and we have followed a much more directed strategy, partially purifying small amounts of these surface antigens using immunopurification techniques. The shotgun approach has generated several interesting antibodies, including antibodies that bind to the surface of specific subsets of axons as well as subsets of cell bodies. The more directed approach of purifying particular antigens has the advantage that it does not continue to depend on the low chance of obtaining particular antibodies using the whole leech cord as antigen. In last year's annual report, we presented evidence that we could identify antigens in specific hand-dissected cell types on immunoblots. We had shown, then, that the antibody, Lan3-2, which recognizes an antigen on the surface of some axons, binds to several high-molecular-weight bands on immunoblots of different species of leech. These antigens all bind to ConA beads, suggesting they are glycoproteins. We chose the leech species, which gave the simplest pattern on immunoblots, and we have used single-step immunopurification procedures to purify these glycoproteins partially. Using the ultrasensitive silver staining procedures, we have been able to see stained protein bands at the molecular weights expected for the Lan3-2 antigens from Lan3-2 immunoblots. These bands are not immunoprecipitated when another control monoclonal antibody is

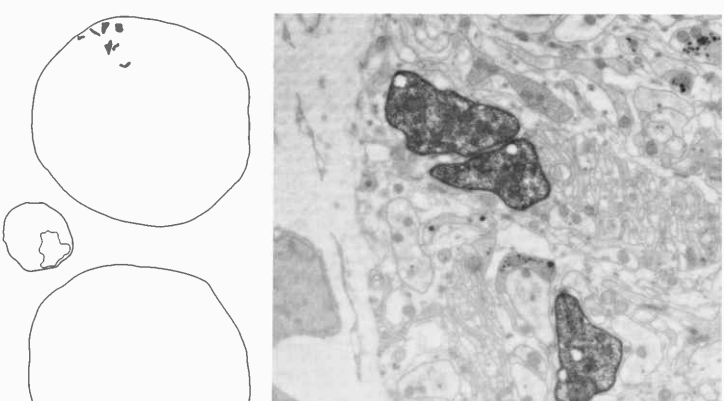


Figure 1
Electron micrograph showing the horseradish peroxidase-labeled processes of mechanosensory cells. The diagram shows the location of these axons in a stereotyped zone of the connective.

used in the purification. The very small amounts of antigen that we obtain from a single leech nerve cord (< 100 ng) restrict the practical use we can make of this purification, but there are new procedures for generating monoclonal antibodies by in vitro immunization against nanogram quantities of antigen, and protein-sequencing techniques have been developed so only small amounts of pure protein are required. We are now using in vitro immunization to generate large numbers of antibodies against neuronal glycoproteins. We hope to use these techniques to obtain a thorough molecular description of those antigens that mark groups of axons. We may then be able to ask whether all axons carry specific surface antigens early in embryonic development and to ask what role these molecules play in axon organization during embryonic development.

The Development of Molecular Diversity in the Vertebrate Nervous System

R. McKay, S. Hockfield, J. Monnier

The application of hybridoma technology to the analysis of the adult vertebrate nervous system has yielded many antibodies that distinguish discrete neuronal types in different areas of the central nervous system (see Anatomical Studies with Monoclonal Antibodies Section). More recently, we have used hybridoma technology to probe the cellular organization of early stages in the development of the vertebrate central nervous system. We already knew that this approach worked on the adult brain. So our next step towards the developmental events we are interested in studying was simply to explore the difficulties and rewards of applying this technique to the analysis of central neural development.

The nervous system is derived from a plate of ectodermal cells (a columnar epithelium) that rolls up in development to form a tube of approximately 10^5 cells, which proliferate and differentiate to generate the 10^{12} cells and possibly hundreds of cell types of the adult vertebrate brain. We chose E15 rat embryo because at this age the nervous system still contains both the rapidly proliferating epithelial cells and the recently differentiated neurons that have just begun to send out processes and receive sensory input. So this stage of development seemed suitable to study the cellular differentiation of the neural plate and the initial events in axon outgrowth and synaptic recognition between neurons using monoclonal antibodies. Although we are still in the early stages of this project, we do have antibodies that bear on both of these questions.

The antibody Rat6-7G12 binds to an antigen which is expressed in a subset of radial cells in the columnar epithelium. These cells have the morphological characteristics of the neuroblast cells first described by the Golgi technique and called radial

glial cells by Cajal. Immunoblots show the antigen to be a doublet of bands of approximately 180,000 daltons. Both biochemical and histochemical assays show the antigen to be absent in the adult rat brain. At the electron microscopic level, the antibody recognizes an internal antigen in a subset of the E15 radial cells—some of these cells contain mitotic figures, so we know they are dividing. The only other cells in the embryo that express a related antigen are located in the region of the developing somites. We hope to use antibodies of this kind to identify the stage in embryonic development when the molecular diversity of neurons first becomes expressed. Embryological transplantation experiments in the developing chick central nervous system and more recent experiments making chimeras with mouse cerebellar mutants suggest that some neuron types may be determined at the neural plate stage. Clearly, antigenic differences between cells at this early stage are consistent with this possibility.

The antibody Rat6-2F2 and a number of other monoclonal antibodies bind to antigens present in the processes of neurons. Immunoblots show that 2F2 binds to the same three bands in the developing and adult central nervous system. Using this antibody, we have been able to follow the growth of single axons from the developing motor neurons of the spinal cord. These studies show that the first axons grow out from several sites on the periphery of the spinal cord and congregate at one point, which we assume to be the site of the future nerve plexus, before radiating towards their peripheral targets. This observation suggests that the site of congregation (the future nerve plexus) is attractive to the first growing axons that find their way to this point by different routes. Although this approach is simple, students of axon pathfinding in invertebrate systems have used similar, simple immunological probes with great success.

Identification of Genes and Gene Products Active in Early Development of the Rat Nervous System

O. Sundin, R. McKay

Monoclonal antibodies have already given us an exciting glimpse into the molecular architecture of the nervous system and in the process have raised new questions concerning the relation of this molecular architecture to neural function. However, the molecular analysis of neural systems using hybridoma technology as our only tool would be an arduous task. Fortunately, recombinant DNA technology may provide new and powerful methods for cloning genes expressed in the nervous system and we hope these methods will simplify our task. For example, once a gene has been cloned, nucleic acid hybridization can be used to isolate additional related genes; nucleic acid sequencing is then a rapid way of establishing the evolutionary relationships amongst a family of genes and the gene can be expressed in bacterial or higher cells to give complementary message RNA and protein. The next question is how to identify a gene of interest?

A common strategy for identifying interesting genes is to take antibodies against the gene product and use these to identify and purify the mRNA or a cDNA clone expressing this protein in bacteria. We are developing cDNA expression systems that may allow us to use this approach to genes, and we also hope these systems will allow us to generate antibodies from cloned cDNAs. The scheme we are devising is, in spirit, the reverse of traditional methods. The idea is first to choose a cDNA clone complementary to a message RNA specifically present in embryonic rat nervous tissue; we then want to obtain protein complementary to this gene, raise antibodies against the protein, and then localize the native gene product to cell types within the developing nervous system.

We have built upon two existing bacterial plasmids to implement this idea. Both of these plasmids were designed to express proteins under the control of the *lac* promoter. One of these plasmids, MR100, which we obtained from Mike Rosbash (Brandeis University), was specifically designed to look for open reading frames in DNA fragments. The plasmid is designed so that bacterial colonies containing plasmid with inserted and expressed cDNA have the lacZ$^+$ phenotype. With insert sizes of over 300 bp, one selects strongly for biologically relevant polypeptides. To use this plasmid as an acceptor of cDNA fragments, it was necessary to increase the efficiency of insertion greatly and to allow strictly only single inserts. To achieve this, a new method was devised in which a *Bam*HI restriction site was introduced into the vector. To prevent self-ligation and multiple inserts of the vector, we filled the two inner nucleotides of the four-base-pair staggered ends of the *Bam*HI site and of the *Xho*I linkers on the double-stranded cDNA. The modified *Xho*I end can still efficiently ligate to the modified *Bam*HI end through the two-base-pair overlap. *Bam-Bam* and *Xho-Xho* self-ligation is blocked by the partial fills so vector and cDNA cannot ligate to themselves but only to each other. In our experience, this method of cloning cDNAs is extremely efficient and we have isolated large numbers of lacZ$^+$ clones; some of these clones express stable fusion proteins. We are currently assessing the stability of the hybrid proteins and raising monoclonal antibodies against these fusion proteins, and we plan to examine the native gene product in the embryonic rat nervous system by immunohistochemistry and protein blotting.

One drawback of a β-galactosidase vector is that the bacterial portion of the fusion protein is at least six times the size of that derived from the cDNA. It would be less complicated to raise insert-specific monoclonals if the "carrier" portion of the protein were smaller in size and not as immunogenic. We have obtained from David Helfman a pUC8-derived plasmid that expresses an unusually stable 17K C-

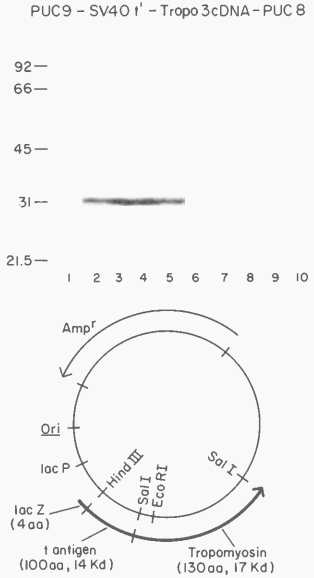


Figure 2
Western blot showing that several bacterial colonies transformed with a plasmid derived from pUC9 express a 31-kD protein that binds monoclonal antibodies against the amino terminus of SV40 large T antigen.

terminal fragment of chick tropomyosin under control of the *lac* promoter. When fused to a 14K N-terminal fragment of SV40 T antigen, which by itself appears highly unstable in *E.coli*, one obtains a 31K fusion protein with considerable stability (Fig. 2). To develop this plasmid to meet our needs, an out-of-phase tropomyosin-coding region is being constructed. cDNA will then be cloned into the *Bam*HI site located between SV40 T antigen and chicken tropomyosin-coding regions. Stable fusion proteins will then be identified by replica-filter screening bacterial colonies with antibody against tropomyosin. Using an affinity column of monoclonal antibodies that bind to T antigen, we hope to purify the fusion protein quickly prior to its use as an antigen.

Our optimism in this enterprise is based on the assumption that genes which are selectively expressed at certain stages of development in specific neuronal types are common rather than rare. Initially, we are only studying a small number of hybrid proteins but we are hopeful that with new methods for generating antibodies against very small amounts of protein, it will be possible to obtain very rapidly sets of antibodies for many different fusion proteins. These antibodies will then be used to find genes expressed in subsets of neurons. The biochemical analysis of these antigens will then be vastly simplified by having the gene precloned.

Publications

Hockfield, S. and R.D.G. McKay. 1983. Monoclonal antibodies demonstrate the organization of axons in the leech. *J. Neurosci. 3*: 369.

——.1983. A surface antigen expressed by a subset of neurons in the vertebrate central nervous system. *Proc. Natl. Acad. Sci.* (in press).

McKay, R.D.G. 1983. Molecular approaches to the nervous system. *Annu. Rev. Neurosci. 6*: 527.

——.1983. Immunoassay for sequence specific DNA-protein interactions. *Meth. Enzymol. 92*: 138.

McKay, R.D.G. and S. Hockfield. 1982. Monoclonal antibodies distinguish antigenically discrete neuronal types in the vertebrate central nervous system. *Proc. Natl. Acad. Sci. 79*: 6747.

McKay, R.D.G., J. Johansen, and S. Hockfield. 1983. A 63,000 dalton antigen found in all central neuronal cell bodies but only a subset of axons in the leech. (Submitted.)

McKay, R.D.G., S. Hockfield, I. Thompson, and J. Johansen. 1983. Specific glycosylated surface antigens associated with axon fascicles during the development of the leech nervous system. (Submitted).

47th Cold Spring Harbor Symposium on Quantitative Biology

Structures of DNA

June 2–June 9

The double helix is deceptively simple. When first found in 1953, it appeared so beautifully clear that for a brief period it seemed that by mere visual inspection we must learn all its mysteries. Now almost 30 years later, DNA structure is no longer a child's game, and those who play with it must be both experienced and of the courage to seek elegance among the almost overwhelming perturbations of its basic double-helical configuration. Not only can DNA be overcoiled or undercoiled, all under strict enzymatic control, it can turn to the left as well as to the right. Since these complexities are not laboratory artifacts but, in fact, provide the molecular underpinnings for the successful functioning of our genetic material, further progress in more firmly establishing the various forms of DNA is likely to be essential for the future of much biological research.

The moment thus was propitious for a high-level meeting in which all aspects of DNA could be presented and analyzed. So, for the topic of our 47th annual Symposium, we chose "The Structures of DNA." From the start we realized that the number of speakers was likely to be very large, and so we sought the advice of many experts as to whom to invite. In particular, I wish to acknowledge the invaluable counsel given by Charles Cantor, Pierre Chambon, Don Crothers, Richard Dickerson, Gary Felsenfeld, Aaron Klug, Alex Rich, and Jim Wang. The formal program contained 114 speakers with many additional, last-minute informal presentations made during the course of the various sessions. Given the inherent complexity of the topics, this was indeed a very demanding Symposium. But because of the consistently high quality of the presentations, this Symposium was also felt by virtually all of the 244 participants to rank among the most stimulating of the scientific meetings that they had ever attended.

The most necessary financial help that let us assemble such a massive program was again provided by the National Institutes of Health, the National Science Foundation, and the Department of Energy.

Opening Remarks: A. RICH, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

SESSION 1 *The Handedness of DNA. I.*

Chairperson: S. ARNOTT, Purdue University, West Lafayette, Indiana

S. IKUTA,[1] A. DUGAICZYK,[2] T. HUANG,[1] R.B. WALLACE,[1] and K. ITAKURA,[1] [1]Dept. of Molecular Genetics, City of Hope Research Institute, Duarte, California; [2]Dept. of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston: Synthetic oligodeoxyribonucleotides as indispensable chemicals in modern biological laboratories.

R.E. DICKERSON,[1] H.R. DREW,[2] B.N. CONNER,[1] and M.L. KOPKA,[1] [1]Molecular Biology Institute, University of California, Los Angeles; [2]MRC Laboratory of Molecular Biology, Cambridge University, England: The anatomy of A, B_1- and Z DNA.

M.A. VISWAMITRA, ICMR Centre of Genetics and Cell Biology, Indian Institute of Science, Bangalore, India: Structural diversity in DNA—From monomer structures to oligonucleotides.

A. H.-J. WANG,[1] S. FUJII,[1] J.H. VAN BOOM,[2] and A. RICH,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Gorlaeus Laboratories, Leiden State University, The Netherlands: The molecular structure of a modified A-DNA octamer, a methylated Z-DNA, and a DNA-RNA hybrid.

SESSION 2 *The Handedness of DNA. II.*

Chairperson: C. CANTOR, Columbia University, New York, New York

V. SASISEKHAREN, Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India: The history of left-handed DNA.

S. ARNOTT, R. CHANDRASEKARAN, I.H. HALL, L.C. PUIGJANER, and J.K. WALKER, Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: DNA secondary structures—Helices, wrinkles, and junctions.

S. ZIMMERMAN and B. PHEIFFER, NIADDK, National Institutes of Health, Bethesda, Maryland: DNA conformations and transitions—X-ray diffraction studies of solvated fibers.

R.D. WELLS,[1,2] P.A. HART,[1] M. KILPATRICK,[1,2] J. KLYSIK,[1,2] J.E. LARSON,[1,2] J.J. MIGLIETTA,[1] C.K. SINGLETON,[1,2] S.M. STIRDIVANT,[1] R.M. WARTELL,[3] and W. ZACHARIAS,[1,2] [1]Dept. of Biochemistry, University of Wisconsin, Madison; [2]Dept. of Biochemistry, University of Alabama, Birmingham; [3]Schools of Physics and Biology, Georgia Institute of Technology, Atlanta: Left-handed DNA in restriction fragments and recombinant plasmids.

T.M. JOVIN,[1] J.H. VAN DE SANDE,[2] and D. ZARLING,[3] [1]Max Planck Institute for Biophysical Chemistry, Göttingen, Federal Republic of Germany; [2]Dept. of Medical Biochemistry, University of Calgary, Canada; [3]Institute of Genetics, University of Cologne, Federal Republic of Germany: Formation, identification, and properties of left-handed DNAs.

M. LENG, E. SAGE, and B. MALFOY, Centre de Biophysique Moléculaire, CNRS, Orleans, France: Interactions between double-stranded nucleic acids and antibodies to Z DNA.

A. NORDHEIM,[1] L.J. PECK,[2] E.M. LAFER,[3] B.D. STOLLAR,[3] J.C. WANG,[2] and A. RICH,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; [3]Dept. of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts: Antibody reactivity with left-handed Z DNA in supercoiled plasmids.

M.L. PARDUE,[1] A. NORDHEIM,[1] E.M. LAFER,[2] B.D. STOLLAR,[2] and A. RICH,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts: Z-DNA and the polytene chromosome.

SESSION 3 *Conformational Analysis. I.*

Chairperson: P. DOTY, Harvard University, Cambridge, Massachusetts

M.J. GAIT, H.W.D. MATTHES, M. SINGH, B. SPROAT, and R.C. TITMAS, MRC Laboratory of Molecular Biology, Cambridge, England: A continuous flow phosphotriester method for solid phase synthesis of oligodeoxyribonucleotides.

J.H. VAN BOOM,[1] G.A. VAN DER MAREL,[1] H. WESTERINK,[1] C.A.G. VAN BOECKEL,[1] J.R. MELLEMA,[1] C. ALTONA,[1] C.W. HILBERS,[2] and C.A.G. HAASNOOT,[2] [1]Dept. of Organic Chemistry, State University of Leiden; [2]Dept. of Biophysics, University of Nijmegen, The Netherlands: Synthesis and conformational analysis of DNA fragments.

M.H. CARUTHERS, P. DEHASETH, E. FISHER, R. GOLDMAN, M. INSLEY, and W. MANDECKI, Dept. of Chemistry, University of Colorado, Boulder: Chemical synthesis and biological studies on mutated gene control regions.

D.J. PATEL,[1] S. KOZLOWSKI,[1] S. IKUTA,[2] A. NORDHEIM,[3] M. WEISS,[4] and K. ITAKURA,[2] [1]Bell Laboratories, Murray Hill, New Jersey; [2]City of Hope National Medical Center, Duarte, California; [3]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [4]Dept. of Biochemistry, Harvard University, Cambridge, Massachusetts: DNA conformation, dynamics, and interactions in solution.

D.M. CROTHERS, N. DATTAGUPTA, and M. FRIED, Depts. of Chemistry and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Transmission of long-range effects in DNA.

M. YANAGIDA,[1] Y. HIRAOKA,[1] and I. KATSURA,[2] [1]Dept. of Biophysics, Kyoto University; [2]Dept. of Biophysics and Biochemistry, University of Tokyo, Japan: Dynamic behaviors of DNA molecules in solution studied by fluorescence microscopy.

V. IVANOV, L. MINCHENKOVA, E. MINYAT, and A. SCHYOLKINA, Institute of Molecular Biology, USSR Academy of Sciences, Moscow: Cooperative transitions in DNA with no separation of strands.

D. SCHWARTZ, W. SAFFRAN, J. WELSH, R. HAAS, M. GOLDENBERG, R. MURPHY, and C.R. CANTOR, Dept. of Human Genetics and Development, Columbia University, New York, New York: New techniques for the study of large DNAs.

SESSION 4 *Conformational Analysis. II.*

Chairperson: D. CROTHERS, Yale University, New Haven, Connecticut

C. BENHAM, Dept. of Mathematics, University of Kentucky, Lexington: Statistical mechanical analysis of competing conformational transitions in superhelical DNA.

W.K. OLSON, Dept. of Chemistry, Rutgers University, New Brunswick, New Jersey: Theoretical probes of DNA conformation.

K. BECHERER, A. COUREY, D. HOROWITZ, L.J. PECK, Y.-C. TSE, and J.C. WANG, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: DNA supercoiling and its effects on DNA structure and function.

D.M.J. LILLEY, Medical Sciences Institute, University of Dundee, Scotland: Dynamic, sequence-dependent DNA structure as exemplified by hairpin extrusion from inverted repeats in negatively supercoiled DNA.

E.N. TRIFONOV, Polymer Dept., Weizmann Institute of Science, Rehovot, Israel: Sequence-dependent variations of B-DNA structure and protein- DNA recognition.

A. KLUG, L.C. LUTTER, D. RHODES, P.J.G. BUTLER, and G.P. LOMONOSSOFF, MRC Laboratory of Molecular Biology, Cambridge, England: Use of nucleases to probe the structure of DNA on and off the nucleosome.

SESSION 5 *Chemically Modified DNA*

Chairperson: J. HEARST, University of California, Berkeley, California

H.M. SOBELL, S.C. JAIN, T.D. SAKORE, and K.K. BHANDARY, Dept. of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, New York: Structural concepts to understand simple vs. complex intercalators.

F. TAKUSAGAWA and H.M. BERMAN, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Some new aspects of actinomycin D—Nucleic acid binding.

J.M. ESSIGMANN, R.C. CROY, G.H. BUCHI, and G.N. WOGAN, Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge: Interactions of aflatoxin B_1 and alkylating agents with DNA—Structural and functional studies.

R.M. SANTELLA, D. GRUNBERGER, and I.B. WEINSTEIN, Division of Environmental Sciences and Institute of Cancer Research, Columbia University, New York, New York: Carcinogens can induce alternate conformations in nucleic acid structure.

P.B. DERVAN and M.W. VAN DYKE, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena: Map of antibiotic binding sites on heterogeneous DNA—Footprinting with MPE•Fe(II).

S.J. LIPPARD, Dept. of Chemistry, Columbia University, New York, New York: Platinum complexes and DNA—Intercalation, Z-DNA, and sequence-specific structures produced by the antitumor drug *cis*-DDP.

S.-H. KIM, Dept. of Chemistry, University of California, Berkeley: 3-D structure of a psoralen-thymine monoadduct formed in photoreaction with DNA and its implications.

R. Axel, J. Steitz, L. Hood

S. Weissman, B. McClintock

G.V. GURSKY, A.A. ZASEDATELEV, A.L. ZHUZE, A.A. KHORLIN, S.L. GROKHOVSKY, S.A. STRELTSOV, S.M. NIKITIN, M.V. MIKHAILOV, A.N. SUROVAYA, V.O. RECHINSKY, R.S. BEABEALASCHVILLY, A.S. KRYLOV, and B.P. GOTTIKH, Institute of Molecular Biology, USSR Academy of Sciences, Moscow: Synthetic sequence-specific ligands—Design, DNA binding properties, and biological activity.

SESSION 6 *DNA-Protein Interactions*

Chairperson: B. ALBERTS, University of California, San Francisco, California

B.W. MATTHEWS,[1] D.H. OHLENDORF,[1] W.F. ANDERSON,[2] R.G. FISHER,[1] and Y. TAKEDA,[3] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]MRC Group on Protein Structure and Function, University of Alberta, Edmonton, Canada; [3]Chemistry Dept., University of Maryland, Baltimore County: *cro* repressor and its interaction with DNA.

T.A. STEITZ, S. ABDEL-MEGUID, P. BRICK, A. GOLDMAN, D. MCKAY, D. OLLIS, I. WEBER, and P. WEBER, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Crystallographic studies of proteins that recognize specific DNA structures—CAP, DNA polymerase I, SSB, and resolvase.

M. LEWIS, J. WANG, A. JEFFREY, and C. PABO, Dept. of Biochemistry, Harvard University, Cambridge, Massachusetts: Structure of the operator-binding domain of the λ repressor and models for operator recognition.

H. NELSON, M. HECHT, and R.T. SAUER, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic and biochemical studies of λ repressor-operator interactions.

J.E. HEARST and H.B. GAMPER, Dept. of Chemistry, University of California, Berkeley: DNA winding and unwinding by transcriptional complexes.

M.T. RECORD, JR.,[1] P. MELANCON,[1] S.L. SHANER,[1] R.R. BURGESS,[2] K.S. LEE,[1] S.J. MAZUR,[1] M. MOSSING,[1] and J.-H. ROE,[1] [1]Dept. of Chemistry and [2]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Ion effects on the interactions of *E. coli* RNA polymerase and DNA.

W.R. MCCLURE, D.K. HAWLEY, B.C. HOOPES, and M.E. MULLIGAN, Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburg, Pennsylvania: DNA determinants of promoter selectivity in *E. coli*.

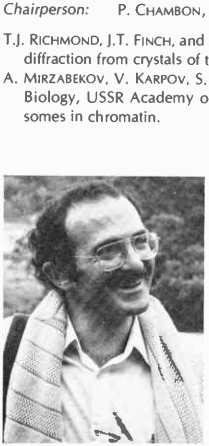
R.S. JACK and W.J. GEHRING, Biozentrum, University of Basel, Switzerland: Sequence-specific DNA binding proteins in eukaryotes.

SESSION 7 *DNA within Nucleosomes*

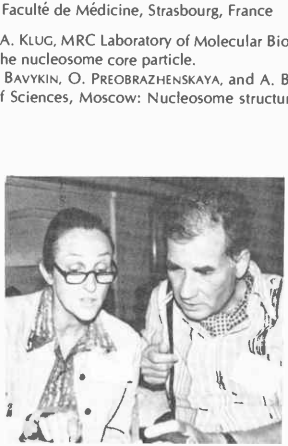
Chairperson: P. CHAMBON, Faculté de Médicine, Strasbourg, France

T.J. RICHMOND, J.T. FINCH, and A. KLUG, MRC Laboratory of Molecular Biology, Cambridge, England: X-ray diffraction from crystals of the nucleosome core particle.

A. MIRZABEKOV, V. KARPOV, S. BAVYKIN, O. PREOBRAZHENSKAYA, and A. BELYAVSKY, Institute of Molecular Biology, USSR Academy of Sciences, Moscow: Nucleosome structure and the alignment of nucleosomes in chromatin.



C. Cantor



D. Skinner, P. Chambon

B. Alberts

A. VARSHAVSKY, L. LEVINGER, O. SUNDIN, J. BARSOUM, E. OZKAYNAK, D. FINLEY, P. SWERDLOW, and K.C. WU, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Cellular and SV40 chromatin—Replication, segregation, ubiquitination, AT-DNA-binding proteins, and functionally significant nucleosome arrangements.

A. LARSEN and H. WEINTRAUB, Dept. of Genetics, Fred Hutchinson Cancer Research Center, Seattle, Washington: An altered DNA conformation detected by S1 nuclease occurs at specific regions in active chick globin chromatin.

S.C.R. ELGIN, I.L. CARTWRIGHT, M.A. KEEN, and K. LOWENHAUPT, Dept. of Biology, Washington University, St. Louis, Missouri: Chromatin structure and DNA sequence organization.

G. GARGIULO, G. GLIKIN, B. JESSEE, and A. WORCEL, Dept. of Biology, University of Rochester, New York: DNA and chromatin structure of the 5000-bp histone gene unit of *D. melanogaster*.

G. FELSENFELD, J. NICKOL, J. MCGHEE, and M. BEHE, NIADDK, National Institutes of Health, Bethesda, Maryland: Chromatin structure and DNA methylation.

P. LABHART,[1] P. NESS,[2] E. BANZ,[2] R. PARISH,[2] and T. KOLLER,[1] [1]Institut für Zellbiologie, ETH-Hönggerberg; [2]Institut für Pflanzenbiologie, Universität Zürich, Switzerland: A model for the structure of the active nucleolar chromatin.

SESSION 8 *The Organization of Genes along DNA*

Chairperson: W. GILBERT, The Biological Laboratories, Harvard University, Cambridge, Massachusetts

J.J. DUNN and F.W. STUDIER, Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Organization and expression of bacteriophage T7 DNA.

D.L. DANIELS,[1] F. SANGER,[2] and A.R. COULSON,[2] [1]Dept. of Genetics, University of Wisconsin, Madison; [2]MRC Laboratory of Molecular Biology, Cambridge, England: Features of bacteriophage λ—The latest analysis of the complete nucleotide sequence.

R.J. ROBERTS, B.D. JIANG, P.A. BULLOCK, R.E. GELINAS, T.R. GINGERAS, M.M. KELLY, K.E. O'NEILL, B.L. PARSONS, D. SCIAKY, and C.E. YEN, Cold Spring Harbor Laboratory, New York: The information content of the adenovirus-2 genome.

V. TATE, H. BOEDTKER, and P. DOTY, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: The procollagen genes—Further sequence studies and interspecies comparisons.

L. HOOD, K. EAKLE, D. FISHER, J. FRELINGER, R. GOODENOW, M. MCMILLAN, A. ORN, M. PECHT, B. SHER, M. STEINMETZ, Y. STROYNOWSKI, H. SUN, and A. WINOTO, Division of Biology, California Institute of Technology, Pasadena: Genes of the major histocompatibility complex.

P.A. BIRO,[1] J. PAN,[1] V.B. REDDY,[2] S.M. WEISSMAN,[1] P. JAGADEESWARAN,[1] M. COHEN-SOLAL,[3] and B.G. FORGET,[4] [1]Dept. of Human Genetics, Yale University Medical School, New Haven, Connecticut; [2]Integrated Genetics, Framingham, Massachusetts; [3]INSERM, Hopital Henri Mondor, Creteil, France; [4]Dept. of Internal Medicine, Yale University Medical School, New Haven, Connecticut: Sequences of human globin and MHC gene complexes.

T. IKEMURA and H. OZEKI, Dept. of Biophysics, Kyoto University, Japan: Codon usage and tRNA contents—Organism-specific codon choice patterns in reference to the isoacceptor contents.

J.C.W. SHEPHERD, Biozentrum, University of Basel, Switzerland: From primeval message to present-day gene.

SESSION 9 *Methylated DNA*

Chairperson: G. FELSENFELD, National Institutes of Health, Bethesda, Maryland

A.P. BIRD, A. LA VOLPE, M. TAGGART, and D. MACLEOD, MRC Mammalian Genome Unit, Edinburgh, Scotland: Methylation and expression of rRNA genes in *X. laevis* and *X. borealis*.

W. DOERFLER, L. VARDIMON, I. KRUCZEK, D. EICK, and I. KUHLMANN, Institute of Genetics, University of Cologne, Federal Republic of Germany: DNA methylation and gene activity—In vitro studies, promoter methylation, and shifts in DNA methylation.

H. CEDAR, A. RAZIN, Y. GRUENBAUM, R. STEIN, and T. NAVEH-MANY, Dept. of Cellular Biochemistry, Hebrew University Medical School, Jerusalem, Israel: The effect of DNA methylation on gene expression.

D. JÄHNER, C.L. STEWART, H. STUHLMANN, K. HARBERS, and R. JAENISCH, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Universität Hamburg, Federal Republic of Germany: De novo methylation, expression, and infectivity of retroviral genomes introduced into early mouse embryo cells.

B.R. MIGEON and S.F. WOLF, Johns Hopkins University Medical School, Baltimore, Maryland: Implications for X chromosome regulation from studies of human X DNA.

L.J. SHAPIRO and T. MOHANDAS, Division of Medical Genetics, Harbor-UCLA Medical Center, UCLA School of Medicine, Torrence, California: DNA methylation and the control of gene expression of the human X chromosome.

R. KAHMANN, Max-Planck-Institut für Biochemie, Munich, Federal Republic of Germany: Methylation regulates the expression of a DNA-modification function encoded by bacteriophage Mu.

S. HATTMAN,[1] M. GORADIA,[2] C. MONAGHAN,[2] and A.I. BUKHARI,[2] [1]Dept. of Biology, University of Rochester: [2]Cold Spring Harbor Laboratory, New York: Regulation of the DNA-modification function *mom* of bacteriophage Mu.

SESSION 10 *Replicating DNA*

Chairperson: J. CAIRNS, Harvard School of Public Health, Boston, Massachusetts

B.M. ALBERTS, J. BARRY, P. BEDINGER, T. FORMOSA, C.V. JONGENEEL, and K.N. KREUZER, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Studies of DNA replication in the T4 bacteriophage in vitro system.

C.W. FULLER, B.B. BEAUCHAMP, M.J. ENGLER, R.L. LECHNER, S.W. MATSON, S. TABOR, J.H. WHITE, and C.C. RICHARDSON, Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Mechanisms for the initiation of bacteriophage T7 DNA replication.

K. MATSUBARA and T. TSURIMORO, Laboratory of Molecular Genetics, Osaka University Medical School, Japan: Replication of bacteriophage λ DNA.

M. STAYTON, L. BERTSCH, S. BISWAS, P. BURGERS, N. DIXON, J. FLYNN, R. FULLER, J. KAGUNI, J. KOBORI, M. KODAIRA, R. LOW, and A. KORNBERG, Dept. of Biochemistry, Stanford University Medical School, California: Enzymatic recognition of DNA replication origins.

D.R. BROWN, D. REINBERG, T. SCHMIDT-GLENEWINKEL, and J. HURWITZ, Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Specific DNA structures required for initiation and termination of ϕX A-protein-directed rolling circle DNA replication.

B. MOSS, B.M. BAROUDY, and S. VENKATESAN, NIAID, National Institutes of Health, Bethesda, Maryland: Flip-flop terminal loops link the two DNA strands of the vaccinia virus genome into the uninterrupted polynucleotide chain.

J. LICHY, T. ENOMOTO, J. FIELD, R. GUGGENHEIMER, J.-E. IKEDA, K. NAGATA, M. HORWITZ, and J. HURWITZ, Depts. of Developmental Biology and Cancer and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Identification of factors involved in adenoviral DNA replication in vitro.

B.W. STILLMAN and F. TAMANOI, Cold Spring Harbor Laboratory, New York: Initiation of adenovirus DNA replication—Structure of the origin and enzymes involved.

C. ASTELL,[1] M. CHOW,[2] M. MERCHLINSKY,[2] J. LEARY,[2] and D. WARD, [1]Dept. of Biochemistry, University of British Columbia, Vancouver, Canada; [2]Dept. of Human Genetics, Yale University, New Haven, Connecticut: Structure and replication of minute virus of mice DNA—Evidence for a type I topoisomerase activity in maturing the genome termini.

P. Doty, A. Klug

A. Rich, G. Georgiev

J. Huberman, G. Eichhorn, K. Matsubara

SESSION 11 *Recombining and Mutating DNA*

Chairperson: F. STAHL, Institute of Molecular Biology, University of Oregon, Eugene, Oregon

M.M. COX, D.A. SOLTIS, and I.R. LEHMAN, Dept. of Biochemistry, Stanford University Medical School, California: Role of single-stranded DNA binding protein in *recA*-protein-promoted DNA strand exchange.

A. STASIAK, E. DiCAPUA, and T. KOLLER, Institut für Zellbiologie, ETH- Hönggerberg, Zurich, Switzerland: The unwinding of duplex DNA in complexes with *recA* protein.

A. WU, R. KAHN, C. DASGUPTA, and C. RADDING, Depts. of Human Genetics and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Nonpolar synapsis vs. polar strand exchange in homologous pairing promoted by *recA* protein.

L.R. BELL and B. BYERS, Dept. of Genetics, University of Washington, Seattle: Homologous association of chromosomal DNA during yeast meiosis.

SESSION 12 *Supercoiled DNA*

Chairperson: J. WANG, Harvard University, Cambridge, Massachusetts

M. GELLERT,[1] R. MENZEL,[1] K. MIZUUCHI,[1] M.H. O'DEA,[1] and D. FRIEDMAN,[2] [1]NIADDK, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Microbiology, University of Michigan, Ann Arbor: Control of DNA supercoiling in *E. coli*.

M. KRASNOW, F. DEAN, R. OTTER, M. PASTORCIC, M.M. MATZUK, and N. COZZARELLI, Dept. of Biochemistry, University of Chicago, Illinois: Catenation, recombination, and the mechanism of type-1 topoisomerases.

A.N. LUCHNIK,[1] V.V. BAKAEV,[2] and V.M. GLASER,[3] [1]Institute of Developmental Biology; [2]Institute of Molecular Biology, USSR Academy of Science, Moscow; [3]Moscow State University, USSR: DNA supercoiling—Changes during cellular differentiation and transcription.

P. MARGOLIN, K.M. OVERBYE, and S. BASU, Public Health Research Institute of the City of New York, Inc., New York: Loss of topoisomerase-I activity in *S. typhimurium* causes alterations in many cellular functions.

J.W. ACKERSON and J.D. GRALLA, Molecular Biology Institute, University of California, Los Angeles: Effects of mutation of *lac* promoter function—Comparison of in vivo and in vitro results.

A.M. ALBERTINI, M.P. CALOS, M. HOFER, and J.H. MILLER, Dépt. de Biologie Moléculaire, Université de Genèva, Switzerland: Spontaneous deletion mutations in *E. coli*.

L.S. RIPLEY and B.W. GLICKMAN, Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: The unique self-complementarity of palindromic sequences provide DNA structural intermediates for mutation.

SESSION 13 *Transcribing DNA*

Chairperson: J. HURWITZ, Albert Einstein College of Medicine, Bronx, New York

L. OLSON, J. DE VILLIERS, J. BANERJI, and W. SCHAFFNER, Institut für Molekularbiologie II der Universität Zürich, Switzerland: Studies on the transcriptional "enhancer" effect.

S. CEREGHINI, P. HERBOMEL, J. JOUANNEAU, S. SARAGOSTI, M. KATINKA, B. BOURACHOT, B. DE CROMBRUGGE, and M. YANIV, Dept. of Molecular Biology, Institut Pasteur, Paris, France: Structure and function of the promoter enhancer region of polyoma and SV40.

P. CHAMBON, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, and Unité 184 de Biologie Moléculaire et de Génie Génétique de l´INSERM, Medical School, Strasbourg, France: Promoter sequences of eukaryotic genes coding for proteins.

B. DAVISON, J.-M. EGLY, E. MULVIHILL, and P. CHAMBON, Laboratoire de Génétique Moléculaire des Eucaryotes and Unité 184 de Biologie Moléculaire et de Génie Génétiquede l'INSERM, Medical School, Strasbourg, France: Formation of stable preinitiation complexes between eukaryotic class-B (II) transcription factors and promoter sequences.

S.L. MCKNIGHT, Developmental Biology Program, Fred Hutchinson Cancer Research Center, Seattle, Washington: Mapping transcriptional control signals of the thymidine kinase gene.

R.H. REEDER[1] and B. SOLLNER-WEBB,[2] [1]Fred Hutchinson Cancer Center, Seattle, Washington; [2]Johns Hopkins University, Baltimore, Maryland: Mapping the *X. laevis* ribosomal gene promoter by use of deletion mutants.

S.G. CLARKSON,[1] R.A. HIPSKIND,[1] and B.D. HALL,[2] [1]Dept. of Microbiology, University of Geneva Medical School, Switzerland; [2]Dept. of Genetics, University of Washington, Seattle: DNA conformation and transcription initiation of eukaryotic tRNA genes.

M. WORMINGTON and D.D. BROWN, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Developmental regulation of *Xenopus* 5S RNA genes.

J. STEITZ, S. MOUNT, M. HINTERBERGER, I. PETTERSSON, E. LERNER, J. RINKE, E. GOTTLIEB, and S. WOLIN, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Small ribonucleoproteins from eukaryotes—Structures and roles in RNA biogenesis.

SESSION 14 *Repetitive DNA Sequences*

Chairperson: S.M. WEISSMAN, Yale University School of Medicine, New Haven, Connecticut

G.P. GEORGIEV, Institute of Molecular Biology, USSR Academy of Sciences, Moscow: On the structure and possible functions of middle repetitive sequences in the eukaryotic genome.

W. JELINEK and S. HAYNES, Dept. of Biochemistry, New York University Medical Center, New York: The mammalian *Alu* family of dispersed repeats.

G.M. FOX,[1] J.F. HESS,[2] C.-K.J. SHEN,[2] and C.W. SCHMID,[1] [1]Dept. of Chemistry and [2]Dept. of Genetics, University of California, Davis: Base sequence studies of Alu family members in the α-globin gene cluster.

L. BERNSTEIN, S. VAN ARSDELL, R. DENISON, S. MOUNT, K. MOWRY, and A. WEINER, Dept. of Molecular Biophysics and Biochemistry, Yale Medical School, New Haven, Connecticut: Genes and pseudogenes for human snRNA species U1, U2, and U3—Evidence that cellular RNA molecules are reverse-transcribed and integrated into new chromosomal sites in germ-line DNA.

J. BROACH, M. JAYARAM, Y-Y. LI, and J. FELDMAN, Dept. of Microbiology, State University of New York, Stony Brook: Analysis of yeast replication origins.

K. BLOOM, M. FITZGERALD-HAYES, and J. CARBON, Dept. of Biological Sciences, University of California, Santa Barbara: Structural analysis and sequence organization of yeast centromeres.

E. BLACKBURN, M. BUDARF, P. CHALLONER, M. CHERRY, E. HOWARD, A. KATZEN, W.C. PAN, and T. RYAN, Dept. of Molecular Biology, University of California, Berkeley: DNA termini in ciliate macronuclei.

J.W. SZOSTAK, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Structural requirements for telomere function in yeast.

SESSION 15 *The Regulation of Eukaryotic DNA Expression*

Chairperson: J. STEITZ, Yale University, New Haven, Connecticut

P. DIERKS, B. WIERINGA, A. VAN OOYEN, M. COCHRAN, J. REISER, F. MEYER, T. KÜHNE, C. DOBKIN, and C. WEISSMANN, Institut für Molekularbiologie I, Universität Zürich, Switzerland: Analysis of the transcription of the rabbit β-globin gene and splicing of the transcripts by reversed genetics.

R. AXEL, Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York: The regulated expression of human genes in mouse cells.

J. Cairns

J. Van Boom, G. Gursky

T. Schopf, M.L. Pardue

R.A. FLAVELL, Laboratory of Gene Structure and Expression, National Institute for Medical Research, London, England, and Biogen, Inc., Cambridge, Massachusetts: Expression of globin and H-2 genes.

K.R. YAMAMOTO, V.L. CHANDLER, G.L. FIRESTONE, B. MALER, and F. PAYVAR, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Analyzing biological activity of cloned mammary tumor virus DNA fragments that bind purified glucocorticoid receptor protein in vitro.

D.T. KURTZ, D.K. BISHOP, M.M. MANOS, and L. MCCULLOUGH, Cold Spring Harbor Laboratory, New York: Hormonal control of α2u globulin synthesis.

A.W. SENEAR and R.D. PALMITER, Dept. of Biochemistry, University of Washington, Seattle: 5′regulatory sites of the mouse metallothionein-I gene are packaged in a special chromatin structure.

J. ABRAHAM, K. NASMYTH, J. FELDMAN, J. HICKS, J. STRATHERN, A. KLAR, and J. BROACH, Cold Spring Harbor Laboratory, New York: Regulation of mating-type genes in yeast—A novel negative control mechanism requiring sequences both 5′and 3′to the regulated region.

Summary: A. KLUG, MRC Laboratory of Molelcular Biology, Cambridge, England

SUMMER MEETINGS

Induction of Heat Shock Proteins

May 5–May 9

Arranged by **Milton J. Schlesinger**, Washington University, St. Louis, **Alfred Tissières**, University of Geneva, **Michael Ashburner**, University of Cambridge

128 participants

This was the first meeting to focus exclusively on heat-shock-induced changes in gene expression. The initial observation that a mild heat shock could dramatically alter an organism's gene activity was reported for *Drosophilia* embryos in 1964, but it was not until 1978 that heat-induced changes in protein synthesis patterns were reported for several other organisms. It was the realization that heat-shock effects on gene expression occurred universally that prompted a call for a meeting that could bring together the diverse groups of scientists engaged in studies of heat shock. The areas of research ranged from cloning of heat-shock genes to studies of thermotolerance in heat- and alcohol-stressed tissue culture cells.

The important conclusions reaffirmed the *universality* of this kind of stress. Several of the major heat-shock genes and proteins were shown to be strongly conserved in sequence and structure from bacteria to man, and a region of the gene controlling expression of the major *Drosophilia* heat-shock gene was reported to function effectively in mammalian cells and regulate non-heat-shock genes. It was also shown that a major function of heat-shock proteins was to protect cells from thermal damage, but the biochemical mechanisms for thermotolerance remain unknown. The 54 papers presented at the meeting were published by Cold Spring Harbor Laboratory in November 1982 in *Heat Shock: From Bacteria to Man.*

This meeting was supported by grants from External Research Development, Monsanto Company; Research Division, Monsanto Agricultural Products Company; National Science Foundation; National Institutes of Health; National Institutes of General Medical Sciences; and Fogarty International Center.

SESSION 1 *Introduction and Overview*

M. ASHBURNER, Cambridge University, Cambridge, England

M. Ashburner, H.R.B. Pelham

F. Ritossa, M.J. Schlesinger

SESSION 2 *Gene Organization*

Chairperson: M. MESELSON, Harvard University, Cambridge, Massachusetts

E. CRAIG, T. INGOLIA, M. SLATER, and L. MANNSEAU, Dept. of Physiological Chemistry, University of Wisconsin, Madison: *Drosophila* and yeast multigene families related to the major *Drosophila* heat-shock inducible gene.

I. TÖRÖK,[1] P.J. MASON,[2] F. KARCH,[2] I. KISS,[1] and A. UDVARDY,[1] [1]Institute of Biochemistry, Hungarian Academy of Sciences, Szeged; [2]Dept. of Molecular Biology, University of Geneva, Switzerland: DNA sequence analysis of the *hsp* 70 heat-shock genes in *D. melanogaster.*

C.G. CORCES,[1] A. PELLICER,[2] R. AXEL,[3] and M. MESELSON,[3] [1]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; [2]Dept. of Pathology, New York University Medical Center, New York; [3]College of Physicians and Surgeons, Columbia University, New York, New York: Localization of sequences responsible for heat shock-induced transcription.

M.-E. MIRAULT, Dept. of Molecular Biology, University of Geneva, Switzerland: The mechanism of heat-shock induction studied by transfection of monkey cells with SV40-derived vectors carrying *D. melanogaster hsp* 70 genes.

H.R.B. PELHAM, MRC Laboratory of Molecular Biology, Cambridge, England: Functional analysis of a heat-shock promoter.

J. LIS, N. COSTLOW, J. DE BANZIE, D. KNIPPLE, and D. O'CONNOR, Dept. of Molecular and Cell Biology, Cornell University, Ithaca, New York: Structure and expression of *Drosophila* heat-shock genes cloned in yeast.

D.B. FINKELSTEIN and S. STRAUSBERG, Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Expression of a cloned yeast heat-shock gene.

SESSION 3 *Chromatin Structure*

Chairperson: S. ELGIN, Washington University, St. Louis, Missouri

M.A. KEENE[1] and S.C.R. ELGIN,[2] [1]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; [2]Dept. of Biology, Washington University, St. Louis, Missouri: Perturbations of chromatin structure associated with gene expression.

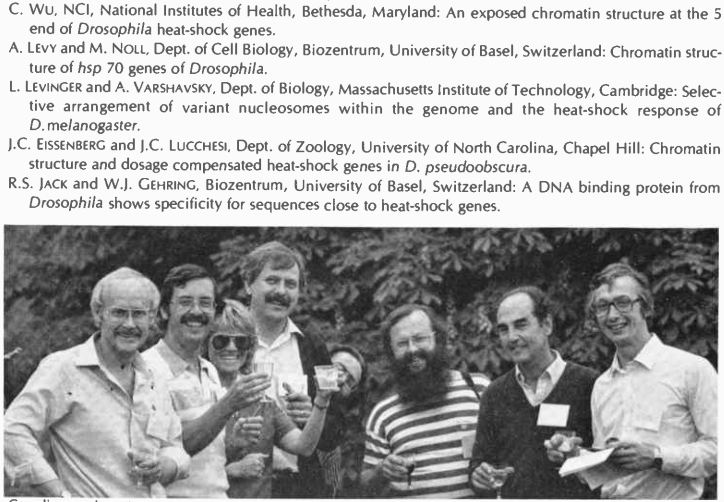
C. WU, NCI, National Institutes of Health, Bethesda, Maryland: An exposed chromatin structure at the 5′ end of *Drosophila* heat-shock genes.

A. LEVY and M. NOLL, Dept. of Cell Biology, Biozentrum, University of Basel, Switzerland: Chromatin structure of *hsp* 70 genes of *Drosophila.*

L. LEVINGER and A. VARSHAVSKY, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Selective arrangement of variant nucleosomes within the genome and the heat-shock response of *D. melanogaster.*

J.C. EISSENBERG and J.C. LUCCHESI, Dept. of Zoology, University of North Carolina, Chapel Hill: Chromatin structure and dosage compensated heat-shock genes in *D. pseudoobscura.*

R.S. JACK and W.J. GEHRING, Biozentrum, University of Basel, Switzerland: A DNA binding protein from *Drosophila* shows specificity for sequences close to heat-shock genes.



Canadian contingent

J.R. PELLON,[1] R.F. GOMEZ,[2] and A.J. SINSKEY,[1] [1]Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge; [2]Genentech, Inc., San Francisco, California: Association of proteins synthesized during heat shock to the *E. coli* nucleoid.

M.A. GOROVSKY, C.V. GLOVER, S.D. GUTTMAN, K.J. VAVRA, S. HOROWITZ, and D.S. PEDERSON, Dept. of Biology, University of Rochester, New York: Stress-induced changes in nuclear proteins of *Tetrahymena*.

K. SIROTKIN, Dept. of Microbiology, University of Tennessee, Knoxville: Detection in embryos of the developmentally regulated transcript in the center of the cluster of small heat-shock genes using end-labeled DNA and nuclease S1.

SESSION 4 *Regulation*

Chairperson: A. TISSIÈRES, University of Geneva, Geneva, Switzerland

T. YAMAMORI, T. OSAWA, T. TOBE, K. ITO, and T. YURA, Institute for Virus Research, Kyoto University, Japan: *E. coli* gene (*hin*) controls transcription of heat-shock operons and cell growth at high temperature.

F.C. NEIDHARDT, R.A. VAN BOGELEN, and E.T. LAU, Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: The high temperature regulon of *E. coli*.

A.A. TRAVERS, MCR Laboratory of Molecular Biology, Cambridge, England: DNA sequences necessary for the shutoff of *E. coli tyrT* transcription at high temperature.

J.J. BONNER, Dept. of Biology, Indiana University, Bloomington: Feedback regulation of the *Drosophila* heat-shock response.

R. MORIMOTO, J. SCHAFFER, and M. MESELSON, Harvard Biochemical Laboratories, Cambridge, Massachusetts: In vitro transcription of *Drosophila* actin and heat-shock genes in HeLa cell-free extracts.

J. MOHLER and M.L. PARDUE, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic analysis of the region containing the 93 D heat-shock locus.

J.F. BURKE and D. ISH-HOROWICZ, Imperial Cancer Research Fund, London, England: Cloning and expression of a mutant, truncated *Drosophila hsp* 70 gene.

SESSION 5 *Regulation*

Chairperson: S. LINDQUIST, University of Chicago, Chicago, Illinois

B.J. DIDOMENICO, G.E. BUGAISKY, and S. LINDQUIST, Dept. of Biology, University of Chicago, Illinois: Feedback regulation in the heat-shock response.

D.G. BALLINGER and M.L. PARDUE, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The subcellular compartmentalization of messenger RNAs in heat-shocked *Drosophila* cells.

C. KRÜGER and B.J. BENECKE, Dept. of Biochemistry, Ruhr-Universität, Bochum Federal Republic of Germany: Translation and turnover of *Drosophila* heat-shock and non-heat-shock mRNA.

M. BIENZ and J.B. GURDON, MRC Laboratory of Molecular Biology, Cambridge, England: The heat-shock response in *Xenopus* oocytes is controlled at the translational level.

C.V. GLOVER, Dept. of Biochemistry, Stanford University School of Medicine, California: Rapid dephosphorylation of ribosomal protein following heat shock of *Drosophila* tissue culture cells.

V. ERNST, E. ZUKOFSKY BAUM, and P. REDDY, Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Heat-shock, eIF-2 phosphorylation, and control of translation in rabbit reticulocytes, reticulocyte lysates and HeLa cells.

E. HICKEY and L. WEBER, Dept. of Biology, University of South Florida, Tampa: Preferential translation of heat-shock mRNAs in HeLa cells.

SESSION 6 *Proteins*

Chairperson: M. SCHLESINGER, Washington University, St. Louis, Missouri

M.J. SCHLESINGER, P.M. KELLEY, and C. MALFER, Dept. of Microbiology and Immunology, Washington University Medical School, St. Louis, Missouri: Properties of three chicken heat-shock proteins and their antibodies.

W.J. WELCH and J.R. FERAMISCO, Cold Spring Harbor Laboratory, New York: Purification of three mammalian stress proteins.

J.M. WILHELM, P. SPEAR, and C. SAX, Dept. of Microbiology, University of Rochester, New York: Heat-shock

proteins in the protozoan *Tetrahymena*—Induction by protein synthesis inhibition and possible role in carbohydrate metabolism.

R.H. BURDON, Dept. of Biochemistry, University of Glasgow, Scotland: Human *hsp* gene expression and the modulation of plasma membrane Na^+K^+ ATPase activity.

H. BIESSMANN,[1] F.G. FALKNER,[2] H. SAUMWEBER,[3] and M.F. WALTER,[4] [1]Dept. of Genetics, University of California, Davis; [2]Dept. of Physiological Chemistry, University of Munich, Federal Republic of Germany; [3]Dept. of Biochemistry, University of California, San Francisco; [4]Dept. of Zoology, University of California, Davis: Disruption of the vimentin cytoskeleton may play a role in heat-shock response.

W. YONEMOTO, L.A. LIPSICH, and J.S. BRUGGE, Dept. of Microbiology, State University of New York, Stony Brook: An analysis of the interaction of the RSV transforming protein, $pp60^{src}$, with a major heat-shock protein.

J.J.-C. LIN, W.J. WELCH, J.I. GARRELS, and J.R. FERAMISCO, Cold Spring Harbor Laboratory, New York: The mammalian 100K heat-shock protein is a Golgi-associated protein.

M.M. SANDERS, Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Heat-shock histone H2b becomes associated with chromatin and the nuclear matrix in heat shock.

SESSION 7 *Inducers*

Chairperson: F. RITOSSA, Institute of Genetics, Bari, Italy

L.E. HIGHTOWER[1] and F.P. WHITE,[2] [1]Microbiology Section, University of Connecticut, Storrs; [2]Faculty of Medicine, Memorial University of Newfoundland, St. John's, Canada: Preferential synthesis of rat heat shock and glucose-regulated proteins in stressed cardiovascular cells.

F.P. WHITE and R.W. CURRIE, Faculty of Medicine, Memorial University of Newfoundland, Canada: The induction of a 71kD protein in various rat organs as a function of postnatal development.

I.R. BROWN and J.W. COSGROVE, Dept. of Zoology, University of Toronto, Canada: Physiologically relevant increases in body temperature induce the synthesis of a heat-shock protein in mammalian brain and other organs.

P.M. KELLEY and M. FREELING, Dept. of Genetics, University of California, Berkley: Comparison of maize anaerobic and heat-shock proteins.

C.H. BUZIN and N. BOURNIAS-VARDIABASIS, Division of Cytogenetics and Cytology, City of Hope Medical Center, Duarte, California: The induction of a subset of heat-shock proteins by drugs that inhibit differentiation in *Drosophila* embryonic cell cultures.

G.P. THOMAS, J.I. GARRELS, and M.B. MATHEWS, Cold Spring Harbor Laboratory, New York: Differential induction of HeLa and CEF stress proteins by alternate inducers.

J.R. NEVINS, Rockefeller University, New York: Induction of synthesis of the HeLa cell 70kD heat-shock protein by the adenovirus E1A gene product.

SESSION 8 *Physiological Response*

Chairperson: H. MITCHELL, California Institute of Technology, Pasadena, California

H.K. MITCHELL and N.S. PETERSEN, Dept. of Biology, California Institute of Technology, Pasadena: Heat-shock induction of abnormal morphogenesis in *Drosophila*.

N.S. PETERSEN and H.K. MITCHELL, Dept. of Biology, California Institute of Technology, Pasadena: Effects of heat shock on mRNA synthesis, stability, and translation in differentiating *Drosophila* wings.

W.F. LOOMIS and S.A. WHEELER, Dept. of Biology, University of California San Diego, La Jolla: The physiological role of heat-shock proteins in *Dictyostelium*.

G.C. LI,[1] D.C. SHRIEVE,[1] and Z. WERB,[2] [1]Dept. of Radiation Oncology; [2]Dept. of Anatomy, University of California, San Francisco: Correlation between synthesis of heat-shock proteins and development of thermotolerance in Chinese hamster fibroblasts.

J.R. SUBJECK and J.J. SCIANDRA, Dept. of Radiation Biology, Roswell Park Memorial Institute, Buffalo, New York: Heat-shock proteins and thermotolerance.

R.R. KLEVECZ,[1] G.A. KING,[1] and C.H. BUZIN,[2] [1]Division of Biology, City of Hope Research Institute; [2]Dept. of Cytogenetics and Cytology, City of Hope National Medical Center, Duarte, California: Cell-cycle phase response and the induction of thermotolerance and heat-shock proteins.

J.L. KEY, C.Y. LIN, E. CEGLARZ, Y.M. CHEN, and F. SCHÖFFL, Dept. of Botany, University of Georgia, Athens: Physiological aspects of the heat-shock response in soybean.

M. ALTSCHULER and J.P. MASCARENHAS, Dept. of Biological Sciences, State University of New York, Albany: The synthesis of heat-shock and normal proteins at high temperatures in plants.

Posters

G. ALIPERTI and M.J. SCHLESINGER, Dept. of Microbiology and Immunology, Washington University Medical School, St. Louis, Missouri: Recovery of normal chicken embryo fibroblast mRNAs after heat shock is inhibited by actinomycin D and cyclohexamide.

B.G. ATKINSON,[1] M. SOMERVILLE,[1] and D.B. WALDEN,[2] [1]Dept. of Zoology and [2]Dept. of Plant Sciences, University of Western Ontario, London, Canada: The effect of heat shock and other stresses on the gene expression of differentiating myogenic cells.

E. BERGER,[1] R. IRELAND,[1] K. SIROTKIN,[2] M.A. YUND,[3] and D. OSTERBUR,[3] [1]Dept. of Biology, Dartmouth College, Hanover, New Hampshire; [2]Dept. of Microbiology, University of Tennessee, Knoxville; [3]Dept. of Genetics, University of California, Berkeley: The regulation of small *hsp* gene activity is under dual control.

N. BOURNIAS-VARDIABASIS and C.H. BUZIN, Division of Cytogenetics and Cytology, City of Hope Medical Center, Duarte, California: Mild heat pretreatment protects *Drosophila* embryonic cells from inhibition of differentiation caused by hyperthermia.

T. BRADY and M. SORLEY, Dept. of Biological Sciences, Texas Tech University, Lubbock: Chromosomal binding of pyridoxine (vitamin B_6) at the tyrosine aminotransferase (TAT) gene site during heat shock and pyridoxine-induced gene activation.

P.A. BROMLEY and R. VOELLMY, Dept. of Molecular Biology, University of Geneva, Switzerland: The effect of a reversible heat shock on the expression of RSV and vesicular stomatitis virus in infected cells.

P.A. BROMLEY and R. VOELLMY, Dept. of Molecular Biology, University of Geneva, Switzerland: Massive heat-shock polypeptide synthesis in late chicken embryos—A convenient system for the study of protein synthesis in highly differentiated organisms.

R. CAIZZI, C. CAGGESE, and F. RITOSSA, Institute of Genetics, Bari, Italy: Modifications of the 70K heat shock protein in *Drosophila*.

R. CAMATO, L. NICOLE, and R.M. TANGUAY, Dept. of Medicine, Université Laval, Ste. Foy, Canada: Histone gene expression and histone modifications during heat shock in *Drosophila* cells.

A. DANGLI,[1] C. GROND,[2] R. KABISCH,[1] and E.K.F. BAUTZ,[1] [1]Molekulare Genetik, Universität Heidelberg, Federal Republic of Germany; [2]Dept. of Genetics, University of Nijmegen, The Netherlands: Heat-shock puff 93 D in *D. melanogaster*—Ultrastructural and immunocytochemical aspects of organization and homology to other species.

K. DYBVIG, G. ALIPERTI, C.D. CLARK, D. LITEANU, and M.J. SCHLESINGER, Dept. of Microbiology and Immunology, Washington University Medical School, St. Louis, Missouri: Cloning of the gene coding for the 24,000 D heat-shock protein (HSP24) from chicken embryo fibroblasts.

G. GRAZIOSI, F. MICALI, A. DI MARCOTULLIO, A. SAVOINI, F. DE CRISTINI, and R. MARZARI, Instituto di Zoologia e Anatomia Comparata, Università di Trieste, Italy: Heat shock of the *Drosophila* egg—Mortality, cellular multiplication, DNA replication, and hsp synthesis.

J.J. HEIKKILA, L. GEDAMU, K. IATROU, and G.A. SCHULTZ, University Biochemistry Group, University of Calgary, Canada: Expression of a set of fish genes following heat or metal ion exposure.

H. IIDA and I. YAHARA, Tokyo Metropolitan Institute of Medical Science, Japan: Durable synthesis of heat-shock proteins in yeast cells and chicken embryonic cells during entering the resting state.

E.W. KHANDJIAN and H. TÜRLEZ, Dept. of Molecular Biology, University of Geneva, Switzerland: Mammalian "heat-shock" proteins are also induced by viral infection.

J.K.C. KNOWLES and K. HEMMINKI, Dept. of Medical Chemistry and Dept. of Genetics, University of Helsinki, Finland: Induction of heat-shock puffs in *D. hydei* salivary gland cells by microinjection of a chromatin fraction.

S. KURTZ, L. PETKO, and S. LINDQUIST, Dept. of Biology, University of Chicago, Illinois: Heat-induced genes of *S. cerevisiae*.

K.W. LANKS, Dept. of Pathology, State University of New York, Downstate Medical Center, Brooklyn: Heat shock induces synthesis of the 85K glucose-regulated protein.

W. LEVINSON, G. LI, H. OPPERMAN, D. JOHNSTON, and J. JACKSON, Dept. of Microbiology, University of California, San Francisco: Chemical induction of heat-shock proteins.

D.L. LOWE, W.D. FULFORD, M.D. PERRY, and L.A. MORAN, Dept. of Biochemistry, University of Toronto, Ontario, Canada: Heat shock in mouse cells.

R.E.J. MITCHEL and D.P. MORRISON, Dept. of Radiation Biology, Atomic Energy of Canada Limited, Chalk River, Ontario: Heat-shock induction of radiation resistance and recombinational repair ability in *S. cerevisiae*.

R. MORIMOTO and M. MESELSON, Harvard Biochemical Laboratories, Cambridge, Massachusetts: The hyperthermal protective role of the mammalian 68K stress-induced protein.

T.S. NOWAK, JR., W.D. LUST, and J.V. PASSONNEAU, NINCDS, National Institutes of Health, Bethesda, Maryland: Metabolic correlates of amphetamine-induced hyperthermia in mouse brain.

F.P.A.M.N. PETERS, C.J. GROND, P.A. SONDERMEIJER, and N.H. LUBSEN, Dept. of Genetics, University of Nijmegen, The Netherlands: Chromosomal arrangement of heat-shock locus 2-48B of *Drosophila hydei*.

J. PLESSET, J.J. FOY, and C.S. MCLAUGHLIN, Dept. of Biological Chemistry, University of California, Irvine: Heat shock in *S. cerevisiae*—Quantitation of transcriptional and translational effects.

O. PONGS, W. KOERWER, and A. POETING, Lehrstuhl für Biochemie, Ruhr-Universität Bochum, Federal Republic of Germany: Two small heat-shock proteins are ecdysterone inducible proteins in salivary glands of *D. melanogaster* larvae.

C.M. PRESTON and E. NOTARIANNI, Institute of Virology, Glasgow, Scotland: HSV immediate-early polypeptides induce heat-shock proteins.

I. RUBIN and H. SWIFT, Dept. of Pathology, University of Chicago, Illinois: Effect of growth conditions and treatment patterns on the heat-shock response of chick embryo cells.

F. SCHÖFFL and J.L. KEY, Dept. of Botany, University of Georgia, Athens: Soybean heat shock—Molecular studies on mRNAs for a group of heat-shock proteins.

V.F. SEMESHIN, I.E. VLASSOVA, E.M. BARICHEVA, E.S. BELYAEVA, and I.F. ZHIMVLEV, Group of Drosophila Cytogenetics, USSR Academy of Sciences, Novosibirsk: Development of heat shock puffs in *D. melanogaster*.

J.C. SILVER and D. PEKKALA, Dept. of Microbiology, University of Toronto, Canada: Effect of heat shock on synthesis and phosphorylation of nuclear and cytoplasmic proteins in the fungus *Achlya*.

R.M. SINIBALDI and P.W. MORRIS, Dept. of Biological Chemistry, University of Illinois Medical Center, Chicago: *D. melanogaster* Kc cell heat-shock proteins and the nucleoskeleton.

T.P. SNUTCH and D.L. BAILLIE, Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Heat-shock induction in *C. elegans*.

R.M. TANGUAY and M. VINCENT, Dept. of Medicine, University Laval, Ste. Foy, Canada: Intracellular distribution and modification of cellular and heat-shock proteins during heat shock in *Drosophila*.

G.P. THOMAS, Cold Spring Harbor Laboratory, New York: Isolation and preliminary characterization of recombinant phage containing the gene for the human 90K stress protein.

G.P. THOMAS and M.B. MATHEWS, Cold Spring Harbor Laboratory: Alterations in gene expression of HeLa cells exposed to amino acid analogs.

J. VELAZQUEZ and S.L. LINDQUIST, Dept. of Biology, University of Chicago, Illinois: Studies with monoclonal antibodies against hsp 70.

R. VOELLMY,[1] P. BROMLEY,[2] and H.P. KOCHER,[3] [1]Dept. of Biochemistry, University of Miami School of Medicine, Florida; [2]Battelle, Geneva Research Center, Switzerland; [3]Dept. of Medical Biochemistry, University of Geneva Medical Center, Switzerland: Structural similarities between heat-shock proteins from different eukaryotes.

R. VOELLMY[1] and D. RUNGGER,[2] [1]Dept. of Biochemistry, University of Miami School of Medicine, Florida; [2]Dept. of Animal Biology, University of Geneva, Switzerland: Heat-induced transcription of *Drosophila* heat-shock genes in *Xenopus* oocytes.

S.C. WADSWORTH, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: A family of related proteins is encoded by the major *Drosophila* heat-shock gene family.

D.B. WALDEN,[1] B.G. ATKINSON,[2] C.L. BASZCZYNSKI,[1] J. BOOTHE,[1] and M.W. KLINCK,[1] [1]Dept. of Plant Sciences; [2]Dept. of Zoology, University of Western Ontario, London, Canada: Temperature shift response in *Zea mays*.

In Vitro Mutagenesis

May 12–May 16

Arranged by **Robert Schleif**, Brandeis University, **David Shortle**, State University of New York, Stony Brook, **R. Bruce Wallace**, City of Hope Research Institute

198 participants

In 1981 progress in genetic engineering had reached the stage at which the ability to generate closely related variants of cloned DNA fragments was rate-limiting to the research of many investigators. Consequently, a meeting on the subject of in vitro mutagenesis was organized to bring together scientists active in the area as well as those interested in the subject.

During the meeting many methods were described for the production of variant DNA molecules by in vitro manipulations. They ranged from direct chemical synthesis of desired variants to clever schemes for specifically modifying bases in a small stretch of a cloned DNA segment. The approaches were applied to a broad range of biological systems and resulted in a meeting unusual in the breadth of its scope. Perhaps the main lesson of the meeting was that no unique best method for variant generation yet exists. Chemical synthesis is valuable when the exact sequence of the desired variant is known, but otherwise, approaches that produce many different types of alteration within small regions are favored.

The subject was of both theoretical and practical interests and a healthy mix of academic and industrial scientists attended. Reflecting this interest, the meeting was supported by the National Science Foundation as well as Pfizer, Inc.; New England Biolabs, Inc.; Applied Molecular Genetics; Cetus Corporation; Merck and Company; Abbott Laboratories, Diagnostics Division; Lilly Research Laboratories; Schleicher and Schuell, Inc.; and New England Nuclear.

SESSION 1 *Chemical Synthesis. I.*

Chairperson: M. SMITH, University of British Columbia, Vancouver, Canada

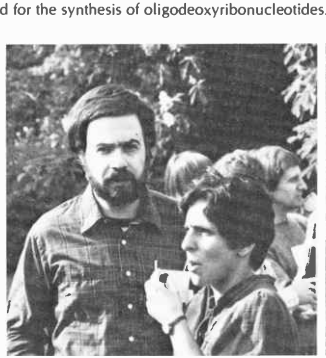
M. SMITH, Dept. of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, Canada: In vitro construction of mutants using oligodeoxyribonucleotides.

L.W. COHEN,[1] K. ITAKURA,[1] A.D. RIGGS,[1] G. DALBADIE-MCFARLAND,[2] and J.H. RICHARDS,[2] [1]City of Hope National Medical Center, Duarte, California; [2]California Institute of Technology, Pasadena: Oligonucleotide-directed mutagenesis as a general and powerful method for studies of protein function.

A.E. GAUTIER,[1] A.D. CHARLES,[1] M.D. EDGE,[2] and J.R. KNOWLES,[1] [1]Dept. of Chemistry, Harvard University, Cambridge, Massachusetts; [2]ICI Pharmaceutical Division, Cheshire, England: Targeted point mutation that creates a unique *Eco*RI site within the signal codons of the β-lactamase gene without altering enzyme secretion or processing.

M. SATO and K. ITAKURA, Dept. of Molecular Genetics, City of Hope Research Institute, Duarte, California: Simple solid phase method for the synthesis of oligodeoxyribonucleotides.

M. Smith



D. Shortle, M. Manos

C. Tibbetts

R.B. WALLACE,[1] M. SCHOLD,[1] B. CONNER,[2] and K. ITAKURA,[1] [1]Dept. of Molecular Genetics; [2]Dept. of Cytogenetics; City of Hope Research Institute, Duarte, California: The use of synthetic DNA for the introduction and identification of site-specific mutations.

J. ESSIGMANN,[1] C. GREEN,[1] E. LOECHLER,[2] and K. FOWLER,[3] [1]Depts. of Nutrition and Food Science; [2]Dept. of Biology; [3]Dept. of Chemistry; Massachusetts Institute of Technology, Cambridge: Synthesis of oligonucleotides containing carcinogen-modified bases and their insertion into viral and plasmid genomes.

M.J. GAIT, H. MATTHES, M. SINGH, and R.C. TITMAS, MRC Laboratory of Molecular Biology, Cambridge, England: Total synthesis of short duplex DNA.

SESSION 2 *Deletions/Insertions/Fusions. I.*

Chairperson: T. SHENK, State University of New York, Stony Brook, New York

D. BOGENHAGEN, Dept. of Pharmacological Sciences, State University of New York, Stony Brook: Control of transcription of *Xenopus* 5S RNA genes.

D. GRASS, D. LEWIS, Y.T. YU, and J. MANLEY, Dept. of Biological Sciences, Columbia University, New York, New York: Construction of specific deletion mutants to analyze gene expression in DNA tumor viruses.

P. HEARING and T. SHENK, Dept. of Microbiology, State University of New York, Stony Brook: Mutational analysis of the adenovirus type 5 E1A transcriptional control region.

M. JASIN and P. SCHIMMEL, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Functional domains in a protein determined by progressive deletions from one end of the gene coding region.

E. COX and D. HORNER, Dept. of Biology, Princeton University, New Jersey: A structural and functional analysis of an *E. coli* mutator, *mutD*.

G.M. SANTANGELO, J. TORNOW, and C.N. COLE, Dept. of Human Genetics, Yale Medical School, New Haven, Connecticut: The role of 3'-end signals in gene expression.

F.A. LASKI,[1] A. FIRE[1], R. BELAGAJE,[2] U. RAJBHANDARY,[1] and P.A. SHARP,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge, [2]Lilly Research Laboratories, Indianapolis, Indiana: Mutagenesis of a tRNA gene to an amber suppressor.

SESSION 3 *Site-directed Mutagenesis. I.*

Chairperson: D. BOTSTEIN, Massachusetts Institute of Technology, Cambridge, Massachusetts

W.R. FOLK[1] and J. HOFSTETTER,[2] [1]Dept. of Biological Chemistry, University of Michigan, Ann Arbor; [2]Institut für Molekularbiologie II, Universität Zürich, Switzerland: Nucleotides within the *X. laevis* tRNA *met I* gene required for transcription by RNA polymerase III.

L.A. LOEB, R.M. SCHAAPER, and T.A. KUNKEL, Joseph Gottstein Memorial Cancer Research Laboratory, University of Washington, Seattle: Infidelity of DNA synthesis as a basis of mutagenesis.

D. SHORTLE,[1] P. GRISAFI,[1] S.J. BENKOVIC,[2] and D. BOTSTEIN,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Chemistry, Pennsylvania State University, University Park: Gap misrepair mutagenesis with α-thiophosphate nucleotides.

E. LORENSEN and J.W. CHASE, Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Bisulfite-induced mutations of *E. coli* single-strand DNA binding protein.

C.-T. CHU, D.M. COEN, S.K. WELLER, and P.A. SCHAFFER, Sidney Farber Cancer Institute, Boston, Massachusetts: In vitro mutagenesis of herpes simplex virus DNA and DNA fragments—Introduction of mutations into selected regions of the viral genome.

W.-C. LEUNG, Dept. of Pathology, McMaster University, Hamilton, Canada: In vitro mutants of a herpes simplex virus regulatory protein ICP4.

K.W.C. PEDEN and D. NATHANS, Dept. of Molecular Biology and Genetics, John Hopkins University School of Medicine, Baltimore, Maryland: Local mutagenesis within deletion loops of DNA heteroduplexes.

SESSION 4 *Allele Replacement—Applications. I.*

Chairperson: A. BERK, University of California, Los Angeles, California

V.R. RACANIELLO and D. BALTIMORE, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Studies on poliovirus using an infectious viral cDNA clone.

C.-J. LAI and M.M. SVEDA, NIAID, National Institutes of Health, Bethesda, Maryland: Requirements for surface expression of the hemagglutinin and the neuraminidase of influenza A virus.

A.J. BERK,[1] C. MONTELL,[1] T. OSBORNE,[1] D. ROSSER,[1] E. TYAU,[1] D. ARVIDSON,[1] E. FISHER,[2] and M. CARUTHERS,[2] [1]Molecular Biology Institute, University of California, Los Angeles; [2]Dept. of Chemistry, University of Colorado, Boulder: Mutagenesis of the adenovirus transforming region.

F. AUSUBEL, W. BUIKEMA, C. EARL, S. GIBBONS, S. LONG, G. RUVKUN, and R. ZUCKERMAN, Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Genetic analysis of symbiotic nitrogen fixation genes.

M. GRUNSTEIN,[1] D. KOLODRUBETZ,[2] M. RYKOWSKI,[2] and J. CHOE,[2] [1]Molecular Biology Institute; [2]University of California, Los Angeles: A genetic test for the association of histone H2A and H2B subtypes in yeast.

D. SHORTLE and D. BOTSTEIN, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Directed mutagenesis of the yeast actin locus—Integrative transformation with actin gene sequences modified in vitro.

R.J. ROTHSTEIN,[1] T.L. ORR-WEAVER,[2] and J.W. SZOSTAK,[2] [1]Dept. of Microbiology, New Jersey Medical School, Newark; [2]Dept. of Biological Chemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Directed integration into the yeast genome.

SESSION 5 *Poster Session*

S.A. BENNER, K.P. NAMBIAR, J. STACKHOUSE, J. ELDREDGE, and D. STAUFFER, Dept. of Chemistry, Harvard University, Cambridge, Massachusetts: Structure-function relationships in catalytic proteins—Ribonuclease.

J.D. BOEKE, M. RUSSEL, and P. MODEL, Rockefeller University, New York: Bisulfite mutagenesis of a signal peptide coding region by deletion heteroduplexing using the single-stranded DNA bacteriophage f1.

W. BUIKEMA, C. EARL, R. ZUCKERMAN, S. GIBBONS, and F. AUSUBEL, Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Cloning and mapping of symbiotic nitrogen fixation genes.

S. BUSBY, M. HERBERT, A. KOLB, and H. BUC, Institut Pasteur, Paris, France: Comparison of the binding sites for the cAMP receptor protein at the galactose and lactose promoters.

M.H. CARUTHERS, P. DE HASETH, R. GOLDMAN, and W. MANDECKI, Dept. of Chemistry, University of Colorado, Boulder: Chemical synthesis and biological studies on mutated promoters.

L. DODSON,[1] R. FOOTE,[2] S. MITRA,[2] and W. MASKER,[2] [1]University of Tennessee School of Biomedical Sciences, Oak Ridge; [2]Biology Division, Oak Ridge National Laboratory, Tennessee: In vitro site-specific mutagenesis of bacteriophage T7 DNA with O^6-methylguanine.

R.C. HAYES and J.E. LECLERC, Dept. of Biochemistry, University of Rochester School of Medicine and Dentistry, New York: A simple method using single-stranded DNA binding protein to preferentially transfect replicated DNA molecules.

R. SALGANIK, G. DYMSHITZ, and L. FRUMGARTZ, Institute of Cytology and Genetics, Siberian Division of the

Poster session

USSR, Academy of Sciences, Novosibirsk: Identification of DNA restricts by complementary polynucleotides carrying fluorescent groupings.

M. SATO,[1] H. TOYODA,[1] Y. KAJUMURA,[1] S. KAPLAN,[1] J. SHIVELY,[2] R.B. WALLACE,[1] and K. ITAKURA,[1] [1]Dept. of Molecular Genetics, [2]Division of Immunology, City of Hope Research Institute, Duarte, California: A screening method using synthetic oligonucleotides as hydridization probes.

N. STOW, M. ANDERSON, J. ENGLER, P. ROSSMAN, and M. ROSSINI, Cold Spring Harbor Laboratory, New York: Construction and characterization of deletion mutants in early region 1 of adenovirus 2.

R.C. THOMPSON[1] and M. YARUS,[2] [1]Dept. of Chemistry, Temple University, Philadelphia, Pennsylvania; [2]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: In vitro mutagenesis of an anticodon—Replacement of U33, the "Universal U."

C. TIBBETTS, L. KOSTURKO, and C. ROBINSON, Dept. of Microbiology, University of Connecticut School of Medicine, Farmington: Evolutionary variants of adenovirus type 3 which delimit the DNA sequence required for left-end polar encapsidation of the viral genome.

J.C. WAY, M.A. DAVIS, D.E. ROBERTS, and N. KLECKNER, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: New tools for transposon mutagenesis in bacteria.

M.J. ZOLLER and M. SMITH, Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: Site-specific mutagenesis of cloned genes.

SESSION 6 *Chemical Synthesis. II.*

Chairperson: R.B. WALLACE, City of Hope Research Institute, Duarte, California

J. ROSSI,[1] G. LARSON,[1] Y. MARUMOTO,[1] X. SOBERON,[2] and K. ITAKURA,[1] [1]Dept. of Molecular Genetics, City of Hope Research Institute, Duarte, California; [2]Instituto de Investigaciones Biomedias, Cuidad Universitario, Mexico: Synthetic oligodeoxyribonucleotide directed site-specific mutagenesis of prokaryotic and eukarotic regulatory regions.

S.A. NARANG,[1] R. BROUSSEAU,[1] and R. WU,[2] [1]Division of Biological Sciences, National Research Council, Ottawa, Canada; [2]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Detection of a clone containing a single base-pair mutation in synthetic human proinsulin gene.

H. HSIUNG,[1] S. INOUYE,[2] J. WEST,[1] B. STURM,[1] and M. INOUYE,[2] [1]Lilly Research Laboratories, Indianapolis, Indiana; [2]State University of New York, Stony Brook: Improvements on phosphotriester synthesis of deoxyribooligonucleotides—Applications to synthesis of oligonucleotide fragments for site-specific mutagenesis of *E. coli* prolipoprotein gene.

M. INOUYE,[1] S. INOUYE,[1] M. INUKAI,[1] G. VLASUK,[1] J. COLEMAN,[1] K. ITAKURA,[2] and H. HSUING,[3] [1]Dept. of Biochemistry, State University of New York, Stony Brook: [2]Division of Biology, City of Hope Research Institute, Duarte, California; [3]Lilly Research Laboratories, Indianapolis, Indiana: Use of site-specific mutagenesis for the study of protein secretion and membrane protein assembly.

C.A. BRENNAN and R.I. Gumport, Dept. of Biochemistry and School of Basic Medical Science, University of Illinois, Urbana: The synthesis of oligodeoxyribonucleotides using T4 RNA ligase.

W.M. BARNES and M. BEVAN, Dept. of Biological Chemistry, Washington University Medical School, St. Louis, Missouri: Kilo-sequencing—A nonrandom strategy for sequencing large amounts of DNA.

G.G. CARMICHAEL,[1] B. SCHAFFHAUSEN,[2] D. DORSKY,[2] D. OLIVER,[3] and T.L. BENJAMIN,[2] [1]Dept. of Microbiology, University of Connecticut Health Center, Farmington; [2]Dept. of Pathology; [3]Dept. of Microbiology; Harvard Medical School, Boston, Massachusetts: Studying polyoma virus gene expression and function by site-directed mutagenesis using synthetic oligonucleotides.

G.F. TEMPLE,[1] A.M. DOZY,[1] K.L. ROY,[2] and Y.W. KAN,[1,3] [1]University of California, San Francisco; [2]University of Alberta, Edmunton, Canada; [3]Howard Hughes Medical Institute, University of California, San Francisco: Construction of human suppressor tRNA gene—Its potential for gene therapy in β-thalassemia.

SESSION 7 *Deletions/Insertions/Fusions. II.*

Chairperson: F. HEFFRON, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

J. MESSING,[1] J. VIEIRA,[1] and R. GARDNER,[2] [1]Dept. of Biochemistry, University of Minnesota, St. Paul; [2]Calgene, Inc., Davis, California: Codon insertion mutagenesis to study functional domains of β-lactamase.

C. TIBBETTS, S. SHARNICK, and C. GREEN, Dept. of Microbiology, University of Connecticut School of Medicine, Farmington: Opportunistic diversions—Mutagenesis and gene transfer with closed circular DNA.

R. HOESS, K. ABREMSKI, and N. STERNBERG, NCI-Frederick Cancer Research Facility, Frederick, Maryland: Deletion analysis of *lox*, the recombining site in P1 site-specific recombination.

B. WASYLYK, C. WASYLYK, A. DIERICH, P. AUGEREAU, and P. CHAMBON, Laboritoire de Génétique Moléculaire des Eucaryotes, CNRS, and Génie Génétique, INSERM, Strasbourg, France: The role of both upstream sequences and the "TATA" box from SV40 and conalbumin genes on transcription and gene expression.

P.F. LAMBERT and R.D. WELLS, Dept. of Biochemistry, University of Wisconsin, Madison: Targeted mutagenesis at nonunique restriction enzyme sites—Alteration of the bacteriophage G4 ori(−).

D.R. BROWN, T. SCHMIDT-GLENEWINKEL, D. REINBERG, and J. HURWITZ, Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Nucleotide sequences required for initiation of icosahedral phage DNA replication.

M. ROSE and D. BOTSTEIN, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Analysis of yeast gene expression by use of *lacZ* gene fusion.

J.D. BOEKE, Rockefeller University, New York, New York: Deletion and fusion analysis of bacteriophage f1 gene III protein, an integral membrane protein.

K. HORIUCHI, G.P. DOTTO, and K.S. JAKES, Rockefeller University, New York, New York: Deletion mutants near the origin of DNA replication of bacteriophage f1.

SESSION 8 *Site-directed Mutagenesis. II.*

Chairperson: L.A. LOEB, University of Washington, Seattle, Washington

T.A. VÖLKER,[1] J. GAFNER,[1] M.K.S. SHOWE,[2] A.U. KUHN,[1] and T.A. BICKLE,[1] [1]Dept. of Microbiology, Biozentrum, University of Basel, Switzerland: [2]Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Site-directed mutagenesis leads to the discovery of a new, essential T4 head gene.

S. BUSBY, Institut Pasteur, Paris, France: Transcription initiation in the galactose operon—Dissection of a complex regulatory region using local mutagenesis.

P. GIZA, D. SCHMIT, and B. MURR, Dept. of Chemistry, John Hopkins University, Baltimore, Maryland: Mutagenesis of gapped plasmids derived from purified single-stranded restriction fragments.

M.D. TOPAL, D. TOORCHEN, and R. REID, Depts. of Pathology and Biochemistry, University of North Carolina Medical School, Chapel Hill: Transversion mutations—Induction by purine deoxynucleoside triphosphates in the syn conformation.

W.A. SAFFRAN, M. GOLDENBERG, C.L. SMITH, and C.R. CANTOR, Dept. of Human Genetics and Development, Columbia University, New York: Formation and repair of site-specific psoralen crosslinks in plasmid pBR322.

R.A. ZAKOUR and L.A. LOEB, Gottstein Memorial Cancer Research Laboratory, University of Washington, Seattle: Infidelity of DNA synthesis as a basis for site-specific mutagenesis.

R. KOSTRIKEN and F. HEFFRON, Cold Spring Harbor Laboratory, New York: Site-directed linker mutagenesis.

J.L. ELIASON and M. PTASHNE, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Mutagenesis of the right operator of bacteriophage lambda by nucleotide misincorporation.

R. CORTESE, C. TRABONI, G. CILIBERTO, and G. CESARENI, European Molecular Biology Laboratories, Heidelberg, Federal Republic of Germany: A general method to select for mutations in a cloned DNA segment without a phenotype in *E. coli*.

SESSION 9 *Allele Replacement—Applications. II.*

Chairperson: F. AUSUBEL, Harvard University, Cambridge, Massachusetts

S.M. ANDERSON and E.M. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: Construction of a murine transforming virus containing the *src* gene of Rous sarcoma virus.

N. MUZYCZKA and D. RAWLINS, Dept. of Immunology and Medical Microbiology, University of Florida, Gainesville: Construction and characterization of a nonsense mutant in the early region of SV40.

B. HOWARD and M. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: λSV2, a plasmid cloning vector that can be stably integrated in *E. coli*.

T.A. VÖLKER,[1] S. IIDA,[2] and T.A. BICKLE,[2] [1]Dept. of Microbiology, Biozentrum, Basel, Switzerland; [2]Dept. of Microbiology, University of Basel, Switzerland: A single gene code for resistance to both chloramphenicol and fusidic acid.

G. CHINNADURAI,[1] K. FUJINAGA,[2] T. SHIMIZU,[2] and K. YOSHIDA,[2] [1]Institute for Molecular Virology, St. Louis University Medical Center, Missouri; [2]Cancer Research Institute, Sapporo Medical College, Japan: Insertion of a human repeated DNA that resembles transposable elements into an adenovirus type 7 genome.

E.R. KANTROWITZ, R.S. SILVER, and H.W. REED, Dept. of Chemistry, Boston College, Chestnut Hill, Massachusetts: Isolation of a series of *E. coli* aspartate transcarbamylase mutants with single amino acid substitutions.

RNA Processing

May 19–May 23

Arranged by **John J. Dunn**, Brookhaven National Laboratory, **Michael Mathews**, Cold Spring Harbor Laboratory, **Joan A. Steitz**, Yale University

236 participants

Genetic information flows from DNA to protein via RNA. In recent years it has become increasingly clear that the role of RNA is far from passive. RNA molecules are synthesized as colinear copies of their genes, but in many (if not most) instances these primary transcripts are substantially modified before accomplishing the functions for which they are designed. These events, collectively termed RNA processing, are much more than merely cosmetic. They are observed in all organisms so far examined and, in different cases, are responsible for the conversion of an inactive primary transcript to a functioning product, for drastically changing its function, or for enhancing its stability. The modifications include alteration of individual nucleotides, the addition or removal of sequences from one or both ends, and, most surprisingly, the deletion of a segment from an RNA chain and the rejoining of the flanking regions (splicing).

The field is a dynamic one and interest in it has increased dramatically since the discovery in 1977 that splicing is involved in the production of most mRNAs of higher cells, and mRNA metabolism figured largely in the meeting. There is much that remains to be learned here, but the shape of things to come was reflected in the reports of the impressive progress that has been made in defining the detailed maturation of rRNA and tRNA. Also of particular interest were sessions on the nature of the ribonucleoprotein complexes and one on a class of small RNA molecules whose functions and properties have recently attracted considerable attention. Providing a backdrop to the meeting as a whole was an enlightening discussion, led by Uhlenbeck, of the nature of interactions between RNA sequences on the one hand and other RNA sequences or proteins on the other.

From a meeting of such broad scope it is difficult to select the high points. The splicing mechanism propounded by Filipowicz was brought into sharp focus by work on tRNA splicing in Abelson's lab. The role of a small RNA in tRNA maturation was demonstrated clearly by Altman and Shimura, and the elegant work presented by Shenk and Walter revealed the function of small RNAs in protein synthesis and secretion. The idea, offered by Steitz, that small RNAs are involved in mRNA splicing was widely debated but remained only an attractive hypothesis. Perhaps most striking of all was Chech's report that the splicing of an rRNA precursor occurs spontaneously and without enzyme mediation.

This meeting was supported by grants from the National Science Foundation, National Institutes of Health, National Institute of General Medical Sciences, and Fogarty International Center.

Introduction: J.E. DARNELL, Molecular Cell Biology Dept., Rockefeller University, New York, New York: RNA processing—Accidental discovery to present-day logic.

SESSION 1 *Large RNP Particles*

Chairperson: R. KORNBERG, Stanford University Medical School, Stanford, California

R. KABISCH,[1] A. DANGLI,[1] K. GRONT,[2] and E.K.F. BAUTZ,[1] [1]Institute of Molecular Genetics, University of

M. Matthews, J. Steitz, J. Dunn

A. Beyer, J. Darnell, E.F.K. Bautz, W. LeStourgeon

Heidelberg, Federal Republic of Germany; [2]Laboratory of Genetics, University of Nijmegen, The Netherlands: Existence of at least two types of nuclear RNP particles in *Drosophila*.

A. BEYER, Dept. of Biology, University of Virginia, Charlottesville: Ultrastructural analysis of the RNP structure of nascent adenoviral RNA.

M. JACOB, R. GATTONI, P. KEOHAVONG, and J. STEVENIN, Laboratoire de Génétique Moléculaire des Eucaryotes, CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique, INSERM, Strasbourg, France: Localization of cellular and adenoviral RNA sequences in the two classes of units of hnRNP.

W. J. VAN VENROOIJ and C.A.G. VAN EEKELEN, Dept. of Biochemistry, University of Nijmegen, The Netherlands: In vivo cross-linking of proteins to RNA by ultraviolet irradiation of intact cells.

T.E. MARTIN, G.P. LESER, and J.M. PULLMAN, Dept. of Biology, University of Chicago, Illinois: Biochemical and immunological studies of nuclear RNP complexes.

L. LOTHSTEIN,[1] J.C. WOOLEY,[2] S.Y. CHUNG,[2] and W.M. LESTOURGEON,[1] [1]Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee; [2]Dept. of Biochemical Sciences, Princeton University, New Jersey: The subunit structure and arrangement of protein and RNA in 40S hnRNP particles.

R. KORNBERG, A.D. LEVINE, R. SPERLING, and J.L. WELIKY, Dept. of Structural Biology, Stanford University Medical School, California: A unitary structure for hnRNP.

SESSION 2 *Small RNAs*

Chairperson: J.E. DAHLBERG, University of Wisconsin, Madison, Wisconsin

A. KROL,[1] C. BRANLANT,[1] E. LAZAR,[2] H. GALLINARO,[2] and M. JACOB,[2] [1]Institut de Biologie Moléculaire et Cellulaire; [2]Laboratoire de Génétique Moléculaire des Eucaryotes, CNRS, Strasbourg, France: The nucleotide sequences of chicken, rat, and man snRNAs.

C. BRANLANT,[1] A. KROL,[1] E. LAZAR,[2] H. GALLINARO,[2] and M. JACOB,[2] [1]Institut de Biologie Moléculaire et Cellulaire; [2]Laboratoire de Génétique Moléculaire des Eucaryotes, CNRS, Strasbourg, France: The snRNA sequences which are good candidates to interact with nuclear proteins or with pre-mRNA.

R. REDDY, D. HENNING, P. EPSTEIN, D. SPECTOR, L. ROTHBLUM, S. CHIRALA, and H. BUSCH, Dept. of Pharmacology, Baylor College of Medicine, Houston, Texas: Nucleotide sequences of snRNAs, Alu snRNA, and 8S RNA.

D. TOLLERVEY, D. MALONEY, J.A. WISE, H. SWERDLOW, and C. GUTHRIE, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Identification of putative snRNAs in yeast.

C. WEINBERGER, R. SCHNEIDER, B. THIMMAPPAYA, and T. SHENK, Dept. of Microbiology, State University of New York, Stony Brook: What is the function of adenoviral VA RNAs?

E. LUND, J.E. DAHLBERG, R.R. BURGESS, and J.T. MURPHY, University of Wisconsin, Madison: Expression and possible processing of human U1 snRNA.

Y. OHSHIMA, N. NAGASU, T. TANI, Y. ITOH, N. KOGA, and N. OKADA, Institute of Biological Sciences, University of Tsukuba, Japan: Structure and expression of mammalian snRNA genes.

T. MANSER and R.F. GESTELAND, Howard Hughes Medical Institute, University of Utah, Salt Lake City: Study of human U1 RNA genes and pseudogenes—Implications regarding the transcription and processing of the RNA and its role in the creation of U1 pseudogenes.

L. BERNSTEIN, S. VAN ARSDELL, R. DENISON, S. MOUNT, K. MOWRY, and A. WEINER, Dept. of Molecular Biophysics and Biochemistry, Yale Medical School, New Haven, Connecticut: Genes and pseudogenes for human snRNA species U1, U2, and U3—Evidence that cellular RNA molecules are reverse-transcribed and integrated into new chromosomal sites in germ line DNA.

POSTER SESSION

P.F. AGRIS,[1] C.W. GEHRKE,[1] C. SMITH,[1] P. THURIAUX,[2] W. HEYER,[2] and J. KOHLI,[2] [1]University of Missouri, Columbia; [2]Institute of General Microbiology, University of Bern, Switzerland: Two mutants of RNA processing—Identification, analyses, and effect of modification-deficient suppressor tRNA mutations.

A. ALONSO,[1] M. GREZ,[1] G. SCHERER,[2] and W. SCHMID,[2] [1]Institute of Experimental Pathology; [2]Institute for Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: The presence of transcripts from a Alu-related DNA sequence in rat hnRNA and mRNA.

A. BALMAIN, R. KRUMLAUF, K. VASS, and G.D. BIRNIE, Beatson Institute for Cancer Research, Glasgow, Scotland: The use of a cloned mouse 7S RNA sequence to study the structure, expression, and genomic organization of 7S RNA in eukaryotic cells.

F.A. BAUTZ, B. HÜGLE, and E. JOCKERS, Institute of Molecular Genetics, University of Heidelberg, Federal Republic of Germany: Monoclonal antibodies directed against RNP proteins.

B. BHAT and D. APIRION, Dept. of Microbiology, Washington University, St. Louis, Missouri: A genetic approach to study RNA processing in RNP particles.

S.G. BONITZ,[1] G. HOMISON,[1] F.G. NOBREGA,[2] B. THALENFELD,[1] and A. TZAGOLOFF,[1] [1]Dept. of Biological Sciences, Columbia University, New York, New York; [2]Instituto de Quimica-Bioquimic, Cadade Universidade, San Paolo, Brazil: Processing of apocytochrome-*b* precursor RNA in yeast.

S. BONITZ, J. HILL, and B. THALENFELD, Dept. of Biological Sciences, Columbia University, New York, New York: 5′and 3′processing of yeast mitochondrial gene products.

D. BOURGAIZE,[1] C. FARRELL,[1] J. MILLER,[1] L.M. HSU,[2] K.H. LANGLEY,[3] and M.J. FOURNIER,[1] [1]Dept. of Biochemistry, University of Massachusetts, Amherst; [2]Dept. of Chemistry, Mount Holyoke College, South Hadley, Massachusetts; [3]Dept. of Physics, University of Massachusetts, Amherst: Physical properties of the *E. coli* 4.5S RNA—First results suggest a near-perfect hairpin helix.

C. BRANLANT,[1] A. KROL,[1] J.P. EBEL,[1] K. EDWARDS,[2] H. KÖSSEL,[2] G. VELDMAN,[3] J. KLOOTWIJK,[3] V. DE REGT,[3] and R. PLANTA,[3] [1]Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France; [2]Institut für Biologie III, Freiburg, Federal Republic of Germany; [3]Biochemisch Laboratorium, Amsterdam, The Netherlands: The secondary structure of the large rRNA —Implication for rRNA processing.

A. CARRASCO, I. MATTAJ, and E. DE ROBERTIS, Biozentrum, University of Basel, Switzerland: RNA transport in microinjected frog oocytes.

C.L. CASTIGLIA, G.A. BELTZ, J.J. PLUNKETT, and S.J. FLINT, Dept. of Biochemical Sciences, Princeton University, New Jersey: Effects of adenoviral infection on synthesis and maturation of cellular RNA.

S.Y. CHUNG, R. CONE, L. ZUKERBERG, and J.C. WOOLEY, Dept. of Biochemical Sciences, Princeton University, New Jersey: snRNP complexes of *D. melanogaster*.

J.-L. DARLIX and P.-F. SPAHR, Dept. of Molecular Biology, University of Geneva, Switzerland: The binding sites of viral protein P19 onto RSV RNA and possible controls of viral functions.

N. DELIHAS,[1] J. ANDERSEN,[1] J. VOURNAKIS,[2] and W.C. CURTISS,[2] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Dept. of Biology, Syracuse University, New York: Generalized structures for prokaryotic, eukaryotic, and organelle 5S rRNAs and phylogenetic relationships.

D.T. DUBIN and C.-C. HSUCHEN, Dept. of Microbiology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Terminal sequences of animal cell mitochondrial rRNA.

M.J. FOURNIER,[1] H. KLEE,[1] C. FARRELL,[1] and L.M. HSU,[2] [1]Dept. of Biochemistry, University of Massachusetts, Amherst; [2]Dept. of Chemistry, Mount Holyoke College, South Hadley, Massachusetts: Structure of an *E. coli* operon containing genes for histidine-, leucine- and proline-tRNAs.

I. GABALDÓN DE KOCH, H.E. WILK, and K.P. SCHAFER, Ruhr-Universität Bochum, Lehrstuhl Biochemie, Federal Republic of Germany: 40S hnRNP particles in resting and concanavalin A-stimulated lymphocytes and their association with snRNP particles.

J.R. GREENBERG, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: A protein associated with mRNA in intact cells is involved in initiation of protein synthesis.

W. GRUISSEM,[1] B.M. GREENBERG,[2] R.B. HALLICK,[2] and D.M. PRESCOTT,[1] [1]Dept. of Molecular, Cellular, and Developmental Biology and [2]Dept. of Chemistry, University of Colorado, Boulder: Processing of polycistronic tRNA transcripts from *E. coli* and *Euglena* chloroplast tRNA gene clusters in a HeLa cell-free system.

M. GUREVITZ, N. WATSON, and D. APIRION, Dept. of Microbiology, Washington University, St. Louis, Missouri: RNase F, a putative RNA processing endoribonuclease from *E. coli* that generates 5′ hydroxyl and 3′ phosphate at the cleavage site.

J.A. HARDIN, Yale University, New Haven, Connecticut: Systemic lupus erythematosus and the "autoimmune" diseases—Clinical, pathophysiologic, and immunologic considerations.

P.C. HEINRICH,[1] G. KRUPP,[2] B. WALCKHOFF,[2] B. KUHN,[1] W. NORTHEMANN,[1] K. SCHNEIDER,[1] and H.J. GROSS,[2] [1]Biochemisches Institut, Freiburg; [2]Institut für Biochemie, Würzburg, Federal Republic of Germany: A new type of small cytoplasmic RNA from rat liver RNP particles—Isolation and structure.

M. HINTERBERGER, I. PETTERSSON, and J. STEITZ, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Fractionation of sRNP particles and identification of anti-Sm and anti-(U1)RNP antigens.

L.M. HSU,[1] D.A. MCKAY,[2] and M.H. FOURNIER,[2] [1]Dept. of Chemistry, Mount Holyoke College, South Hadley, Massachusetts; [2]Dept. of Biochemistry, University of Massachusetts, Amherst: Cloning and sequence analysis of the gene for the 4.5S RNA of *E. coli*.

L. IRELAND, J. SZYSZKO, and M.O. KRAUSE, Dept. of Biology, University of New Brunswick, Fredericton, Canada: Differential patterns in the biosynthesis and turnover of snRNAs in *Drosophila* K_c cells and in SV40(TsA)-transformed mouse cells.

A. JACOBSON and M. FAVREAU, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester: Possible involvement of poly(A) in protein synthesis.

S. JAIN, M. GUREVITZ, and D. APIRION, Dept. of Microbiology, Washington University, St. Louis, Missouri: A small stable RNA with a role in RNA processing.

S. JINKS-ROBERTSON, D.M. BEDWELL, and M. NOMURA, Institute for Enzyme Research, University of Wisconsin, Madison: Regulation of α operon protein synthesis in *E. coli*.

SESSION 3 *Small RNP Particles*

Chairperson: H. BUSCH, Baylor College of Medicine, Houston, Texas

J. STEITZ, S. MOUNT, M. HINTERBERGER, I. PETTERSSON, J. RINKE, E. GOTTLIEB, S. WOLIN, M. LERNER, M. ROSA, C. HASHIMOTO, J. HENDRICK, and C. BERG, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Studies of small RNPs from mammalian cells.

R. ZELLER, T. NYFFENEGGER, and E.M. DE ROBERTIS, Biozentrum, University of Basel, Switzerland: snRNPs in frog oocytes.

D.J. FORBES, T.B. KORNBERG, and M.W. KIRSCHNER, Dept. of Biochemistry and Biophysics, University of California, San Francisco: snRNA in the early *Xenopus* embryo.

P.F. AGRIS,[1] Y. KIKUCHI,[2] H.J. GROSS,[2] M. TAKANO,[1] and G.C. SHARP,[1] [1]University of Missouri, Columbia; [2]University of Wurzburg, Federal Republic of Germany: Biochemical definition of an autoimmune antigenic determinant of a small RNP particle.

S.O. HOCH, P.B. BILLINGS, and P.J. WHITE, Dept. of Cellular Biology, Research Institute of Scripps Clinic, La Jolla, California: Characterization of RNP complexes recognized by autoimmune sera.

C.S. KINLAW, S.D. SWARTZ, and S.M. BERGET, Dept. of Biochemistry, Rice University, Houston, Texas: U1 snRNP versus U2 snRNP polypeptides.

J. SRI-WIDADA, C. ASSENS, J.P. LIAUTARD, C. BRUNEL, and P. JEANTEUR, Laboratoire de Biologie Moléculaire et Laboratoire de Biochimie, Montpellier, France: Fine structure and antigenicity of snRNPs.

J.H. SMITH and G.L. ELICEIRI, St. Louis University School of Medicine, Missouri: Anti-N^2,N^2,7-trimethylguanosine antibodies as probes for snRNAs and RNPs.

E.D. WIEBEN, S. MADORE, and T. PEDERSON, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: In vitro assembly of snRNP.

A.M. FRANCOEUR and M.B. MATHEWS, Cold Spring Harbor Laboratory, New York: The lupus antigen, La, binds the termini of VA RNA and is not required for transcription.

R.H. GROSS and M.S. CETRON, Dept. of Biological Sciences, Dartmouth College, Hanover, New Hampshire: Behavior of liposome-introduced snRNAs in *Drosophila* cells.

P. THOMPSON, Dept. of Zoology, University of Georgia, Athens: Localization of snRNPs with the U1 splicing function on intact *Drosophila* chromosomes.

SESSION 4 *RNA:RNA and RNA:Protein Interactions*

Chairperson: O.C. UHLENBECK, University of Illinois, Urbana, Illinois

I. TINOCO, JR., Laboratory of Chemical Biodynamics, University of California, Berkeley: Can thermodynamics be used to predict correct secondary structures in RNA?

H. SWERDLOW and C. GUTHRIE, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Probing the conformation of spliced tRNA precursors.

H.F. NOLLER,[1] R.R. GUTELL,[1] J. KOP,[1] and C.R. WOESE,[2] [1]Thimann Laboratories, University of California, Santa Cruz; [2]Dept. of Genetics and Development, University of Illinois, Urbana: Secondary structures of rRNAs—Implications for assembly, structure, and function.

P. WOLLENZIEN,[1] C. CANTOR,[1] D. GRANT,[2] and A. LAMBOWITZ,[2] [1]Dept. of Human Genetics and Development, Columbia University College of Physicians and Surgeons, New York, New York; [2]Dept. of Biochemistry, St. Louis University Medical School, Missouri: Structure of the unspliced 35S pre-rRNA in *N. crassa* mitochondria detected by psoralen cross-linking.

B.K. KLEIN, T.C. KING, and D. SCHLESSINGER, Division of Biology and Biomedical Sciences, Washington

University School of Medicine, St. Louis, Missouri: Electron microscopy of secondary structure in *E. coli* rRNA and ribosomes.

F.R. KRAMER and D.R. MILLS, Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York: Secondary structure formation during RNA synthesis.

F.M. RICHARDS, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Ribonuclease and catalysis—An early example of a detailed structure for a protein-nucleotide complex.

P.J. ROMANIUK, J. CAREY, and O.C. UHLENBECK, Dept. of Biochemistry, University of Illinois, Urbana: Single nucleotide substitution alters the R17 coat-protein–RNA interaction.

POSTER SESSION

A. JOACHIMIAK, V. KLINGHOFER, E.J. ACKERMAN, and P.B. SIGLER, Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: Nucleotides involved in the photo-induced covalent joining of *E. coli* $tRNA_1^{Tyr}{}_2$ with *E. coli* Tyr•tRNA synthetase.

P.F. JOHNSON and J. ABELSON, Dept. of Chemistry, University of California San Diego, La Jolla: A role for the intervening sequence in the biosynthesis of yeast tyrosine tRNA.

R. JOVE and J.L. MANLEY, Dept. of Biological Sciences, Columbia University, New York: mRNA processing at the 5′end—Methylation of the cap structure as required for protective transcription initiation.

J.M. KEITH, M. CASTLE, D. GALER, and L. WESTREICH, New York University, New York: mRNA biosynthesis—Initiation of transcription and post-transcriptional modification.

A.K. KINNIBURGH,[1] L.E. MAQUAT,[1] T. SCHEDL,[1] E.A. RACHMILEWITZ,[2] and J. ROSS.[1] [1]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; [2]Dept. of Hematology, Hadassah University Hospital, Jerusalem, Israel: Unstable β-globin mRNA in mRNA-deficient β^0 thalassemia.

R. LAST and J.L. WOOLFORD, Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Cloning and characterization of genes implicated in processing of ribosomal protein mRNAs in *S. cerevisiae*.

C. LAWRENCE, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Effect of adenovirus-2 infection on nuclear matrix.

R. MACKAY, Dept. of Biochemistry, Dalhousie University, Halifax, Canada: Hypothesis concerning the role in 5S rRNA function of helices containing looped-out nucleotide residues.

E.C. MARIMAN and W.J. VAN VENROOIJ, Dept. of Biochemistry, University of Nijmegen, The Netherlands: Adenoviral hnRNA is associated with the host nuclear matrix during processing.

E.S. MAXWELL and T.E. MARTIN, Dept. of Biology, University of Chicago, Illinois: Complementarity of sequences in low-molecular-weight RNAs to regions of mRNAs and rRNAs.

P. MCGRAW and A. TZAGOLOFF, Dept. of Biological Sciences, Columbia University, New York, New York: A nuclear gene product required for processing the apocytochrome-*b* transcript.

A. MICZAK and D. APIRION, Dept. of Microbiology, Washington University, St. Louis, Missouri: A new gene that affects endonucleolytic RNA processing in *E. coli*.

K.S. MILLER[1,2] and A.G. SAPONARA,[1] [1]Los Alamos National Laboratory, New Mexico; [2]Lindsley F. Kimball Research Institute of the New York Blood Center, New York: Small RNA containing RNPs from cytoplasmic and nuclear extracts of CHO cells.

G. MORRIS, D. BROWN, C. CARD, and W.F. MARZLUFF, Dept. of Chemistry, Florida State University, Tallahassee: Organization and expression of sea urchin snRNAs.

C.M. NEBIOLO and G.H. DIXON, Dept. of Medical Biochemistry, University of Calgary, Canada: Characterization and subcellular localization of low-molecular-weight RNAs in developing trout testis.

L.K. PAPE,[1] S.G. BONITZ,[2] C.L. DIECKMANN,[1] J.E. HILL,[1] G. HOMISON,[1] P. MCGRAW,[1] B.E. THALENFELD,[3] and A. TZAGOLOFF.[1] [1]Dept. of Biological Sciences, Columbia University, New York, New York; [2]Genentech, South San Francisco, California; [3]Enzo Biochem, Inc. New York, New York: Processing of *S. cerevisiae* mitochondrial mRNAs.

B. PRAGAI, M. GUREVITZ, and D. APIRION, Dept. of Microbiology, Washington University, St. Louis, Missouri: Processing of RNA from the T4 tRNA cluster by host enzymes.

M. ROY, B. SINGH, A. RAY, B. RAY, and D. APIRION, Dept. of Microbiology, Washington University Medical School, St. Louis, Missouri: Processing of rRNA by ribonuclease E.

O. SAMARINA, O. BORISOVA, and A. KRICHEVSKAYA, Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow: Physical properties of nuclear hnRNP particles—Evidence in favor of surface localization of RNA.

O. SAMARINA, I. LEKACH, and A. RYSKOV, Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow: The sequences B1 and B2 in hnRNA, cytoplasmicpoly(A)⁺ and polysomal poly(A)-containing RNAs.

K. SCHERRER, Institut de Recherche Biologie Moléculaire, Paris, France: Globin pre-mRNA processing in normal and AEV-transformed avian erythroblasts.

J.C. SCHMIT,[1] T.J. SEBO,[1] and P.J. RUSSELL,[2] [1]Dept. of Chemistry and Biochemistry, Southern Illinois University, Carbondale; [2]Reed College, Portland, Oregon: rRNA processing in *N. crassa*.

B. SETYONO,[1] J. VAN STEEG,[2] C. VAN DER MAST,[2] M. KASPERAITIS,[2] M. SALIMANS,[2] and H.O. VOORMA,[2] [1]Institute of Biology, University of Stuttgart, Federal Republic of Germany; [2]Molecular Cell Biology Dept., State University of Utrecht, The Netherlands: RNA-protein interaction—Ultraviolet crosslinking in vitro of eukaryotic initiation factors (eIFs) to mRNA and rRNA.

A. SOLARI and M.P. DEUTSCHER, Dept. of Biochemistry, University of Connecticut Health Center, Farmington: Subcellular localization of the tRNA processing enzyme, tRNA nucleotidyltransferase, in *Xenopus* oocytes and in somatic cells.

M.J.R. STARK,[1] R.L. GOURSE,[1] D.L. THURLOW,[2] R.J. GREGORY,[2] R.A. ZIMMERMANN,[2] and A.E. DAHLBERG,[1] [1]Section of Physiological Chemistry, Brown University, Providence, Rhode Island; [2]Dept. of Biochemistry, University of Massachusetts, Amherst: Site-specific mutations in *E. coli* 16S rRNA that perturb the binding of ribosomal proteins.

J. STEVENIN, P. KEOHAVONG, R. GATTONI, and M. JACOB, Laboratoire de Génétique Moléculaire des Eucaryotes, CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique, INSERM, Strasbourg, France: A specific test of ligation for the analysis of in vivo and in vitro splicing of pre-mRNA.

J.O. THOMAS, RAZIUDDIN, and W. SZER, Dept. of Biochemistry, New York University School of Medicine, New York: The hnRNPs from brine shrimp, beef, and wheat both unwind and condense single-stranded nucleic acids.

N.S.B. THOMAS, P.D. BUTCHER, and H.R.V. ARNSTEIN, Dept. of Biochemistry, King's College, London, England: The importance of eukaryotic mRNA-RNP interaction in protein synthesis. I. Discovery of a role for a rabbit reticulocyte polysomal mRNP in the formation of the 40S-mRNA preinitiation complex.

P. THOMAS and R. REEDER, Fred Hutchinson Cancer Research Center, Seattle, Washington: Nucleolar antibodies from patients with scleroderma recognize a novel small RNP complex.

J.F. THOMPSON and J.E. HEARST, Dept. of Chemistry, University of California, Berkeley: Function and structure of *E. coli* 16S rRNA probed by psoralen photochemistry.

P. THOMPSON, Dept. of Zoology, University of Georgia, Athens: The immunofluorescent labeling of the *Drosophila* 5S rRNA locus and an associated nucleolus with lupus serum against La snRNPs.

A. VINCENT and K. SCHERRER, Institut de Recherche Biologie Moléculaire, Paris, France: Biological activity and structure of mRNP complexes.

G. WESTIN, H.-J. MONSTEIN, J. ZABIELSKI, K. HAMMERSTRÖM, L. PHILIPSON, and U. PETTERSSON, Biomedical Center, Uppsala University, Sweden: Genes and pseudogenes for human snRNAs.

H.E. WILK, G. ANGELI, and K.P. SCHÄFER, Abteilung Chemie, Ruhr-Universität Bochum, Federal Republic of Germany: In vitro reconstitution of RNP particles containing 30S hnRNP proteins and exogenous RNAS.

J.A. WISE and C. GUTHRIE, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Effects of rna1 and rna2 mutations on small RNAs in yeast.

S.B. ZAIN,[1] R. CHANDA,[1] N. STOW,[2] and C.M. I. AHMED,[1] [1]Microbiology and Cancer Center, University of Rochester Medical Center, New York; [2]Institute of Virology, Glasgow, Scotland: mRNA processing from early gene block III of adenovirus 2.

M. ZASLOFF,[1] M. ROSENBERG,[2] D. HAMER,[2] and T. SANTOS,[1] [1]NIADDK and [2]NCI, National Institutes of Health, Bethesda, Maryland: Transcription and precursor processing of normal and mutant human initiator methionine tRNA genes.

G. ZIEVE, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Progressive association of snRNA species C and D with the cytoplasmic and nuclear skeleton.

SESSION 5 *tRNA Processing*

Chairperson: C. GUTHRIE, University of California, San Francisco, California

L. COOLEY, B. APPEL, and D. SOLL, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: The 5′ terminus of mature histidine tRNA species is formed by posttranscriptional nucleotide addition.

M. BAER,[1] H. DONIS-KELLER,[2] P. FURDON,[1] C. GUERRIER-TAKADA,[1] R. REED,[1] and S. ALTMAN,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Biogen, Cambridge, Massachusetts: Structure and function of RNase P from *E. coli*.

P.Y. SHIMURA, N. KIMURA, F. NAGAWA, N. NAKAJIMA, H. SAKAMOTO, and J. OZEKI, Dept. of Biophysics, Kyoto University, Japan: Structure and function of the RNA component of ribonuclease P.

N.C. MARTIN, D.L. MILLER, T.A. VIDA, and N.A. DOBRIAN, Division of Molecular Biology, University of Texas Health Science Center, Dallas: Is an RNase P required for mitochondrial tRNA biosynthesis?

A.K. HOPPER,[1] N.C. MARTIN,[2] N.S. ATKINSON,[1] and A. FURUKAWA,[1] [1]Hershey Medical Center, Pennsylvania; [2]University of Texas Health Science Center, Dallas: Mutations of yeast affecting tRNA synthesis.

C.L. PEEBLES,[1] P. GEGENHEIMER,[2] and J.ABELSON,[2] [1]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; [2]Dept. of Chemistry and Agouron Institute, University of California, San Diego, La Jolla: Enzymatic mechanism of pre-tRNA splicing—Cleavage by the purified splicing RNase of yeast produces phosphatase-resistant "3'" termini.

P. GEGENHEIMER, C.L. PEEBLES, C. GREER, R.C. SCHWARTZ, and J.N. ABELSON, Dept. of Chemistry, University of California, San Diego, La Jolla: Formation of a novel phosphodiester bond by tRNA-splicing ligases from yeast and wheat germ.

D.S. COLBY and C. GUTHRIE, Dept. of Biochemistry, University of California, San Francisco: In vivo processing pathway of wild-type and mutant yeast $tRNA^{Tyr}$.

M.P. DEUTSCHER, G. ADAMI, P. ROY, and R. ZANIEWSKI, Dept. of Biochemistry, University of Connecticut Health Center, Farmington: *E. coli* exoribonucleases and their roles in tRNA processing.

T. BARKAY,[1] A. GOLDFARB,[2] and V. DANIEL,[1] [1]Dept. of Biochemistry, Weizmann Institute of Science, Rehovot, Israel; [2]College of Physicians and Surgeons, Columbia University, New York: An *E. coli* endoribonuclease responsible for primary cleavage of tRNA precursors.

F.J. SCHMIDT, K. LEE, H. MOTAMEDI, and L. NICHOLS, Dept. of Biochemistry, University of Missouri, Columbia: Cloning and characterization of an RNA component of *E. coli* RNase P.

SESSION 6 *rRNA Processing*

Chairperson: N.R. PACE, University of Colorado Medical School, Denver, Colorado

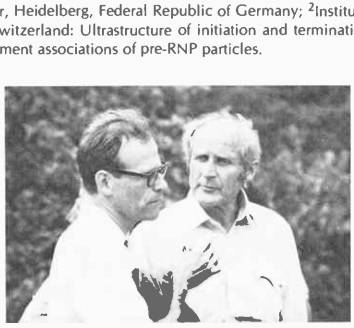
D.A. STAHL,[1] B. PACE,[1] and N.R. PACE,[1,2] [1]National Jewish Hospital and Research Center, Denver, Colorado; [2]University of Colorado Health Sciences Center, Denver: The RNP substrate for an rRNA-processing endonuclease.

L.H. BOWMAN, G.I. GOLDBERG, W.E. GOLDMAN, and D. SCHLESSINGER, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri: Processing of pre-rRNA in cultured mouse cells.

R.J. CROUCH, P. EARL, S. KANAYA, and R. FELDMANN, National Institutes of Health, Bethesda, Maryland: Processing of chick rRNA—Conversion of 32S RNA to 5.8S rRNA and 28S rRNA.

M.F. TRENDELENBURG,[1] W.W. FRANKE,[1] and M.L. BIRNSTIEL,[2] [1]Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany; [2]Institute of Molecular Biology II, University of Zurich, Switzerland: Ultrastructure of initiation and termination sites of *Xenopus* rDNA transcription and of filament associations of pre-RNP particles.

T. Martin



D. Schlessinger, A. Tissières

Y. Shimura

R.J. PLANTA, J. KLOOTWIJK, and G.M. VELDMAN, Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands: Processing of yeast rRNA precursor.

K. KRUGER, P. GRABOWSKI, A. ZAUG, S. BREHM, and T.R. CECH, Dept. of Chemistry, University of Colorado, Boulder: Autoexcision and autocyclization of the intervening sequence of the *Tetrahymena* rRNA precursor.

H. KÖSSEL, K. EDWARDS, W. KOCH, P. LANGRIDGE, Z. SCHWARZ, G. STRITTMATTER, and G. ZENKE, Institute für Biologie III, Universität Freiburg, Federal Republic of Germany: The rRNA operon and its flanking tRNA genes from *Zea mays* chloroplasts.

A. LAMBOWITZ,[1] H. BERTRAND,[2] P. BRIDGE,[2] R. COLLINS,[1] G. GARRIGA,[1] and D. GRANT,[1] [1]Dept. of Biochemistry, St. Louis University Medical School, Missouri; [2]Dept. of Biology, University of Regina, Canada: RNA splicing in *Neurospora* mitochondria.

A.E. DAHLBERG, M.J.R. STARK, and R.L. GOURSE, Section of Physiological Chemistry, Brown University, Providence, Rhode Island: Effects of site-directed mutations on processing of 16S and 23S rRNA.

S. JAIN and D. APIRION, Dept. of Microbiology, Washington University Medical School, St. Louis, Missiouri: A "processome"—A processor nightmare or pipedream.

SESSION 7 *mRNA Splicing*

Chairperson: J. FLINT, Princeton University, Princeton, New Jersey

C.L. DIECKMANN and A. TZAGOLOFF, Dept. of Biological Sciences, Columbia University, New York, New York: DNA sequence of a nuclear gene involved in the processing of the mitochondrial apocytochrome *b* gene.

P.Q. ANZIANO,[1] M.L. HALDI,[1] M.R. LAMB,[2] H.R. MAHLER,[2] and P.S. PERLMAN,[1] [1]Dept. of Genetics, Ohio State University, Columbus; [2]Dept. of Chemistry, Indiana University, Bloomington: *cis*-acting point mutations that block RNA splicing in yeast mitochondria.

P.S. PERLMAN,[1] H.R. MAHLER,[2] P.Q. ANZIANO,[1] M.R. LAMB,[2] D.K. HANSON,[2] and M.A. NICHOLAS,[1] [1]Dept. of Genetics, Ohio State University, Columbus; [2]Dept. of Chemistry, Indiana University, Bloomington: *trans*-acting point mutations that block RNA splicing in yeast mitochondria.

B. WIERINGA, F. MEYER, J. REISER, T. KÜHNE, and C. WEISSMANN, Institut für Molekularbiologie I, Universität Zurich, Switzerland: Splicing of transcripts from rabbit β-globin genes modified in vitro.

C. BENYAJATI, NCI, Frederick Cancer Research Facility, Maryland: Deletions splitting the GT- and AG-mRNA splice sites cause an *adh*-mRNA-negative phenotype in *Drosophila*.

D. GALLWITZ, W. NELLEN, and C. LANGFORD, Institut für Physiologische Chemie I, Universität Marburg, Federal Republic of Germany: Requirements for mRNA splicing and splicing selectivity in yeast.

H. ESUMI,[1] Y. TAKAHASHI,[1] R. MAKINO,[1] S. SATO,[1] S. NAGASE,[2] and T. SUGIMURA,[1] [1]National Cancer Center Research Institute; [2]Sasaki Institute, Tokyo, Japan: An intron mutation at a splicing junction blocks albumin mRNA splicing in analbuminemic rats.

M. ROSBASH, N. ABOVICH, T. LIN, J. RODRIGUEZ, and J. TEEM, Rosenstiel Research Center, Brandeis University, Waltham, Massachusetts: Effect of the temperature-sensitive mutation *rna2* on splicing.

V.E. AVVEDIMENTO[1] and B. DE CROMBRUGGHE,[2] [1]C.E.O.S. CNR Instituto di Patologia Generale, Naples, Italy; [2]NCI, National Institutes of Health, Bethesda, Maryland: Splicing pathway in a segment of the chick collagen gene.

R. ZIELINSKI, S. KAMIJO, and P. LIZARDI, Rockefeller University, New York, New York: Multiple sites for splicing in the intervening sequence of fibroin mRNA.

T. CREFELD, N. HERNANDEZ, K. SASS, A. SERGEANT, D. SOLNICK, B. WEINGARTNER, and W. KELLER, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Transcription and processing of mRNAs in vitro.

R. KOLE and S.M. WEISSMAN, Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Accurate splicing of human β-globin mRNA in vitro.

SESSION 8 *mRNA Processing*

Chairperson: H.D. ROBERTSON, Rockefeller University, New York, New York

J.J. DUNN, A.H. ROSENBERG, and F.W. STUDIER, Dept. of Biology, Brookhaven National Laboratory, Upton, New York: RNase-III cleavage sites in bacteriophage T7 RNA.

Y. Furuichi, R. Smith, M. Yamakawa, and A.J. Shatkin, Roche Institute of Molecular Biology, Nutley, New Jersey: Biosynthesis of G(5′)pppp(5′)G and G(5′)pppp(5′)A and effects on in vitro transcription by double- stranded RNA viruses.

R.M. Krug, I. Ulmanen, J. Braam, and B. Broni, Molecular Biology and Genetics Unit, Memorial Sloan-Kettering Cancer Center, New York: Influenza viral mRNA synthesis—The role of individual viral proteins in cap recognition, transcription initiation, and elongation.

T.W. Nilsen,[1] P.A. Maroney,[1] C. Baglioni,[1] and H. Robertson,[2] [1]Dept. of Biological Sciences, State University of New York, Albany; [2]Rockefeller University, New York, New York: Is the 2′,5′oligo(A)-activated endonuclease involved in mRNA processing?

J.C. Alwine, University of Pennsylvania, Philadelphia: Evidence that the SV40-associated small RNA is synthesized by specific cleavage from large viral transcripts.

W. Filipowicz,[1,2] M. Konarska,[1] H.J. Gross,[3] and A.J. Shatkin,[2] [1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw; [2]Roche Institute of Molecular Biology, Nutley, New Jersey; [3]Institut für Biochemie, Würzburg, Federal Republic of Germany: RNA ligase activities in extracts of plant and animal cells.

C. Greer, P. Gegenheimer, R.C. Schwartz, C.L. Peebles, and J.N. Abelson, Dept. of Chemistry, University of California, San Diego, La Jolla: Mechanism of ligation during tRNA splicing.

A.D. Branch and H.D. Robertson, Rockefeller University, New York, New York: RNA processing of plant viroid RNA as a model system for eukaryotic RNA splicing.

I. Winicov and J.D. Button, Dept. of Biochemistry, University of Nevada, Reno: Nuclear ligation of 5′OH polynucleotide kinase products in endogenous RNA.

RNA Tumor Viruses

May 26–May 30

Arranged by **Erwin Fleissner,** Memorial Sloan-Kettering Cancer Center, **Hidesaburo Hanafusa,** Rockefeller University

436 participants

This was the seventh meeting devoted to discussion of progress in RNA tumor virus research. The meeting's continuing popularity is reflected by the increase in attendance and number of presentations.

Many speakers talked of efforts to construct vectors derived from retrovirus sequences and incorporation of various eukaryotic genes in the retrovirus genome. Sequence determination of v-*onc* genes has been extended to many strains of virus, and from these analyses have emerged unexpected similarities among several oncogenes, particularly those encoding protein kinases, suggesting the existence of a family of genes. The relationship between cellular and viral oncogenes was also discussed. Identification of cellular oncogenes and their activation by promoter insertion reported at last year's meeting suggested the involvement of oncogene activation in human cancer. Several reports this year described the elevated expression of cellular oncogenes in human tumor tissues. One exciting discovery related to this topic was the identification of human cellular transforming genes detected by transfection to 3T3 cells as cellular *ras*^{H} and *ras*^{K} genes. This was the first demonstration that the oncogenes originally identified in the viral genome are involved in human cancer. Human adult T-cell leukemia virus was the retrovirus isolated recently from human leukemic patients. In addition to further biological characterization, a molecular clone of this leukemia virus was reported and some unique features of the genome of the first human retrovirus were described.

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

SESSION 1 *Reconstructed Viral Genomes*

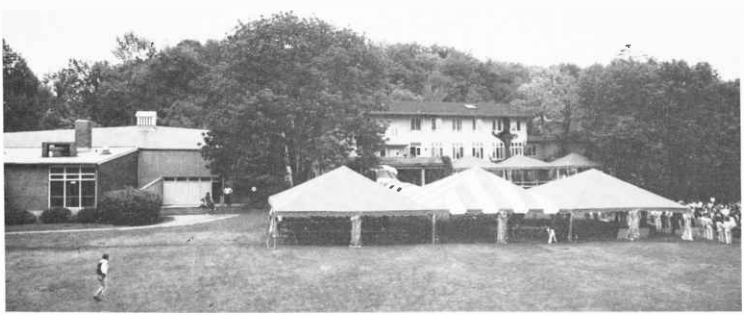
Chairperson: J.M. COFFIN, Tufts University School of Medicine, Boston, Massachusetts

A. PERKINS, P. KIRSCHMEIER, S. GATTONI-CELLI, and I.B. WEINSTEIN, Dept. of Human Genetics and Development and Cancer Center, Columbia University, New York, New York: Development of a new transfection vector containing LTR sequences.

E. GILBOA, J. PARK, S. HWANG, and M. KOLBE, Dept. of Biochemical Sciences, Princeton University, New Jersey: Transduction and expression of nonselectable genes using retrovirus-derived vectors.

M. KRIEGLER, C.F. PEREZ, and M. BOTCHAN, Dept. of Molecular Biology, University of California, Berkeley: Construction and analysis of Ha-MSV/SV40 hybrid retrovirus proviruses—Biological activities and structural analysis.

B. MERMER, M. MALAMY, and J.M. COFFIN, Dept. of Molecular Biology and Microbiology, Tufts University



Vannevar Bush Lecture Hall on left, Blackford Hall on right. Tents on rear lawn provided outdoor dining area.

School of Medicine, Boston, Massachusetts: RSV DNA as a bacterial gene—Synthesis and processing of *gag*-related proteins.

I.S.Y. CHEN, S. WATANABE, K. SHIMOTOHNO, and H.M. TEMIN, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Mapping the regions of reticuloendotheliosis viruses necessary for encapsidation of virus and expression of eukaryotic genes.

R. MANN, R. MULLIGAN, and D. BALTIMORE, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: A Mo-MLV mutant that cannot package its own RNA.

J.J. KOPCHICK and D.W. STACEY, Roche Institute of Molecular Biology, Nutley, New Jersey: Localization and biological analyses of genetic lesions in cloned retroviral DNA.

M.L. MCGEADY, R. ASCIONE, G.F. VANDE WOUDE, NCI, National Institutes of Health, Bethesda, Maryland: The effect of methylation on the transforming ability of MoSV.

S.M. ANDERSON and E.M. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: Construction of a murine transforming virus containing the *src* gene of RSV.

L.-H. WANG and C. GRANDORI, Rockefeller University, New York, New York: Replicative function and site of integration affect transcription and processing of ASV mRNAs.

J. SORGE and S.H. HUGHES, Cold Spring Harbor Laboratory, New York: The splicing of intervening sequences introduced into an infectious retrovirus vector.

E. HUNTER,[1] G. DAVIS,[1] M. HARDWICK,[1] G. DAVIS,[1] M.J. GETHING,[2] and J. SAMBROOK.[2] [1]Dept. of Microbiology, University of Alabama, Birmingham; [2]Cold Spring Harbor Laboratory, New York: Expression of the RSV *env* gene products from an SV40-RSV hybrid vector.

SESSION 2 *Oncogenes*

Chairperson: I.M. VERMA, The Salk Institute, San Diego, California

J.Y.J. WANG, S. GOFF, C. QUEEN, and D. BALTIMORE, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Structure and function of the Abelson virus transforming gene and its normal cellular homolog.

T. TAKEYA and H. HANAFUSA, Rockefeller University, New York, New York: Comparison of DNA sequences of the *src* genes of two strains of ASV and the cellular homolog.

R.C. PARKER, R. SWANSTROM, H.E. VARMUS, and J.M. BISHOP, Dept. of Microbiology and Immunology, University of California, San Francisco: Cellular gene capture by retroviruses—Evidence for involvement of DNA recombination.

M. YOSHIDA,[1] N. KITAMURA,[1] K. TOYOSHIMA,[2], Y. HIRAYAMA,[1] and A. KITAMURA.[1] [1]Cancer Institute, Kami-Ikebukuro, Toshima-ku, Tokyo; [2]Institute for Medical Sciences, University of Tokyo, Japan: Nucleotide sequence of ASV Y73—Similarity of its transforming gene product to that of RSV.

M. SHIBUYA and H. HANAFUSA, Rockefeller University, New York, New York: Similarities exist between transforming genes of RSV and FSV in nucleotide and amino acid sequences.

C. VAN BEVEREN, F. VAN STRAATEN, T. CURRAN, and I.M. VERMA, Tumor Virology Laboratory, Salk Institute, San Diego, California: A novel oncogene (*fos*) of an osteosarcoma virus—Complete molecular analysis.

K.H. KLEMPNAUER,[1] T.J. GONDA,[2] and J.M. BISHOP.[1] [1]Dept. of Microbiology and Immunology, University of California, San Francisco; [2]Ludwig Institute for Cancer Research, Victoria, Australia: Nucleotide sequence of the oncogene of AMV and its cellular homolog.

E.P. REDDY, K. REYNOLDS, D.K. WATSON, J. LAUTENBERGER, and T. PAPAS, NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence analysis of the AMV (MC29).

Y. LI, C. MAGARIAN and E. STAVNEZER, Graduate School of Medical Sciences, Sloan-Kettering Institute, New York, New York: The SKVs have a unique transforming gene.

P. BESMER,[1] H.W. SNYDER, JR.,[1] J.E. MURPHY,[1] M.C. SINGHAL,[1] W.D. HARDY JR.,[1] and A. PARODI.[2] [1]Memorial Sloan-Kettering Cancer Center, New York, New York: [2]École Nationale Vétérinaire, Maisons, Alfort, France: P1-FeSV—Biological and biochemical characterization.

S. JOSEPHS, R. DALLA-FAVERA, E.P. GELMANN, R.C. GALLO, and F. WONG-STAAL, NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence of the transforming gene (*v-sis*) of SSV and comparison with 5′ human c-*sis* sequences.

S.G. DEVARE, P.E. REDDY, K.C. ROBBINS, P.R. ANDERSON, S.R. TRONICK, and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence of the transforming gene of SSV.

N.R. RICE,[1] R.R. HIEBSCH,[1] R.M. STEPHENS,[1] H.R. BOSE,[2] and R.V. GILDEN.[1] [1]NCI- Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Microbiology, University of Texas, Austin: Sequence analysis of the *rel* gene of reticuloendotheliosis virus.

J.M. ONG and K.F. WATSON, Dept. of Chemistry, University of Montana, Missoula: A ribonucleoprotein complex from AMV efficient in reverse transcription.

C.A. OMER and A.J. FARAS, Dept. of Microbiology, University of Minnesota Medical School, Minneapolis: Mechanism of release of the $tRNA^{trp}$ primer molecule from viral DNA during reverse transcription—Site-specific endonucleolytic RNase H.

J. SUMMERS, J. TAYLOR, and W. MASON, Institute for Cancer Research, Philadelphia, Pennsylvania: Replication of a "DNA" virus via reverse transcription—Studies on a hepatitis B virus of ducks.

E. KESHET, G. ROTMAN, and A. ITIN, Dept. of Virology, Hadassah Medical School, Hebrew University, Jerusalem, Israel: Unusual genomic arrangements of murine "retroviruslike" (VL30) sequences.

S.V.S. KASHMIRI, R. MEHDI, B. ALTLAND, and J.F. FERRER, University of Pennsylvania, Kennett Square: Detection and characterization of two species of covalently closed circular proviral DNA molecules of BLVO.

T.S. HOROWIT and P.R. SHANK, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Instability of RSV proviral DNA in quail cells.

M. HILL,[1] J. HILLOVA,[1] R. MARIAGE-SAMSON,[2] and J. BELEHRADEK,[2] [1]Institut de Cancérologie et d' Immunogénétique, CNRS, Villejuif; [2]Institut Gustave-Roussey, Villejuif, France: Provirus amplification in RSV-transformed Chinese hamster cells and its segregation in Chinese hamster-mouse cell hybrids.

V. PARSONS,[1] M. GOLOMB,[1] T. MISRA,[2] P. HIPPENMEYER,[2] and D. GRANDGENETT,[2] [1]Division of Biological Sciences, University of Missouri, Columbia; [2]St. Louis University, Missouri: Binding selectivity of the avian retrovirus pp32 protein to cloned retroviral DNA.

E. CANAANI,[1] E. SHTIVELMAN,[1] and P. ARTHUR,[2] [1]Weizmann Institute, Rehovot, Israel; [2]Hazelton Laboratories, Vienna, Virginia: Mo-MSV DNA in clonally derived nonproduce transformants—1) High frequency generation of mutants defective in virion RNA packaging. 2) Presence of subpopulations of cells with different viral integration patterns.

M.S. KAHN and D.W. STACEY, Roche Institute of Molecular Biology, Nutley, New Jersey: Characterization of an unusually large *src* region in an RSV clone.

P. LUCIW,[1] M. CAPECCHI,[2] J.M. BISHOP,[1] and H. VARMUS,[1] [1]University of California, San Francisco; [2]University of Utah, Salt Lake City: Enhancement of tk transformation by retroviral LTR sequences.

D. SCHOLL,[1] R. MALAVARCA,[1] S. ASTRIN,[2] and A.M. SKALKA,[1] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Institute for Cancer Research, Fox Chase, Pennsylvania: Comparison of the nucleotide sequences of LTRs and adjacent regions of integrated endogenous virus 2 (ev 2) and unintegrated RAV-0.

J. SORGE and S.H. HUGHES, Cold Spring Harbor Laboratory, New York: The polypurine tract adjacent to the U_3 region of the RSV genome provides a *cis*-acting function.

D.W. STACEY, M. KAHN, B. CULLEN, and J. KOPCHICK, Roche Institute of Molecular Biology, Nutley, New Jersey: Interrelationship of viral and exogenous DNA signals in microinjected chimeric clones.

G. PETERS and C. DICKSON, Imperial Cancer Research Fund, London, England: Conservation of coding potential in the LTRs of exogenous and endogenous MMTV.

E.P. REDDY, K.C. ROBBINS, S.R. TRONICK, and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence analysis of retroviral deletion mutants—Localization of sequences required for efficient transforming retrovirus rescue.

E.C. WOODLAND and P.R. SHANK, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Analysis of two spontaneous deletion mutants of RSV missing 2/3 of the viral genome.

A. HONIGMAN and A. PANET, Depts. of Microbiological Chemistry and Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: A transcription termination signal in the LTR of Mo-MLV.

E. TISCHER and A.P. CZERNILOFSKY, Austrian Academy of Sciences, Salzburg, Austria: Generation of partially deleted *src*-specific DNA via M13 miniphages.

C.Y. OU,[1] L.R. BOONE,[2,3] F.E. MEYER,[2] and W.K. YANG,[2] [1]School of Biomedical Sciences, University of Tennessee, Oak Ridge; [2]Biology Division, Oak Ridge National Laboratory, Tennessee; [3]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Sequence characterization of a cloned proviral DNA endogenous of BALB/c mouse.

T. PUGATSCH and D.W. STACEY, Roche Institute of Molecular Biology, Nutley, New Jersey: Biological properties associated with virus replication, packaging, or transcription localized in three defined regions of avian retrovirus clones.

G.M. STOLTZFUS and T.A. FICHT, Dept. of Microbiology, University of Iowa, Iowa City: Stability of ASV RNAs—Comparison of genome size and subgenomic mRNAs.

N. SARKAR,[1] P. ETKIND,[1] E. LASFARGUES,[2] and E. WHITTINGTON.[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York, [2]Institute for Medical Research, Camden, New Jersey; Towards an understanding of the pathogenesis of spontaneous mammary tumors in BALB/c mice—Cell culture and MMTV proviral DNA analysis.

A.J.J. VAN OOYEN,[1] A. ARNBERG,[2] R. MICHALIDES,[1] N. KENNEDY,[3] B. GRODNER,[3] and N.E. HYNES,[3] [1]The Netherlands Cancer institute, Amsterdam; [2]Biochemical Laboratory, University of Groningen, The Netherlands; [3]Kernforschungszentrum Karslruhe, Institute of Genetics, Federal Republic of Germany: Structure and function of the LTRs of MMTV.

M. KESSEL, M. OSTROWSKI, G. HAGER, G. KHOURY, and P. GRUSS, NCI, National Institutes of Health, Bethesda, Maryland: Hormone induction mediated by MMTV LTR sequences analyzed in a transient assay.

D. OWEN and H. DIGGELMANN, Swiss Institute for Experimental Cancer Research, Laussane, Switzerland: MMTV DNA clones transfected into nonmurine cells exhibit glucocorticoid-dependent expression.

J.J. MERMOD,[1] S. BOURGEOIS,[1] and M. CRÉPIN,[2] [1]Salk Institute, San Diego, California; [2]Dept. of Molecular Biology, Institute Pasteur, Paris, France: MMTV mRNA synthesis and methylation of proviral DNA in dexamethasone-sensitive, -supersensitive, and -resistant mouse thymoma cell lines.

S.M.S. REDMOND and C. DICKSON, Imperial Cancer Research Fund, London, England: Nucleotide sequence analysis of the *env* region of MMTV.

J.W. GAUTSCH and M.C. WILSON, Research Institute of Scripps Clinic, La Jolla, California: Restriction of Mo-MLV growth in teratocarcinoma—Involvement of DNA methylation.

O. NIWA, Y. YOKOTA, and H. ISHIDA, Dept. of Experimental Radiology, Kyoto University, Japan: Mo-MLV genome in mouse teratocarcinoma cells is methylated but can be activated by 5-AzaCyd only after differentiation of the cells.

C. HAMMOND,[1] J.M. BISHOP,[1] and G.R. MARTIN,[2] [1]Dept. of Microbiology and Immunology, and [2]Dept. of Anatomy, University of California, San Francisco: Mo-MLV viral expression in undifferentiated and differentiated teratocarcinoma cells.

L. D'AURIOL,[1] W.K. YANG,[2] F. SAAL,[1] F. CAVALIERI,[1] J. PÉRIÉS,[1] and R. EMANOIL-RAVICOVITCH,[1] [1]Unité 107 INSERM, Hôspital Saint Louis, Paris, France; [2]O.R.N.L. Biology Division, Oak Ridge, Tennessee: DNA integration in undifferentiated teratocarcinoma cell lines restrictive to type-C retrovirus replication.

H. STUHLMANN, C. STEWART, D. JÄHNER and R. JAENISCH, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Universität Hamburg, Federal Republic of Germany: De novo methylation, infectivity, and expression of retroviral genomes introduced into embryonal carcinoma cells.

J. LEIS,[1] G. DUYK,[1] M. LONGIARU,[2] and A. SKALKA,[2] [1]Case Western Reserve University Medical School, Cleveland, Ohio, [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Mechanism of action of the DNA endonuclease associated with the $\beta\beta$ and the $\alpha\beta$ forms of ASV reverse transcriptase.

T.M. SHINNICK, R.A. LERNER, and J.G. SUTCLIFFE, Dept. of Immunopathology, Research Institute of Scripps Clinic, La Jolla, California: Mo-MLV *pol* gene encodes three polypeptides.

W.P. TSAI, T.D. COPELAND, and S. OROSZLAN, Biological Carcinogenesis Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland: Avian reticuloendotheliosis virus (REV) *gag* and *env* gene products—Organization, structural and immunological relatedness to mammalian type-C and type-D viruses.

R.V. SRINIVAS and R.W. COMPANS, Dept. of Microbiology, University of Alabama, Birmingham: Glycosylation and intracellular transport of SFFV glycoproteins.

R.B. PEPINSKY and V.M. VOGT, Section of Biochemistry, Cornell University, Ithaca, New York: Low-molecular-weight proteins of ASV and ALV.

H.L. NIMAN, J.W. GAUTSCH, and J.H. ELDER, Dept. of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: Neutralizing properties of monoclonal antibodies directed against Rauscher gp70.

R. NASO, C. EDBAUER, and Y.-H. WU, M.D. Anderson Hospital and Tumor Institute, University of Texas System Cancer Center, Houston: Retrovirus assembly and the antiretroviral effects of interferon and temperature-sensitive mutations.

M.S. MCGRATH,[1] L. CHEN,[2] J.M. BISHOP,[2] M.D. COOPER,[3] G. GASIC,[4] W. HAYWARD,[5] and I.L. WEISSMAN,[1] [1]Stanford University, California; [2]University of California, San Francisco; [3]University of Alabama, Birmingham; [4]Rockefeller University, New York, New York; [5]Sloan-Kettering Cancer Center, New York, New York; Avian bursal lymphoma cell-surface IgM recognizes ALV.

D. MATHIEU-MAHUL,[1] J.M. HEARD,[2] S. FICHELSON,[2] M. MAUCHAUFFÉ,and C.J. LARSON,[1] [1]Laboratoire Phar-

macologie Expérimentale, Hôpital Saint Louis, and [2]Laboratoire Virologie et Immunologie des Tumeurs, Hôpital Cochin, Paris, Frange: gp66—a particular specific *env* gene expression common to MLV-infected myelomonocytes and mastocytes.

T.H. LEE, J. MCCARTY, and M. ESSEX, Dept. of Microbiology, Harvard School of Public Health, Boston, Massachusetts: Expression of FeLV proteins at the surface of virus-producer lymphoma cells.

N.C. KAN and T.S. PAPAS, NCI, National Institutes of Health, Bethesda, Maryland: The predicted carboxy termini of reverse transciptase are different in AMV and MAV.

H. SCHETTERS,[1] R. HEHLMANN,[1] and V. ERFLE,[2] [1]Medizinische Poliklinik, Universität Munchen, Munich, and [2]Gesellshaft für Strahlen und Umwelforschung, Neuherberg, Federal Republic of Germany: Detection of group and interspecies reactivities of mammalian type-C-virus p30 proteins by the ELISA method.

R. FRIEDRICH, W. KOCH, and G. HUNSMANN, Institute of Tumor Immunology, University of Freiburg, Federal Republic of Germany: Nucleotide sequence of the *env* gene of Fr-MLV.

T.A. FICHT and C.M. STOLTZFUS, Dept. of Microbiology, University of Iowa, Iowa City: Translation products and efficiencies of hybrid-selected avian retrovirus polysomal RNAs.

T.C. COPELAND, M.A. MORGAN, and S. OROSZLAN, NCI-Frederick Cancer Research Facility, Frederick, Maryland: BLV and FeLV nucleic acid binding proteins—Primary structure and immunological characterization.

J.E. CLEMENTS and L. BHADURI, Johns Hopkins University, Baltimore, Maryland: Presence of a 15,000-dalton protein in the envelope of visna virus and the role of this protein in the antibody response in persistently infected sheep.

J.G. LEVIN,[1] B.F. HUGHES,[2] J.S. GRAETER,[1] A. REIN,[3] E. RANDS,[4] and A.B. MUKHERJEE,[2] [1]Laboratory of Molecular Genetics; [2]NICHHD, National Institutes of Health; [3]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland; [4]NCI, National Institutes of Health, Bethesda, Maryland: Chromosome-mediated transfer of MLV and MSV genes.

A. PANET, G. ARAD, I. GLOGER, and H. FALK, Dept. of Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Inhibition of Mo-MLV production by interferon.

SESSION 4 *Poster Session: Structure of Cellular and Viral Oncogenes*

H.W. SNYDER, JR., P. BESMER, E.E. ZUCKERMAN, M.C. SINGHAL, L. LEDERMAN, and W.D. HARDY, JR., Memorial Sloan-Kettering Cancer Center, New York, New York: Translation products of three new isolates of FeSV.

W.D. HARDY, JR., E.E. ZUCKERMAN, R. MARKOVICH, P. BESMER, and H.W. SNYDER, JR., Memorial Sloan-Kettering Cancer Center, New York, New York: Isolation of two new FeSVs, one of which contains a new cat *onc* sequence.

U.R. RAPP,[1] F.H. REYNOLDS, JR.,[2] and J.R. STEPHENSON,[1] [1]National Cancer Institute and [2]Frederick Cancer Research Facility, Frederick, Maryland: Isolation of a new mammalian type-C transforming virus.

Y. YUASA, J.H. PIERCE, A. SRINIVASAN, S.G. DEVARE, E.P. REDDY, and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Ab-MLV mutants constructed from molecularly cloned DNA—Deletion mutants of *Bgl*II fragments in the transforming gene.

N. SAGATA, H. AMANUMA, T. MATSUGI, and Y. IKAWA, Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: Specific sequences and biological activities of molecularly cloned genomes of FrLV complex (K-I strain).

P.N. TSICHLIS,[1] L. DONNEHOWER,[1] G. HAGER,[1] N. ZELLER,[1] R. MALAVARCA,[2] S. ASTRIN,[3] and A.M. SKALKA,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland, [2]Roche Institute of Molecular Biology, Nutley, New Jersey, [3]Fox Chase Institute for Cancer Research, Philadelphia, Pennsylvania; The U_3 region of the LTR determines the growth rate and the oncogenic potential of avian retroviruses.

T.C. WONG, R.C. COHEN, A. HIRANO, P.K. VOGT, and M. LAI, Dept. of Microbiology, University of Southern California Medical School, Los Angeles: Comparative analysis of genome structure of class-II defective ASVs.

C.I. HAMMOND, C.-C. HUANG, and J.M. BISHOP, Dept. of Microbiology and Immunology, University of California, San Francisco: Analysis of the genomic structure of PrCII sarcoma virus and its homologous cellular loci (c-*fps*) from chickens.

T.G. WOOD, D.G. BLAIR, M.L. MCGEADY, and C.F. VANDE WOUDE, NCI, National Institutes of Health, Bethesda, Maryland: The structural arrangement of integrated DNAs from cells transformed by *mos*.

R. HAMELIN, M.-A. AUGER-BUENDIA, and A. TAVITIAN, INSERM, Faculté de Médecine Lariboisière-Saint Louis, Paris, France: Moloney sarcoma virus expression in MSV-transformed nonproducer mouse cells.

M.A. GONDA,[1] H.A. YOUNG,[2] S. RASHEED,[3] J.E. ELSER,[1] K.NAGASHIMA,[1] C.C. LI,[1] and R.V. GILDEN,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Bethesda Research Laboratories, Gaithersburg, Maryland; [3]University of Southern California Medical School, Los Angeles: Molecular cloning, genomic analysis, and biological properties of rat leukemia virus and the *v-onc* sequences of Rasheed rat sarcoma virus.

P.R. ANDERSON, E.P. REDDY, S.R. TRONICK and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence analysis of transforming gene of BALB-MSV and its human analog.

D.K. WATSON,[1] E.P. REDDY,[1] P. DUESBERG,[2] and T. PAPAS,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland: [2]Dept. of Molecular Biology, University of California, Berkeley: Nucleotide sequence analysis of the chicken c-*myc* gene.

K.C. WILHELMSEN, I.S.Y. CHEN, and H.M. TEMIN, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The organization of c-*rel* in chicken and turkey DNAs.

S.S. YANG, R. MODALI, J. TAUB, and N.A. WIVEL, NCI, National Institutes of Health, Bethesda, Maryland: Conservation of a rat leukemia helper virus subgenomic DNA sequence critical to the expression of the helper biological activity among various rat tropic retroviruses.

P.R. SHANK, M.GOYETTE, C.J. PETROPOULOS, and N. FAUSTO, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Activation of cellular oncogenes during liver regeneration.

W.L. HSIAO, S. GATTONI-CELLI, P. KIRSCHMEIER, and I.B. WEINSTEIN, Dept. of Human Genetics and Development and Cancer Center, Columbia University, New York, New York: Induction of hypomethylation and transcription of the c-mos locus in mouse cells following exposure to 5-AzaCyd.

B. GAYATHRI DEVI,[1] L.H. SOE,[1] M.P. BUSCH,[1] B. PERBAL,[2] and P. ROY-BURMAN,[1] [1]University of Southern California Medical School, Los Angeles; [2]University of California Medical School, Los Angeles: Transcriptional activity of cellular FeLV *myb* and *myc* sequences in normal and tumor tissues of the cat.

J.H. CHEN, Dept. of Virology, Showa University Research Institute, Clearwater, Florida: Expression and methylation of c-*myb* sequences.

A. BARNEKOW,[1] M. SCHARTL,[2] F. ANDERS,[2] and H. BAUER,[1] [1]Institut für Virologie, Fachbereich Humanmedizin, Giessen [2]Genetisches Institut, Giessen, Federal Republic of Germany: Comparative studies on the expression of the cellular homolog of the RSV *src* gene.

R. BASSIN,[1] M. NODA,[1] Z. SELINGER,[2] and E. SCOLNICK,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Life Sciences Institute, Hebrew University, Jerusalem, Israel: Biological properties of flat revertants isolated from KiSV-transformed NIH/3T3 cells.

S. RUSCETTI and E. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: Expression of a transformation-related protein (p53) in erythroleukemia cell lines derived from mice infected with Fr-MLV or FVP.

D.J. CHISWELL, J.A. WYKE, and D.A.F. GILLESPIE, Imperial Cancer Research Fund, London, England: Morphological reversion of ASV-transformed rat cells is accompanied by changes in the structure of proviral chromatin and in the pattern of proviral methylation.

B. MATHEY-PREVOT, M. SHIBUYA, and H. HANAFUSA, Rochefeller University, New York, New York: Isolation of revertants and their retransformants of rat cells transformed by FuSV.

M. GRIASER and D. DINA, Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Characterization of revertant cell lines with multiple integrated Mo-MSV genomes.

M. OSKARSSON, R. WATSON, M.L. MCGEADY, T. WOOD, D. BLAIR, and G.F. VANDE WODE, NCI, National Institutes of Health, Bethesda, Maryland: A human homolog of the mouse c-*mos* gene.

J. GROFFEN,[1] N. HEISTERKAMP,[1] M. SHIBUYA,[2] and J.R. STEPHENSON,[1] [1]National Cancer Institute, Frederick, Maryland; [2]Rockefeller University, New York, New York; Transforming genes of avian (*v-fps*) and mammalian (*v-fes*) retroviruses correspond to a common cellular locus.

J.G. SODROSKI, M. TRUS, and W. HASELTINE, Dept. of Pathology, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachussetts: Structure and expression of c-*fes* in human cell lines.

D. ROSSON and A. TEREBA, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Transcription of oncogenes in human primary tumors.

V. KRUMP, K.J. VAN DEN BERG, and P. BENTVELZEN, Radiobiological Institute, Rijswijk, The Netherlands: Normal BALB/c mouse transforming DNA fragment—Characterization and promoting effect of LTS on its expression.

N. HANANIA, D. SHAOOL, and J. HAREL, Groupe de Recherche nº8 du CNRS, Institut Gustave-Roussy, Villejuif, France: Multigenic activation in human neoplasia.

D.J. FUJITA,[1] J. RADUL,[1] J. BAAR,[1] C. GIBBS,[2] and H.-J. KUNG,[2] [1]Cancer Research Laboratory, University of Western Ontario, London, Canada; [2]Dept. of Biochemistry, Michigan State University, East Lansing:

Isolation and characterization of molecular clones containing human genomic DNA sequences homologous to the transforming gene (*src*) of RSV.

G. FRANCHINI,[1] R. DALLA-FAVERA,[1] S. JOSEPHS,[1] R.C. GALLO,[1] C.M. CROCE,[2] M.A. BALUDA,[3] and F. WONG-STAAL,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Wistar Institute, Philadelphia, Pennsylvania; [3]University of Calaifornia Medical School, Los Angeles: Isolation, nucleotide sequencing, and chromosomal localization of human DNA sequences related to the *onc* gene of AMV.

C.-K. SHIH,[1] C. MOSCOVICI,[2] and W.S. HAYWARD,[3] [1]Rockefeller University, New York, New York; [2]Tumor Virology Laboratory, Veterans Administration Hospital, Gainesville, Florida; [3]Sloan-Kettering Institute for Cancer Research, New York, New York: Induction of lymphoma by the *v-myc* gene of MC29 virus.

R. KETTMANN,[1,2] J. DESCHAMPS,[1] Y. CLEUTER,[1] D. COUEZ,[1] A. BURNY,[1,2] and G. MARBAIX,[1] [1]Dept. of Molecular Biology, University of Brussels; [2]Faculty of Agronomy, Gembloux, Belgium: Leukemogenesis by BLV—Proviral DNA integration and lack of RNA expression of viral LTR and 3' proximate cellular sequences.

G. LEMAY and P. JOLICOEUR, Institut de Recherches Cliniques de Montréal, Université de Montréal, Canada: Molecular studies of Mo-MLV induced thymoma in rats.

A. SCHÖN,[1] V. ERFLE,[1] and R. HEHLMANN,[2] [1]Abteilung für Pathologie, Gesellschaft für Strahlen - und Umweltforschung, Munich; [2]Medizinische Poliklinik der Universität München, Federal Republic of Germany: Cellular Moloney sarcoma sequences (MOS) are transposed in spontaneous murine osteosarcomas but not in radiation-induced osteosarcomas.

S. GATTONI-CELLI, W.L. HSIAO, P. KIRSCHMEIER, and I.B. WEINSTEIN, Dept. of Human Genetics and Development and Cancer Center, Columbia University, New York, New York: Induction of transcription of the *c-mos* gene in NIH/3T3 cells following infection with Mo-MLV.

E.C. MURPHY, Dept. of Tumor Virology, M.D. Anderson Hospital and Tumor Institute, University of Texas, Houston: Mo-MSV ts110 viral RNAs—The 4.0-kb and 3.5-kb RNAs code for different polypeptides.

G. WEINMASTER, J. INGMAN-BAKER, and T. PAWSON, Dept. of Microbiology, University of British Columbia, Vancouver, Canada: Localization of phosphorylation sites on the Fujinami ASV transforming protein.

G.R. ANDERSON, Dept. of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: K-asp56—KiSV *onc* product and its human counterpart.

S. IWASHITA, N. KITAMURA, and M. YOSHIDA, Laboratory of Viral Oncology, Cancer Institute, Tokyo, Japan: Mechanism of fusiform morphological transformation by partial *src* deletion mutant of RSV.

T.Y. SHIH,[1] R. DHAR,[1] and S. OROSZLAN,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Frederick Cancer Research Center, Frederick, Maryland: Radiochemical sequencing and determination of the phosphorylation site of the p21 protein of Ha-MSV.

F. VERONESE,[1] G.J. KELLOFF,[1] F.H. REYNOLDS, JR.,[2] and J.R. STEPHENSON,[1] [1]National Cancer Institute and [2]Frederick Cancer Research Facility, Frederick, Maryland: Monoclonal antibodies specific to transforming polyproteins encoded by independent isolates of FeSV.

J. EVEN,[1] G. KHOURY,[1] F. GALIBERT,[2] A. HAMPE,[2] S.J. ANDERSON,[1] D. LOWY,[1] and C.J. SHERR,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Hôpital Saint Louis, Paris, France: Retroviral mutants encoding the transforming polyprotein of Gardner-Arnstein FeSV.

S. WATANABE, S. ZIEGLER, and O. WITTE, Molecular Biology Institute, University of California, Los Angeles: Site directed mutagenesis of Ab-MLV.

M. NUNN,[1] K. BISTER,[1] C. MOSCOVICI,[2] B. PERBAL,[3] M.A. BALUDA,[3] and P.H. DUESBERG,[1] [1]Dept. of Molecular Biology and Virus Laboratory, University of California, Berkeley; [2]Virus Research Laboratory, Veterans Administration Hospital, Gainesville, Florida; [3]University of California Medical School, Los Angeles: An erythroblastosis virus shares transformation-specific sequences with AMV but expresses them differently.

L. STANKER, G. GALLICK, W. KLOETZER, E. MURPHY, and R. ARLINGHAUS, Dept. of Tumor Virology, University of Texas and M.D. Anderson Hospital and Tumor Institute, Houston: Detection of a Mo-MSV transforming-gene product by antibody directed against a synthetic peptide.

G.W. NOTANI, University of Minnesota, Minneapolis: Nucleotide sequencing of reticuloendotheliosis virus genome-related sequences.

SESSION 5 *Functions of the LTR*

Chairperson: E. HUNTER, University of Alabama, Birmingham, Alabama

G.M. GILMARTIN and J.T. PARSONS, Dept. of Microbiology, University of Virginia Medical School, Charlottesville: Analysis of the RSV promoter region by site-directed mutagenesis.

S.A. MITSIALIS, S. MALIK, J.L. MANLEY, and R.V. GUNTAKA, Columbia University, New York, New York: Studies on the localization of a functional promoter for eukaryotic RNA polymerase II in the LTR of ASV DNA.

B. CULLEN,[1] A.M. SKALKA,[2] and G. JU,[1] [1]Dept. of Molecular Genetics, Hoffmann-La Roche Inc.; [2]Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: Comparison of the promoter activity of LTRs from exogenous and endogenous avian retroviruses.

A. SRINIVASAN, E.P. REDDY, C.Y. DUNN, and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Molecular dissection of Ab- MLV LTR by in vitro mutagenesis and its role in viral gene expression.

L.R. BOONE,[1,2] F.E. MYER,[1] M. YANG,[1] J.O. KIGGANS,[1] C. KOH,[1] R.W. TENNANT,[2] and W.K. YANG,[1] [1]Biology Division, Oak Ridge National Laboratory, Tennessee; [2]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Analysis of recombinant DNA clones of the endogenous BALB/c MLV WN1802N—Variation in LTR size.

O. PRAKASH,[1] S.A. MITSIALIS,[2] R.V. GUNTAKA,[2] and N.H. SARKAR,[1] [1]Memorial Sloan-Kettering Cancer Center and [2]Columbia University, New York, New York: The MMTV LTR contains both a eukaryotic and a prokaryotic promoter.

M.J.F. ROUSSEL, M.C. OSTROWSKI, and G.L. HAGER, NCI, National Institutes of Health, Bethesda, Maryland: Association of transcriptionally active MMTV LTR promoters with *cis*-acting regulatory elements.

N.E. HYNES, H. PONTA, N. KENNEDY, U. RAHMSDORF, P. HERRLICH, and B. GRONER, Kernforschungszentrum Karlsruhe, Institute of Genetics, Federal Republic of Germany: A 400-nucleotide MMTV LTR subfragment containing the proviral promoter confers hormone inducibility to proviral genes and tk chimeras.

J.E. MAJORS and H.E. VARMUS, Dept. of Microbiology and Immunology, University of California, San Francisco: Glucocorticoid regulation by the MMTV LTR—Less than 190 bp of U^3 is required for steroid control of a linked heterologous gene.

N. FASEL, K. PEARSON, E. BUETTI, and H. DIGGELMANN, Swiss Institute for Experimental Cancer Research, Laussane, Switzerland: Coding potential and signals for hormonally regulated transcription in the region of MMTV DNA containing the LTR.

M.C. OSTROWSKI, H. RICHARD-FOY, and G.L. HAGER, NCI, National Institutes of Health, Bethesda, Maryland: Hormone-regulated expression from the MMTV LTR mobilized on high-copy extrachromosomal BPV minichromosomes.

D.A. WHEELER,[1] R.D. CARDIFF,[2] J.S. BUTEL,[3] D. MEDINA,[4] and G.L. HAGER,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Pathology, University of California, Davis; [3]Dept. of Virology and Epidemiology and [4]Dept. of Cell Biology, Baylor University, Houston, Texas: Identification of a candidate mRNA for the MMTV LTR gene product (p-LTR).

SESSION 6 *Structure and Expression of Oncogenes*

Chairperson: M. LINIAL, Fred Hutchinson Cancer Research Center, Seattle, Washington

L. SEALY,[1] J.M. BISHOP,[1] G. MOSCOVICI,[2] and C. MOSCOVICI,[2] [1]Dept. of Microbiology, University of California, San Francisco; [2]Veterans Administration Hospital, Gainesville, Florida: In vitro mutagenesis of AEV.

T. YAMAMOTO,[1] S. SAKAMOTO,[1] T. KOYAMA,[1] S. KAWAI,[1] H. HIHARA,[2] and K. TOYOSHIMA,[1] [1]Institute of Medical Science, University of Tokyo; [2]National Institute of Animal Health, Ibaraki, Japan: A characteristic structure of transforming gene in a newly isolated AEV.

K. TOYOSHIMA,[1] S. KAWAI,[1] T. KOYAMA,[1] T. YAMAMOTO,[1] H. HIHARA,[2] H. YANAMOTO,[2] and T. SHIMIZU,[2] [1]Institute of Medical Science, University of Tokyo; [2]National Institute of Animal Health, Ibaraki, Japan: Isolation and characterization of a new strain of AEV.

S. SAULE, J. COLL, D. LEPRINCE, A. BEGUE, and D. STEHELIN, Oncologie Molécularie, INSERM, Institut Pasteur, Lille, France: Structure of the *erb* domain of AEV and its cellular homolog in chicken and man.

G. MARDON and H.E. VARMUS, Dept. of Microbiology and Immunology, University of California, San Francisco: Analysis of an RSV frameshift mutant suggests *src* contains two functional sites for the initiation of protein synthesis.

Y.-K.T. FUNG,[1] D. ROBINSON,[1] L.B. CRITTENDEN,[2] and H.-J. KUNG,[1] [1]Dept. of Biochemistry, Michigan State University, East Lansing; [2]US Dept. of Agriculture, Science and Education Administration, Regional Poultry Research Laboratory, East Lansing, Michigan: Tumor induction in chickens with cloned v-*src* DNA.

S.J. ANDERSON, L.A. FEDELE, M. FURTH, S.K. RUSCETTI, and C.J. SHERR, NCI, National Institutes of Health, Bethesda, Maryland: The feline retroviral oncogene (*v-fms*), its encoded glycoprotein products, and its human proto-oncogene (*c-fms*).

B. SHILO[1] and M. HOFFMANN,[2] [1]Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel; [2]Dept. of Biology, Harvard University, Cambridge, Massachusetts: Conservation of vertebrate oncogenes in *D. melanogaster*.

B. BIEGALKE, C. PACHL, and M. LINIAL, Fred Hutchinson Cancer Research Center, Seattle, Washington: Subgenomic expression of *v-myc* in MH2-transformed cells.

W.H. SCHUBACH and P.E. NEIMAN, Fred Hutchinson Cancer Research Center, Seattle, Washington: Anti–complementary-strand transcription from the chicken *c-myc* region.

R. MÜLLER,[1] D.J. SLAMON,[2] J.M. TREMBLAY,[1] M.J. CLINE,[2] and I.M. VERMA,[1] [1]Tumor Virology Laboratory, Salk Institute, San Diego, California; [2]Dept. of Medicine, University of California, Los Angeles: Expression of cellular oncogenes.

B. OZANNE, T. WHEELER, B. DALE, J. ZACK, and R.G. SMITH, University of Texas Health Science Center, Dallas: Arrangement and expression of Ab- MLV-related nucleic acid sequences in a novel human pre-B lymphoblastic leukemia.

R. DALA FAVERA, E. GELMANN, S. MARTINOTTI, G. FRANCHINI, T. PAPAS, R.C. GALLO, and F. WONG-STAAL, NCI, National Institutes of Health, Bethesda, Maryland: *c-myc* sequences—Pseudogene generation during evolution and gene amplification in the human leukemic cell line HL-60.

S. COLLINS[1] and M. GROUDINE,[2] [1]Veterans Administration Hospital, Seattle; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Amplification of endogenous human retrovirus-related oncogene sequences in cultured human myeloid leukemia cell lines.

SESSION 7 *Poster Session: Transforming Proteins,Differentiation, and Human Retroviruses*

J. NEIL, Beatson Institute for Cancer Research, Glasgow, Scotland: Structural properties of ASV transforming proteins.

L.E. GENTRY,[1] L.R. ROHRSCHNEIDER,[1] J.E. CASNELLIE,[2] and E.G. KREBS,[2] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Dept. of Pharmacology, University of Washington, Seattle: Antibodies to a synthetic peptide fragment of the *src* gene product identifies $pp60^{src}$ in transformed cells.

S. AMINI, A. TANAKA, N. KOBAYASHI, and A. KAJI, University of Pennsylvania, Philadelphia: Relationship between stimulation of growth rate of chondrocytes by RSV and phosphorylation level of $pp60^{src}$ and its presumed target pp36.

C. BLAT,[1] L. HAREL,[1] J. VILLAUDY,[2] and A. GOLDE,[2] [1]Institut Recherches Scientifiques sur le Cancer, Villejuif; [2]Institut Curie, Pavillon Regaud, Paris, France: Modifications of the in vitro phosphorylation of nonhistone chromosomal proteins after infection of chicken fibroblasts by RSV.

M. JULLIEN,[1] L. HAREL,[1] J. VILLAUDY,[2] and A. GOLDE,[2] [1]Institut Recherches Scientifiques sur le Cancer, Villejuif, France; [2]Institut Curie, Paris, France: Early effect of the expression of *src* gene on the phosphate uptake and metabolism in chicken cells.

S. ITO, N.D. RICHERT, and I.H. PASTAN, NCI, National Institutes of Health, Bethesda, Maryland: Phospholipids stimulate vinculin phosphorylation by the *src* kinase.

N.D. RICHERT, D. BLITHE, and I. PASTAN, NCI, National Institutes of Health, Bethesda, Maryland: Highly purified *src* kinase from SR-ASV tumors in rats.

C.B. BOSCHEK, A. BARNEKOW, T. TAMURA, R.R. FRIIS, R. BACK, and H. BAUER, Institut für Virologie, Justus-Liebig-Universität, Giessen, Federal Republic of Germany: Subcellular localization and action of the *src*-gene product of ASV.

R. MARTINEZ, K.D. NAKAMURA, and M.J. WEBER, Dept. of Microbiology, University of Illinois, Urbana: Identification of phosphotyrosine-containing proteins in normal and RSV-transformed cells.

J. NAWROCKI,[1] A. LAU,[2] E. ERIKSON,[3] and A. FARAS,[1] [1]Dept. of Microbiology, University of Minnesota, Minneapolis; [2]Cancer Center, University of Hawaii, Honolulu; [3]Dept. of Pathology, University of Colorado Medical School, Denver: Correlation of 34K phosphorylation and $pp60^{src}$- associated protein kinase activity with tumorigenicity in transformed and revertant vole cells.

H. RUBSAMEN,[1] R.R. FRIIS,[2] and E. EIGENBRODT,[3] [1]Paul-Ehrlich-Institut, Frankfurt, Federal Republic of Germany; [2]Institut für Virologie; [3]Institut fur Biochemie, University of Geissen, Federal Republic of Germany: Comparison of cytosolic malic dehydrogenase activity between normal and RSV-transformed chicken embryo fibroblasts.

K. MOELLING, P. DONNER, M.-K. OWADA, I. GREISER-WILKE, and T. BUNTE, Max- Planck-Institut fur Molekulare Genetik, Berlin, Federal Republic of Germany: Biochemical characterization of transformation-specific proteins of acute ALV and ASV.

S.H. BENEDICT and P.K. VOGT, Dept. of Microbiology, University of Southern California Medical School, Los Angeles: A DNA-binding protein specific for cells transformed by defective ALV.

S. WRIGHT,[1,3] S. HARMON,[2,3] D. SMITH,[3] J. HAYES,[3] P. ROBERTSON,[3] and A. WAYNE,[3] [1]Depts. of Medicine and Cellular, Viral and Molecular Biology, University of Utah Medical School; [2]Dept. of Biology, University of Utah, Salt Lake City; [3]Veterans Administration Medical Center, Salt Lake City, Utah: In vitro translation of AMV genomic RNA yields nonstructural proteins.

L. SCHIFF-MAKER, K. MURTAGH, and N. ROSENBERG, Cancer Research Center, Tufts University Medical School, Boston, Massachusetts: Monoclonal antibodies against Abelson protein.

V. ROTTER and D. WOLF, Dept. of Cell Biology, Weizmann Institute of Science, Rehovot, Israel: Regulation of the expression of the P50 host- encoded protein in Ab-MLV transformed cells.

K. GANGULY and M. ESSEX, Dept. of Microbiology, Harvard School of Public Health, Boston, Massachusetts: FeSV-transformed cat and mink cells shed FeLV-related soluble polypeptides into culture medium.

A. CHEN and M. ESSEX, Dept. of Microbiology, Harvard University School of Public Health, Boston, Massachusetts: Tumor-associated antigens and transformation proteins of feline retroviruses.

N. PEDERSEN and L. JOHNSON, School of Veterinary Medicine, University of California, Davis: Dichotomy and interrelationship of tumor and Fe-LV "helper" virus immunity in cats infected with ST-FeSV.

W. PRENSKY, S. JHANWAR, and S.S. VEDBRAT, Memorial Sloan-Kettering Cancer Center, New York, New York: Feline oncornavirus-associated cell membrane antigen (FOCMA) expression—Current concepts.

S. VEDBRAT,[1] M.B. GARDNER,[2] S. RASHEED,[3] S. RUSCETTI,[4] H. LUTZ,[5] and W. PRENSKY,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]University of California, Davis; [3]University of Southern California Medical School, Los Angeles; [4]NCI, National Institutes of Health, Bethesda, Maryland; [5]University of Zurich, Switzerland: Feline oncornavirus-associated cell membrane antigen (FOCMA) expression in virus-negative lymphosarcoma (LSA) cells.

J.H. PIERCE and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: BALB- and Ha-MSV transformation of a novel lymphoid progenitor cell.

J.P. BLANCHET,[2] J. SAMARUT,[2] I. PARK,[1] L. GAZZOLO,[1] and V. KRSMANOVIC,[1] [1]Unite de Virologie, INSERM-CNRS, Lyon; [2]Dept. de Biologie Générale et Appliquée, Université Claude-Bernard, Villeurbanne, France: Differentiation and age-related antigens expressed on AEV-transformed erythroid cells.

M. OLSEN and D. BOETTIGER, Dept. of Microbiology, University of Pennsylvania, Philadelphia: Interaction of AMV with differentiating hematopoietic cells.

A.E. BARKAS,[1] L.A. BRENNAN,[1] I. BALAZS,[1] and E. STAVNEZER,[1] [1]Genetics and Molecular Biology Unit, Sloan-Kettering Institute School of Medical Sciences, New York, New York; [2]Dept. of Biology, New York University, New York: Induction of differentiation and transformations by SKV.

Publication party for authors of *RNA Tumor Viruses*

C. KRYCÈVE-MARTINERIE, D.A. LAWRENCE, J. CROCKET, P. JULLIEN, and P. VIGIER, Institut Curie, Centre Universitaire, Orsay, France: Cells transformed by RSV release transforming growth factors.

A. TANAKA,[1] J. SABRAN,[2] K. HSIA,[1] Y. IWASAKI,[3] T. FURUSE,[3] and A. KAJI,[1] [1]University of Philadelphia Medical School, Pennsylvania; [2]University of Wisconsin, Madison; [3]National Center for Nervous, Mental and Muscular Disorders, Tokyo, Japan: Production of RSV in terminally differentiated myotubes without intergration of viral DNA.

R.L. FARRELL, L. SCHNAPP, and V.M. INGRAM, Massachusetts Institute of Technology, Cambridge: Globin expression in RSV-transformed quail myoblasts.

D. BOETTIGER and M. PACIFICI, Dept. of Microbiology, University of Pennsylvania, Philadelphia: Infection of limb bud cells by ts LA24A accelerates chondrogenic differentiation.

P. CRISANTI-COMBES,[1] A. GIRARD,[1] B. PESSAC,[1] F. POIRIER,[2] and G. CALOTHY,[2] [1]CNRS, INSERM, Hôpital Broussais, Paris; [2]Institut Curie-Biologie, Orsay, France: Stimulation of glutamate and malate decarboxylase activities in quail neuroretinal cells infected with avian retroviruses.

S. OROSZLAN,[1] T.D. COPELAND,[1] V.S. KALYANARAMAN,[2] M.G. SARNGADHARAN,[2] and R.C. GALLO,[3] [1]NCI-Frederick Cancer Research Facility, Frederick; [2]Dept. of Cell Biology, Litton Bionetics, Inc., Kensington; [3]NCI, National Institutes of Health, Bethesda, Maryland: Human T-cell leukemia virus (HTLV) core proteins are structurally and immunologically related to BLV protein homologs.

K. SUGAMURA, Y. GOTOH, and Y. HINUMA, Institute for Virus Research, Kyoto University, Japan: Healthy carriers of a human retrovirus, adult T-cell leukemia virus (ATLV)—Demonstration by clonal culture of ATLV- carrying T cells from peripheral blood.

M.S. REITZ, JR.,[1] M. POPOVIC,[1] V.S. KALYANARAMAN,[2] M.G. SARNGADHARAN,[2] M. ROBERT-GUROFF,[1] Y. NAKAO,[3] I. MIYOSHI,[4] Y. ITO,[5] J. MINOWADA,[6] and R.C. GALLO,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland; [3]Dept. of Medicine, Kobe University Medical School, Japan; [4]Dept. of Internal Medicine, Kochi Medical School, Japan; [5]Dept. of Microbiology, Kyoto University, Japan; [6]Dept. of Immunology, Roswell Park Memorial Institute, Buffalo, New York: HTLV is the virus of Japanese adult T-cell leukemia.

T. BONNER, C. O'CONNELL, and M. COHEN, NCI- Frederick Cancer Research Facility, Maryland: Cloning of endogenous human retroviral sequences.

E.P. GELMANN, A. CETTA, E. PETRI, S. JOSEPHS, R.C. GALLO, and F. WONG-STAAL, NCI, National Institutes of Health, Bethesda, Maryland: Cloning and characterization of different classes of human genomic sequences homologous to the baboon endogenous virus.

E. GELMANN, F. WONG-STAAL, R. KITTMAN, A. BURNEY, and R.C. GALLO, NCI, National Institutes of Health, Bethesda, Maryland: Human T-cell leukemia virus (HTLV) has a high degree of homology to a specific sequence of the BLV and has regions homologous to other retroviruses.

N. YAMAMOTO, M. OKADA, Y. KOYANAGI, T. CHOSA, Y. TANAKA, and Y. HINUMA, Institute for Virus Research, Kyoto University, Japan: Transformation of human leukocytes by cocultivation with an adult T-cell leukemia virus (ATLV)-producer cell line.

V.S. KALYANARAMAN,[1] M.G. SARNGADHARAN,[1] M. ROBERT-GUROFF,[2] Y. ITO,[3] Y. NAKAO,[4] and R.C. GALLO,[2] [1]Litton Bionetics, Inc., Kensington, Maryland; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]Kyoto University, [4]Kobe University, Japan: Natural antibodies to human leukemia lymphoma virus (HTLV) in Japanese sera.

L. THIRY, J. COGNIAUX-LECLERC, S. SPRECHER-GOLDBERGER, and R. OLISLAGER, Institut Pasteur du Bragant, Brussels, Belgium: Monoclonal antibodies against specific cell receptors for baboon endogenous virus.

J. SUNI,[1,2] L. PARTANEN,[1] T. WAHLSTRÖM,[3] P. LEHTOVIRTA,[3] and A. VAHERI,[1] [1]Dept. of Virology, University of Helsinki; [2]Dept. of Virology, Aurora Hospital, Helsinki; [3]Dept. of Obstetrics and Gynecology, Helsinki University Central Hospital, Finland: p28 and precursor polypeptides of human endogenous retrovirus in placental syncytiotrophoblastic cells.

R.B. NASO and S.A. FUQUA, M.D. Anderson Hospital and Tumor Institute, University of Texas System Cancer Center, Houston: On the origin of SSAV.

J. JORE, R. DUBBES, and P. BENTVELZEN, Radiobiological Institute TNO, Rijswijk, The Netherlands: Sequences related to SSAV in normal cellular DNA of several mammals.

E. HEFTI, A. HARDEN, and S. PANEM, Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Genomic DNA rearrangements in patients with leukemia.

G. CARLONI, A. LE PATEZOUR, M.L. MICHEL, C. BRECHOT, A. FRITSCH, P. TIOLLAIS, and S. WAIN-HOBSON, INSERM, Institut Pasteur, Paris, France: Cellular transformation of cloned and uncloned sequences of hepatocellular carcinoma DNA.

M.J. KOURY,[1] I.B. PRAGNELL,[1] J. LANG,[2] J. NEIL, and N.M. WILKIE,[1] [1]Beatson In- stitute for Cancer Research,

Glasgow; [2]Institute of Virology, Glasgow, Scotland: Induction of granulocyte-macrophage colony stimulation factor (GM-CSF) by murine retroviruses.

SESSION 8 *Poster Session: Biology of Endogenous Viruses, Control of Viral Gene Expression*

M. MARX,[1] R. MARIAGE-SAMSON,[1] J. HILLOVA,[1] M. HILL,[1] and A. SARGEANT,[2] [1]Institute of Cancerology and Immunogenetics, CNRS, Villejuif; [2]Oncologie Moleculaire and INSERM, Institut Pasteur, Lille, France: Host- cell-induced restriction of the *src* gene during RSV replication on transformed and mutagenized quail cell lines.

M. KOTLER and H. MOSKOWITZ, Dept. of Molecular Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Factors involved in the expression of ASV proviruses in productive and non- productive cells.

D.L. EWERT and M.S. HALPERN, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Endogenous retrovirus antigen expression by B cells.

R. TO, W. SCHUBACH, and P. NEIMAN, Fred Hutchinson Cancer Research Center, Seattle, Washington: Oncogenicity of ALV 3′regions.

J.H. MORGAN and R.E. SMITH, Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Construction and characterization of recombinants between RAV-0 and MAV-2(0).

H.L. ROBINSON,[1] P.N. TSICHLIS,[2] and J.M. COFFIN,[3] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]Tufts University Medical School, Boston, Massachusetts: ALVs target for specific forms of non- acute disease.

P. SCHATZ and P.R. SHANK, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Construction of in vitro recombinants between molecular clones of two ALVs with distinct disease tropisms.

C.L. OW, J.K. CARTER, and R.E. SMITH, Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Hyperlipidemia induced by an avian retrovius.

A.S. KHAN and M.A. MARTIN, NIAID, National Institutes of Health, Bethesda, Maryland: Characterization of *env* and LTR segments of endogenous MLV proviruses.

F.S. PEDERSEN, H.D. ANDERSEN, M. ETZERODT, J. FRYDENBERG, P. JØRGENSEN, N.O. KJELDGAARD, and H. SKJØDT, Dept. of Molecular Biology, University of Aarhus, Denmark: Analysis of MLV-specific mRNAs in cell lines derived from lymphomas of AKR mice.

J. LENZ,[1] R. CROWTHER,[1] S. KLIMENKO,[2] A. SHELDON,[1] E. ELFASSI,[1] and W. HASELTINE,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]D.I. Ivanosky Institute, Moscow, USSR: Leukemogenic determinants of the SL3-3 virus.

R. VILLEMUR, E. RASSART, and P. JOLICOEUR, Institut de Recherches Cliniques de Montréal, Université de Montréal, Canada: Restriction analysis and sequencing of the leukemogenic Gross Passage A MLV cloned DNA reveals significant changes at the 3′end of the genome and on a short portion of its LTR.

D.L. BUCHHAGEN and J.D. MORRISSEY, Dept. of Microbiology and Immunology, State University of New York, Downstate Medical Center, Brooklyn: Molecularly-cloned Gross proviral DNA contains sequences homologous to xenotropic AKR virus.

J.H. WOLFE, E.P. BLANKENHORN, and K.J. BLANK, University of Pennsylvania Medical School, Philadelphia: Virus protein processing in *H-2*-congenic Gross-virus-induced tumor cell lines.

K.E. FRY, R.A. GRYMES, H.S. KAPLAN, and J.P. KIM, Cancer Biology Research Laboratory, Stanford University Medical School, California: Interrelations between the viruses inhabiting the C57BL/Ka mouse.

E. RASSART, P. SANKAR, and P. JOLICOEUR, Institut de Recherches Cliniques de Montreal, Universite de Montreal, Canada: Molecular studies of the ecotropic MLV recovered from primary x-ray-induced thymoma in C57BL/6 mice.

M. ZIJLSTRA,[1] R.E.Y. DE GOEDE,[1] H.J. SCHOENMAKERS,[1] A.H. SCHINKEL,[1] W.G. HESSELINK,[1] J.L. PORTIS,[2] and C.J.M. MELIEF,[1] [1]Laboratory of Experimental and Clinical Research, University of Amsterdam, The Netherlands; [2]NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Isolation and characterization of different host-range classes of MLV from virus-induced C57BL lymphomas.

M. MOWAT and A. BERNSTEIN, Ontario Cancer Institute, Toronto, Canada: Association of the *Fv-2* locus with an endogenous ecotropic provirus in C57BL/6J.S mice.

A. REIN, Biological Carcinogenesis Program, NCI-Frederick Cancer Research Facility, Maryland: A distinct receptor for MCFs on mouse cells.

A. HABARA, E.P. REDDY, C. DUNN, and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Construction of recombinants between Rauscher MLV and Mo-MLV.

T. SHIBUYA and T.W. MAK, Ontario Cancer Institute, Toronto, Canada: Erythroleukemia induction by Friend leukemia virus—A host gene in the control of early anemia and polycythemia induction and the rate of proliferation of late (post-CFU-E) erythroid precursor cells.

D. BARBIERI-WEILL and F. GAY, CNRS, Villejuif, France: Genesis of a SFFV —Possible role of Fr-MCF viruses.

A.M. SCHULTZ, L.E. HENDERSON, A. REIN, G.W. SMYTHERS, and S. OROSZLAN, NCI-Frederick Cancer Research Facility, Maryland: Structural characterization of Rauscher-MCF virus proteins.

L. EVANS, B. CHESEBRO, and M. CLOYD, NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Comparison of MCF viruses derived from Fr-MLV or Mo-MLVs.

L. WOLFF and S. RUSCETTI, NCI, National Institutes of Health, Bethesda, Maryland: Mapping of endogenously acquired portion of Fr-MCF gp70 to its N terminus and a structural comparison of MCF gp70 with SFFV gp52.

W. BRITT and B. CHESEBRO, NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Cells from the late stages of FV leukemia express a non-ecotropic MLV gp70 closely related to a gp70 expressed on fetal hematopoietic cells.

N.M. TEICH and J. ROWE, Imperial Cancer Research Fund, London, England: Studies of BSB, a murine erythroleukemia virus complex distinct from Friend virus.

T.W. MAK and T. SHIBUYA, Ontario Cancer Institute, Toronto, Canada: Host control of the types of leukemia (erythroid granulocytic and T-lymphoid) induced by helper-independent MLVs and isolation of erythroid and myeloid cell lines.

J.M. HEARD,[1] F. WENDLING,[2] R. BERGER,[3] S. FICHELSON,[1] and S. GISSELBRECHT.[1] [1]Laboratoire Immunologie et Virologies de Tumeurs, INSERM, Paris; [2]Unité de Recherches de Physiologie Cellulaire, INSERM, Orsay; [3]Laboratoire de Cytogénétique, Hopital Saint-Louis, Paris, France: Establishment of in vitro myelomonocytic permanent cell lines from in vivo virus-induced chloroleukemias in mice.

E. PILLEMER,[1] D. KOOISTRA,[1] O. WITTE,[2] and I. WEISSMAN.[1] [1]Stanford University, California; [2]Molecular Biology Institute, University of California, Los Angeles: Identification of MuLV-related nonvirion antigens on the surface of murine T- and B-cell lymphomas.

D.E. LEVY,[1] R.A. LERNER,[2] and M.C. WILSON.[2] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Dept. of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: Control of endogenous retrovirus mRNA expression by a separate regulatory gene.

S. RASHEED and M. LAI, Depts. of Pathology and Microbiology, University of Southern California, Los Angeles: Genomic analysis of a thymoma- inducing amphotropic virus.

S. MOHAN and B.K. PAL, Dept. of Biological Sciences, California State Polytechnic University, Pomona: Wild mouse retrovirus-induced paralysis—Role of virus infection, replication, and gene expression in CNS.

J. Coffin, P. Tsichlis

Lecture Hall

B.R. BROOKS,[1] P. BOWMAN,[2] and G. GOLDSTEIN, [1]Dept. of Neurology, Johns Hopkins University Medical Center, Baltimore, Maryland; [2]Dept. of Neurology, University of Michigan Medical School, Ann Arbor: Murine neutrotropic retrovirus infection is not restricted in purified CNS capillary endothelial cells in vitro.

K.B. ANDERSEN and B.A. NEXØ, Fibiger Laboratory, Copenhagen, Denmark: The pathway of entry of retrovirus into mouse fibroblasts.

J. MCCUBREY, P. GREEN, J. HOROWITZ, C. SINAIKO, and R. RISSER, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: DNA transfection studies of endogenous ecotropic MLV.

M.-A. AUGER-BUENDIA, J. DEVAUX, and A. TAVITIAN, INSERM, Faculte de Medecine, Paris, France: Mo-MSV of large genomic size produced by a transformed rat-cell line.

C. DE GIULI MORGHEN, A. RADAELLI, M. RIGHI, and E. LIBOI, CNR Center for Cytopharmacology, Milano, Italy: Ultrastructural and biochemical analysis of defectiveness of L929 cell retrovirus.

G.L.C. SHEN-ONG and M.D. COLE, Dept. of Biochemistry, St. Louis University Medical School, Missouri: Differing populations of intracisternal A-particle genes in myeloma tumors and mouse subspecies.

D.K. HAAPALA,[1] K.J. DUNN,[1] and J.H. GILBERT,[2] [1]NCI, Frederick Cancer Research Center, Frederick, Maryland; [2]Michigan State University, East Lansing: Studies of the nature and control of RD-114-like viruses in cat cells.

J.H. GILBERT,[1] J. DUNN,[2] and D.K. HAAPALA,[2] [1]Dept. of Microbiology and Public Health, Michigan State University, East Lansing; [2]NCI, Frederick Cancer Research Center, Maryland: Differential expression of RD-114 virus genetic information by cells producing FeLV.

M.A. MINK, A. HABERMAN, and L.F. VELICER, Dept. of Microbiology and Public Health, Michigan State University, East Lansing: A putative envelope glycoprotein gene recombinant between FeLV and RD-114—Immunoprecipitation analysis and comparison of tryptic and chymotryptic peptide maps.

C. BRUCK,[1] D. PORTETELLE,[1] A. BURNY,[1] and J. ZAVADA,[2] [1]Dept. of Molecular Biology, University of Brussels, Belgium; [2]Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia: Molecular dissection of BLV envelope glycoprotein gp51 by monoclonal antibodies.

S. MOLINEAUX and J.E. CLEMENTS, Johns Hopkins University, Baltimore, Maryland: Characterization of visna virus mRNAs using cloned viral DNAs.

M.S. REITZ, JR., E.P. GELMANN, C.D. TRAINOR, and F. WONG-STAAL, NCI, National Institutes of Health, Bethesda, Maryland: Construction and analysis of molecular clones of oncogenic (SEATO) and non- oncogenic (Brain) strains of gibbon ape leukemia virus.

S. BROOME, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: A retroviral promoter located in the 3′ end of the *env* gene.

G.L. FIRESTONE, F. PAYVAR, and K.R. YAMAMOTO, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Glucocorticoid- dependent processing and compartmentalization of MMTV proteins in infected HTC cells.

SESSION 9 *Transforming Proteins*

Chairperson: C.J. SHERR, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

R. PRYWES and D. BALTIMORE, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Site-directed mutagenesis of the Ab-MLV genome and its expression in SV40 vectors.

D. BRYANT,[1] V. WILKERSON,[1] J. BRUGGE,[2] and J.T. PARSONS,[1] [1]Dept. of Microbiology, University of Virginia Medical School, Charlottesville; [2]State University of New York, Stony Brook: Site-directed mutagenesis in the *src* gene of RSV.

J. BRUGGE and D. DARROW, Dept. of Microbiology, State University of New York, Stony Brook: Analysis of the turnover and distribution of the protein complex consisting of $pp60^{src}$ and two cellular phosphoproteins.

S. COURTNEIDGE[1] and J.M. BISHOP,[2] [1]National Institute for Medical Research, Mill Hill, London, England; [2]Dept. of Microbiology and Immunology, University of California Medical Center, San Francisco: The subcellular location of the transforming protein of RSV and its effect on phenotype.

E.A. GARBER, J.G. KRUEGER, H. HANAFUSA, and A.R. GOLDBERG, Rockefeller University, New York, New York: Phosphorylation of $pp60^{src}$ in membrane vesicles from RSV-transformed cells.

R.A. FELDMAN, E. WANG, and H. HANAFUSA, Rockefeller University, New York, New York: Subcellular localization of the transforming protein of FuSV.

P. MOSS, T. GILMORE, and G.S. MARTIN, Dept. of Zoology, University of California, Berkeley: Membrane association of the transforming protein of FuSV.

B. ADKINS,[1] R. MORGAN,[2] and K. BEEMON,[2] [1]Institut für Virusforshung, Im Neuenheimer Feld, Heidelberg, Federal Republic of Germany; [2]Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Two structurally and functionally distinct forms of the PRCII ASV transforming protein.

J. PAPKOFF, I.M. VERMA, and T. HUNTER, Salk Institute, San Diego, California: Detection of a transforming gene product in Mo-MSV-transformed cells.

M.L. PRIVALSKY,[1] A.D. LEVINSON,[2] J.P. MCGRATH,[2] and J.M. BISHOP,[1] [1]University of California, San Francisco; [2]Genentech, Inc., South San Francisco, California: Identification of the protein product of the AEV *erb*-B domain.

B. VENNSTROM,[1] L. FRYKBERG,[1] H. BEUG,[2] S. PALMIERI,[2] and M. HAYMAN,[3] [1]Dept. of Microbiology, University of Uppsala, Sweden; [2]Deutsche Krebsforschungszentrum, Heidelberg, Federal Republic of Germany; [3]Imperial Cancer Research Fund, London, England: Analysis of the transforming capacity of mutants of AEV generated by site-directed mutagenesis in vitro.

K.C. ROBBINS, S.G. DEVARE, E.P. REDDY, and S.A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Detection of the transforming gene product of SSV—Preliminary analysis of its functional properties.

M.O. WEEKS, C.M. WEI, and E.M. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: Molecular and biological characterization of Ha-MSVtk, a retrovirus containing the p21 *ras* gene and the *tk* gene.

P. KAPLAN, J. ZACK, and B. OZANNE, Dept. of Microbiology, University of Texas Health Science Center, Dallas: Transforming growth factor production and use by Ki-MSV transformed cells.

SESSION 10 *Leukemogenesis*

Chairperson: P.V.O. O'DONNEL, Memorial Sloan-Kettering Cancer Center, New York, New York

L. DESGROSEILLERS and P. JOLICOEUR, Institut de Recherches Cliniques de Montréal, Université de Montréal, Canada: Construction and characterization of in vitro recombinants of *N*- and *B*-tropic endogenous BALB/c MLV DNA—Gene mapping of Fv-l tropism determinant and thymotropism.

R. SOEIRO,[1] J. CHINSKY,[1] and J. KOPCHICK,[2] [1]Albert Einstein College of Medicine, Bronx, New York; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Fv-l host restriction of Fr-MLV—Studies of non- integrated DNA.

C.A. HOLLAND,[1] J.W. HARTLEY,[2] W.P. ROWE,[2] and N. HOPKINS,[1] [1]Massachusetts Institute of Technology, Cambridge; [2]National Institutes of Health, Bethesda, Maryland: Genetic analysis of leukemogenicity and tissue tropism by construction of in vitro recombinants between molecular clones of AKV and MCF 247 viruses.

C.Y. THOMAS and M. COFFIN, Tufts University Medical School, Boston, Massachusetts: Independent origin of the non-AkV p15E gene/U3 region and gp70 gene sequences in recombinant viruses of AKR/J mice.

N.G. FAMULARI, C.F. KOEHNE, and P.V. O'DONNELL, Memorial Sloan- Kettering Cancer Center, New York, New York: Leukemogenesis by Gross Passage of A-MLV—Expression of viruses with recombinant *env* genes in transformed cells.

R.A. BOSSELMAN,[1] F. VAN STRAATEN,[2] C. VAN BEVEREN,[2] M. VOGT,[2] and I.M. VERMA,[2] [1]Applied Molecular Genetics, Inc., Newbury Park; [2]Salk Institute, San Diego, California: Analysis of the *env* gene of a molecularly cloned and biologically active Moloney mink cell focus-forming (MCF) proviral DNA.

R. REPASKE, R.P. O'NEILL, C.E. BUCKLER, and M.A. MARTIN, NIAID, National Institutes of Health, Bethesda, Maryland: Nucloetide sequence of the *env*-specific region of NFS-Th-1 xenotropic proviral DNA.

J. DUDLEY and R. RISSER, McArdle Laboratory for Cancer Research, Univer- sity of Wisconsin, Madison: Alterations in genomic DNA of murine T cell lymphomas—Detection of novel restriction enzyme fragments containing endogenous MMTV proviruses.

J. KAMINCHIK, W.D. HANKINS, and E.M. SCOLNICK NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning of biologically active proviral DNA of the anemia-inducing strain of SFFV.

A. OLIFF[1] and S. RUSCETTI,[2] [1]Memorial Sloan-Kettering Cancer Center, New York; [2]NCI, National Institutes of Health, Bethesda, Maryland: Localization of the pathogenic functions of Fr-MLV and Friend mink cells focus-inducing virus (Fr-MCF) to the *env* gene by in vitro construction of recombinant viruses.

D. KABAT, C. MACHIDA, M. RUTA, and R. BESTWICK, Dept. of Biochemistry, Oregon Health Sciences University Medical School, Portland: Glycoprotein structural gene mutants of SFFV are nonleukemogenic.

K. RADKE, H. BEUG, and T. GRAF, Institute for Virology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Transformation of both erythroid and myeloid cells by E26, an avian erythroleukemia virus which contains the *myb* gene.

G. WANECK and N. ROSENBERG, Cancer Research Center, Tufts University Medical School, Boston, Massachusetts: Erythroid target cells for Abelson virus and Harvey virus.

SESSION 11 *Activation of Host Genes*

Chairperson: G.F. VANDE WOUDE, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

B.E. NEEL,[1] C.-K. SHIH,[1] G.P. GASIC,[1] and W.S. HAYWARD,[2] [1]Rockefeller University, New York, New York; [2]Sloan-Kettering Institute for Cancer Research, New York, New York: Localization of potential initiation sites for RNA polymerase II in the c-*myc* gene.

D. WESTAWAY,[1] G.S. PAYNE,[1] C. MOSCOVICI,[2] J.M. BISHOP,[1] and H.E. VARMUS,[1] [1]Dept. of Microbiology and Immunology, University of California, San Francisco; [2]Tumor Virology, Veteran's Administration Hospital, Gainesville, Florida: Induction of lymphoid and renal tumors by Rous and myeloblastosis-associated viruses.

G. GOUBIN,[1] P.A. NEIMAN,[2] and G.M. COOPER,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Molecular cloning and characterization of a cellular transforming gene of chicken B-cell lymphoma.

A.D. ZELENETZ and G.M. COPPER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: LTR-mediated activation of endogenous transforming genes of NIH/3T3 mouse cells.

Y.-K. FUNG,[1] L. CRITTENDEN,[2] and H.-J. KUNG,[1] [1]Dept. of Biochemistry, Michigan State University, East Lansing; [2]United States Dept. of Agriculture, Sciences, and Education Administration, Regional Poultry Research Laboratory, East Lansing, Michigan: Involvement of c-*erb* in avian leukemogenesis.

J.K. CARTER,[1] S.J. PROCTOR,[2] and R.E. SMITH,[1] [1]Dept. of Microbiology and Immunology, Duke University Medical Center, Durham; [2]Experimental Pathology Laboratories, Inc., Raleigh, North Carolina: Induction of sarcomas by an ALV.

M.C. SIMON,[1] R.E. SMITH,[2] and W.S. HAYWARD,[3] [1]Rockefeller University, New York, New York; [2]Duke University Medical Center, Durham, North Carolina; [3]Sloan-Kettering Institute for Cancer Research, New York, New York: Pheasant viruses may induce lung tumors by activating a cellular gene.

R. NUSSE[1] and H.E. VARMUS,[2] [1]Dept. of Virology, Antoni van Leeuwen- hoekhuis, Amsterdam, The Netherlands; [2]Dept. of Microbiology, University of California, San Francisco: Acquired proviruses of the MMTV are integrated in a common domain of tumor DNA.

P.R. ETKIND and N.H. SARKAR, Memorial Sloan-Kettering Cancer Center, New York, New York: New proviral integration and differential hypomethylation of the endogenous MMTV proviral DNA sequences in tissues of the C3Hf mouse.

F. YOSHIMURA and K. LEVINE, Fred Hutchinson Cancer Research Center, Seattle, Washington: Characterization of MLV proviral DNA and RNA from AKR thymic lymphomas.

P.N. TSICHLIS, L. HU, and P.G. STRAUSS, NCI, National Institutes of Health, Bethesda, Maryland: Mo-MLV-induced rat thymomas.

M. BOCCARA, M. SOUYRI, C. MAGARIAN, E. STAVNEZER and E. FLEISSNER, Memorial Sloan-Kettering Cancer Center, New York, New York: A novel species of MLV env-related RNA in radiation leukemias from BALB/c mice.

E. ATHAN, M. GOODENOW, F. LILLY, and D. DINA, Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Expression of virus- related sequences in carcinogen-induced lymphomas.

SESSION 12 *Viruses, Oncogenes, and Human Tumors*

Chairperson: J.M. BISHOP, University of California, San Francisco, California

P. STEELE, R. REPASKE, T. BRYAN, A. RABSON, R. O'NEILL, and M. MARTIN, NIAID, National Institutes of Health, Bethesda, Maryland: Human DNA contains endogenous type-C retrovirus sequences.

C. O'CONNELL, M. COHEN, and T. BONNER, NCI-Frederick Cancer Research Facility, Frederick, Maryland: Characterization of a cloned retrovirus- like genome from human DNA.

R. CALLAHAN, W. DROHAN, S. TRONICK, and J. SCHLOM, Laboratory of Cellular and Molecular Biology, NCI,

National Institutes of Health, Bethesda, Maryland: Identification and molecular cloning of novel MMTV-related- DNA sequences in mouse and human cellular DNA.

M. ROBERT-GUROFF,[1] V.S. KALYANARAMAN,[1] M.G. SARNGADHARAN,[1] W.A. BLATTNER,[1] D. CATOVSKY,[2] F. MERINO,[3] and R.C. GALLO,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Royal Postgraduate Medical School, London, England; [3]Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela: Serologic studies show HTLV is associated with aggressive T-cell malignancies in various geographic locations.

M. POPOVIC,[1] P.S. SARIN,[1] V.S. KALYANARAMAN,[2] M. ROBERT-GUROFF,[1] M.G. SARNGADHARAN,[2] J. MINOWADA,[3] T. AOKI,[4] D. MANN,[1] W. BLATTNER,[1] S. BRODER,[1] D. GOLDE,[5] and R.C. GALLO,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Litton Bionetics, Inc., Kensington, Maryland; [3]Roswell Park Memorial Institute, Buffalo, New York; [4]Shimrakuen Hospital, Niigata, Japan; [5]University of California, Los Angeles: New HTLV isolates from geographically different parts of the world and their infectivity of human T cells.

M. YOSHIDA, M. SEIKI, and S. HATTORI, Cancer Institute, Kami-Ikebukuro, Toshima-ku, Tokyo, Japan: Human retrovirus ATLV—Characterization of the viral genome and association with ATL.

F. WONG-STAAL, V. MANZARI, E.P. GELMANN, E. WESTIN, G. FRANCHINI, S. JOSEPHS, R. DALLA-FAVERA, and R.C. GALLO, NCI, National Institutes of Health, Bethesda, Maryland: Integration and expression of HTLV in infected cells and molecular cloning of the 5-proximal viral sequences.

L.F. PARADA, C. TABIN, C. SHIH, and R.A. WEINBERG, Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Homology between the EJ human bladder carcinoma oncogene and the HaSV oncogene, *ras*.

C.J. DER, T.G. KRONTIRIS, and G.M. COOPER, Sidney Farber Cancer Institute, Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of HaSV and KiSV.

M. BARBACID, E. SANTOS, and S. PULCIANI, NCI, National Institutes of Health, Bethesda, Maryland: A transforming gene involved in human bladder carcinomas.

E.H. CHANG,[1] M.A. GONDA,[2] R.W. ELLIS,[1] M.E. FURTH,[1] E.M. SCOLNICK,[1] and D.R. LOWY,[1] [1]NCI, National Institutes of Health, Bethesda; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: Characterization of four members of the p21 gene family isolated form normal human genomic DNA and demonstration of their oncogenic potential.

M.A. LANE, A.C. SAINTEN, and G.M. COOPER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Isolation of a molecular clone containing the human mammary tumor transforming sequence.

U.G. ROVIGATTI, R. DIEHL, and S.M. ASTRIN, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Oncogene expression in human tumors.

E.H. WESTIN,[1] F. WONG-STAAL,[1] E.P. GELMANN,[1] G. FRANCHINI,[1] S.K. ARYA,[1] R. DALLA-FAVERA,[1] T.S. PAPAS,[1] J.A. LAUTENBERGER,[1] A. EVA,[1] P. REDDY,[1] S.R. TRONICK,[1] M.A. BALUDA,[2] S.A. AARONSON,[1] and R.C. GALLO,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Pathology, University of California Medical School, Los Angeles: Human hematopoietic cells express cellular homologs of retroviral *onc* genes.

SV40, Polyoma, and Adenoviruses

August 18–August 22

Arranged by **Terri Grodzicker**, Cold Spring Harbor Laboratory, **Michael Botchan**, University of California, Berkeley

378 participants

The annual meeting on the molecular biology of SV40, polyoma, and adenoviruses is now held in alternate years at the Cold Spring Harbor Laboratory. That over 350 scientists attended the 1982 meeting, attests to the active interest in the study of the molecular biology and transforming properties of small DNA tumor viruses. Areas of active research that were discussed include: development of in vitro systems for DNA replication and transcription; purification of viral and cellular proteins active in in vitro systems; characterization of sequences that enhance gene expression; viral regulatory proteins and RNAs involved in the control of transcription and translation; the role of different viral T antigens in transformation; and the purification and properties of transforming proteins. It was clear that the use of recombinant DNA technology, prokaryotic and viral expression vectors, and sets of monoclonal antibodies and peptides to be used as antigens have become routine and valuable tools in this area of research.

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

SESSION 1 *SV40, Polyoma, and Adenoviruses: Replication and Recombination*

Chairperson: D. NATHANS, John Hopkins University School of Medicine, Baltimore, Maryland

J.M. OSTROVE,[1] P. ROSENFELD,[1] J. WILLIAMS,[2] and T.J. KELLY, JR.,[1] [1]Dept. of Molecular Biology and Genetics, Johns Hopkins University Medical School, Baltimore, Maryland; [2]Dept. of Biological Sciences, Mellon Institute, Pittsburgh, Pennsylvania: In vitro complementation as an assay for the purification of adenovirus replication proteins.

B. FRIEFELD, M. KREVOLIN, J. LICHY, R. KORN, J. HURWITZ, and M. HORWITZ, Albert Einstein College of Medicine, Bronx, New York: Studies on adenovirus H5ts107 and H5ts149 DNA synthesis defective mutants in vitro.

F. TAMANOI, M.B. MATHEWS, and B.W. STILLMAN, Cold Spring Harbor Laboratory, New York: Enzymes and DNA sequence required for initiation of adenovirus DNA replication.

R.T. HAY, E.A. HENDRICKSON, and M.L. DEPAMPHILIS, Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Comparison of initiation events at the origin of SV40 DNA replication with Okazaki fragments.

G. MAGNUSSON, H. LUTHMAN, and M. ÖSTERLUND, Dept. of Biochemistry, Karolinska Institute, Stockholm, Sweden: Altered rate of DNA replication in *cis*-active mutants of polyoma virus.

L. DANDOLO, J. AGHION, and D. BLANGY, Institut de Recherches Scientifiques sur le Cancer, Villijuif, France: Replication of polyoma virus DNA in embryonal carcinoma cells.

P. Bourgaux, Y. Aloni

P. Sharp, T. Grodzicker

M. Botchan

E. WINOCOUR and D. DORSETT, Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: SV40 recombination in monkey cells.

M. RUBEN,[1] S. BACCHETTI,[1] and F.L. GRAHAM,[1,2] [1]Dept. of Pathology; [2]Dept of Biology, McMaster University, Hamilton, Canada: Evidence for head to tail joining of human Ad5 DNA molecules.

R. GAHLMANN, S. STABEL, R. DEURING, U. WINTERHOFF, and W. DOERFLER, Institute of Genetics, University of Cologne, Federal Republic of Germany: Integration sites of adenovirus DNA in transformed and tumor cells.

D. HUBERDEAU, B.S. SYLLA, D. BOURGAUX-RAMOISY, and P. BOURGAUX, Dept. de Microbiologie, Université de Sherbrooke, Canada: Excision of polyoma DNA in transformed mouse cells.

SESSION 2 *SV40 and Polyoma: Transcription*

Chairperson: R. TJIAN, University of California, Berkeley, California

E. RULEY and M. FRIED, Imperial Cancer Research Fund Laboratories, London, England: Duplicated regions of polyoma which enhance gene expression.

B. FARMERIE, S. TRIEZENBERG, and W. FOLK, Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Isolation of polyoma mutants with altered expression of the early region.

B. BYRNE, J. YAMAGUCHI, D. BERGSMA, and K. SUBRAMANIAN, Dept. of Microbiology and Immunology, University of Illinois Medical Center, Chicago: The SV40 promoter regions—a) location of the early promoter within the 21-bp repeat; and b) the 72-bp repeat exhibits a pronounced host range in its enhancement effect.

M. BOTCHAN, P. ROBBINS, M. LUSKY, L. BERG, and H. WEIHER, Dept. of Molecular Biology, University of California, Berkeley: Characterization of the SV40 enhancer/activator sequence and other related experiments.

J. DE VILLIERS,[1] C. TYNDALL,[2] L. OLSON,[1] R. KAMEN,[3] and W. SCHAFFNER,[1] [1]Institut für Molekularbiologie II der Universität Zürich, Switzerland; [2]Biochemistry Division, MRC, London; [3]Imperial Cancer Research Fund, London, England: A functional analysis of polyoma virus-SV40 hybrid recombinants.

S. LORD, J. SAFFER, M. LERMAN, and M. SINGER, NCI, National Institutes of Health, Bethesda, Maryland: Functional characterization of a monkey DNA segment homologous to the SV40 regulatory region.

J. BRADY, M. RADONOVICH, M. VODKIN, V. NATARAJAN, M. THOREN, G. DAS, and N.P. SALZMAN, NIAID, National Institutes of Health, Bethesda, Maryland: Site specific mutations which enhance or suppress transcription of the SV40 major late RNA.

J.C. ALWINE, Dept. of Microbiology, University of Pennsylvania, Philadelphia: SV40 late transcriptional control.

Y. ALONI, N. HAY, H. SKOLNIK-DAVID, R. PRUZAN, and P. PFEIFFER, Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Attenuation in the control of SV40 gene expression.

A. BARKAN, S. SEDMAN, and J.E. MERTZ, MCARDLE Laboratory for Cancer Research, University of Wisconsin, Madison: Effect of alternative leader region sequences on the relative rates of translation of genes encoded by the SV40 late mRNAs.

M. GRAESSMANN and A. GRAESSMANN, Institut für Molekularbiologie und Biochemie der Freien Universitat Berlin, Federal Republic of Germany: SV40 cRNA is processed into functional mRNA in microinjected cells.

SESSION 3 *Poster Session: Adenoviruses*

P. ALESTRÖM, G. AKUSJÄRVI, M. PETTERSSON, and U. PETTERSSON, Dept. of Medical Genetics, University of Uppsala, Sweden: The DNA sequence of the region encoding the terminal protein and the hypothetical N-gene of Ad2.

K.P. ANDERSON and D.F KLESSIG, Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: Ad2 fiber message isolated from abortively infected monkey cells is translatable in vitro.

G. ANTOINE, T. DÖRPER, R. SCHILLING, and E.-L. WINNACKER, Dept of Biochemistry, University of Munich, Federal Republic of Germany: Defective expression of mouse adenovirus strain FI in human cells.

F.A.M. ASSELBERGS, M.B. MATHEWS, and J.E. SMART, Cold Spring Harbor Laboratory, New York: Functional domains and complexity of the adenovirus early region 2A proteins.

A. BABICH and J.R. NEVINS, Rockefeller University, New York, New York: Control of mRNA stability by the Ad-5 72K DNA binding protein and evidence for its presence in mRNP.

K.L. BERKNER and P.A. SHARP, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Adenovirus as a mammalian cloning vector.

P.E. BRANTON,[1] S.-P. YEE,[1] and D.T. ROWE,[2] [1]Dept. of Pathology; [2]Dept of Biology, McMaster University, Hamilton, Canada: Identification of E1 polypeptides of Ad5 using a serum directed against the predicted carboxy termini.

K.-C. CHOW, R.E. ENNS, K.G. AHERN, C.Z. MATHEWS, and G.D. PEARSON, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Mapping the adenovirus origin.

B.M.M. DEKKER and J. VAN ORMONDT, Dept. of Medical Biochemistry, State University of Leiden, The Netherlands: Structure and organization of the Ad5 genome between map positions 17 and 31.5 (fragment *Hind*-C).

G.J. DUIGOU and S.G. ZIMMER, Dept. of Pathology, University of Kentucky Medical Center, Lexington: Recombination of superinfecting Ad5 DNA with the integrated Ad2 DNA sequences of a rat cell genome.

F. EGGERDING and W. PIERCE, Dept. of Pathology, University of California, Los Angeles: Construction of a cloned library of restriction enzyme fragments from the Ad2 genome utilizing bacteriophage M13.

H. ESCHE, [1] B. SIEGMANN,[1] and E. BAUSE,[2] [1]Institute of Genetics, University of Cologne; [2]Dept. of Biochemistry, University of Cologne, Federal Republic of Germany: Antibodies to chemically synthesized peptides localized on the amino-and carboxy-terminal regions of Ad12 tumor antigens.

L. FELDMAN and J. NEVINS, Rockefeller University, New York, New York: Antisera to adenovirus E1A proteins.

T.R. GINGERAS, D. SCIAKY, R.E. GELINAS, B.-D. JIANG, C.E. YEN, M.M. KELLY, P.A BULLOCK, B.L. PARSONS, K.E. O'NEILL, and R.J. ROBERTS, Cold Spring Harbor Laboratory, New York: Sequences from the Ad2 genome.

C.R. GODING and W.C. RUSSELL, National Institute for Medical Research, London, England: Characteristics of adenovirus replication in vitro.

R.A. GUILFOYLE, W. OSHEROFF, and M. ROSSINI, Cold Spring Harbor Laboratory, New York: Analysis of the regulatory interaction between early regions 1A and 2A.

D.N. HALBERT and T.E. SHENK, Dept. of Microbiology, State University of New York, Stony Brook: Analysis of deletion and insertion mutations within early region 4 of Ad5.

P. HEARING and T. SHENK, Dept. of Microbiology, State University of New York, Stony Brook: Mutational analysis of Ad5 E1A transcriptional control region.

D.R. HURWITZ and G. CHINNADURAI, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Expression of Ad2 early gene region E1A lacking the intervening sequences for the 13S mRNA.

M.J. IMPERIALE and J.R. NEVINS, Rockefeller University, New York, New York: Definition of transcriptional control sequences of early adenovirus genes.

I. KRUCZEK and W. DOERFLER, Institute of Genetics, University of Cologne, Federal Republic of Germany: Unmethylated DNA sequences in the promoter/leader and 5'-regions of integrated adenovirus genes correlate with gene expression.

H.J. LAANEN, B. ZELLE, J.S. SUSSENBACH, and T.H. ROZIJN, Laboratory for Physiological Chemistry, University of Utrecht, The Netherlands: Integration of adenovirus sequences in human cells.

F.A. LASKI,[1] A. FIRE,[1] R. BELAGAJE,[2] U. RAJBHANDARY,[1] and P.A. SHARP,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Lilly Research Laboratories, Indianapolis, Indiana: Mutagenesis of a tRNA gene to an amber suppressor.

J.B. LEWIS[1] and C.W. ANDERSON,[2] [1]Molecular Biology Program, Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Dept. of Biology, Brookhaven National Laboratory, Upton, New York: The Ad2 "i-leader" encodes a 13.6K protein.

P. MALETTE, S.-P. YEE, and P.E. BRANTON, Dept. of Pathology, McMaster University, Hamilton, Canada: Phosphorylation of the 58K E1B antigen of Ad5.

V. MAUTNER, MRC Institute of Virology, Glasgow, Scotland: Recombination in adenovirus—Construction and analysis of interserotypic recombinants with crossing-over sites in a defined region of the genome.

E.A. OOSTEROM-DRAGON and C.W. ANDERSON, Dept of Biology, Brookhaven National Laboratory, Upton, New York: Polypeptide structure and encoding location of the Ad2 late, non-structural, phosphoprotein—33K.

T.F. OSBORNE, D. ROSSER, and A.J. BERK, Molecular Biology Institute, University of California, Los Angeles: Upstream adenovirus DNA sequence required for E1A transcription in vivo.

J. OVERHAUSER and N.C. JONES, Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Splicing mutants in the transformation region of Ad5.

J. PÜNTER,[1] E. LESCHINSKI,[1] and E.-L. WINNACKER,[2] [1]Dept. of Biochemistry, Hoechst A. G., Pharma Research,

Frankfurt; [2]Dept. of Biochemistry, University of Munich, Federal Republic of Germany: Monoclonal antibodies against proteins from adenovirus type infected rat cells.

R.L. RADNA,[1] L.A. FELDMAN,[2] and H.L. OZER,[1] [1]Dept. of Biological Sciences, Hunter College, City University of New York, New York; [2]Dept. of Microbiology, College of Medicine and Dentistry, Newark, New Jersey: Restriction of Ad2 replication in CHO.

W.D. RICHARDSON[1] and H. WESTPHAL,[2] [1]Biochemistry Division, National Institute for Medical Research, London, England; [2]Laboratory of Molecular Genetics, NICHD, National Institutes of Health, Bethesda, Maryland: Regulatory interactions among adenovirus early genes and adeno-associated virus.

A.W.M. RIJNDERS and J.S. SUSSENBACH, Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands: Immunological analysis of Ad5 terminal protein.

J.A. ROSE and J.E. JANIK, NIAID, National Institutes of Health, Bethesda, Maryland: Adenovirus-associated virus polypeptide synthesis requires the adenovirus DNA-binding protein and VAI RNA.

M.L. SOPORI,[1] J.M. SHEIL,[1] G.J. DUIGOU,[2] and S.G. ZIMMER,[2] [1]Dept. of Medical Microbiology Immunology; [2]Dept. of Pathology, University of Kentucky Medical Center, Lexington: Susceptibility of Ad2 transformed rat cell lines to natural killer cells—Lack of correlation between natural cytotoxicity and tumorigenesis.

D.J. SPECTOR and M.J. TEVETHIA, Dept. of Microbiology, College of Medicine, Pennsylvania State University, Hershey: Transformation of mouse embryo fibroblasts by DNA from adenovirus transformed human cell line 293.

C. SVENSSON, U. PETTERSSON, and G. AKUSJÄRVI, Dept. of Microbiology, University of Uppsala, Sweden: Experimental evidence that RNA splicing proceeds over intermediate stages.

H.E. TAKIFF and S.E. STRAUS, NIAID, National Institutes of Health, Bethesda, Maryland: Enteric adenovirus (EAd) DNAs—Cloning, mapping, and homology studies.

M. TOTH and J. WEBER, Dépt. de Microbiologie, Faculté de médecine, Centre Hospitalier Universitaire, Sherbrooke, Canada: Nuclease sensitivity of Ad2 chromatin.

M. TREMBLAY, C.V. DÉRY, and J. WEBER, Dépt. de Microbiologie, Centre Hospitalier Universitaire de Sherbrooke, Canada: In vitro cleavage specificity of the Ad2 protease.

B.G.M. VAN BERGEN, P.A. DER LEIJ, W. VAN DRIEL, and P.C. VAN DER VLIET, Laboratory for Physiological Chemistry, University of Utrecht, The Netherlands: Replication of adenovirus DNA and various plasmids in vitro using nuclear extract of Ad5, H5ts125 and H5ts36.

M.P. VAN BREE and J.A. ENGLER, Cold Spring Harbor Laboratory, New York: Comparable regulatory signals encoded in DNAs from different classes of adenoviruses are usually conserved.

P. VAN DEN ELSEN, A. HOUWELING, and A. VAN DER EB, Dept. of Medical Biochemistry, State University of Leiden, The Netherlands: Studies on the role of region E1b of Ad5 in cell transformation.

L. VARDIMON, D. RENZ, and W. DOERFLER, Institute of Genetics, University of Cologne, Federal Republic of Germany: Methylation of specific DNA sequences inhibits expression of a viral gene.

M.E. VAYDA, K. LEONG, and S.J. FLINT, Dept. of Biochemical Sciences, Princeton University, New Jersey: The structure and transcription in vitro of nucleoprotein cores isolated from adenovirions.

A. VIRTANEN, P. ALESTRÖM, N. BALGOBIN, J. CHATTOPADHYAYA, and U. PETTERSSON, Depts. of Medical Genetics and Microbiology, University of Uppsala, Sweden: Analysis of adenovirus 5′termini by reverse transcription.

R.J. VOSATKA, A.R. SHAW, and E.B. ZIFF, Dept. of Biochemistry, New York University Medical Center, New York: Ad2 chromatin and transcriptional control.

K. YOSHIDA and K. FUJINAGA, Dept of Molecular Biology, Cancer Research Institute, Sapporo Medical College, Japan: Structure and expression of integrated viral DNA in a rat cell line transformed by the leftmost 7.8% fragment of Ad7 DNA.

SESSION 4 *Adenoviruses: Transcription*

Chairperson: D. KLESSIG, University of Utah Medical Center, Salt Lake City, Utah

P. SASSONE-CORSI, J. CORDEN, R.HEN, R. ELKAIM, T. LEFF, C. KÉDINGER, and P. CHAMBON, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS Unité 184 de Biologie Moléculaire et de Génie Génétique de INSERM, Faculté de Médecine, Strasbourg, France: Sequence elements controlling the transcription of adenovirus protein coding genes.

M. SAMUELS, A. FIRE, and P.A. SHARP, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Protein fractions required for transcription of adenovirus genes in vitro.

T. BURGESS, T. CREFELD, N. HERNANDEZ, K. SASS, D. SOLNICK, B. WEINGÄRTNER, and W. KELLER, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Transcription and processing of mRNAs in vitro.

C. AHMED, R. CHANDA, R. WATKINS, and S. ZAIN, Microbiology and Cancer Center, University of Rochester Medical Center, New York: Transcription and RNA processing of mRNA in vivo and in vitro from early gene block III of Ad2.

C. MONTELL,[1] E. FISHER,[2] M. CARUTHERS,[2] and A. BERK,[1] [1]Molecular Biology Institute, University of California, Los Angeles; [2]Dept. of Chemistry, University of Colorado, Boulder: Site specific mutagenesis into adenovirus mRNA processing signals.

J.S. LOGAN, P.D. SOLOWAY, and T. SHENK, Dept. of Microbiology, State University of New York, Stony Brook: Sequences involved in the control of the production and translation of late mRNA in adenovirus infected cells.

G. AKUSJÄRVI and C. SVENSSON, Dept. of Microbiology, University of Uppsala, Sweden: Cloning of the complex arrangement of 5'-leaders attached to the fiber mRNA of Ad2.

G.A. FREYER, Y. KATOH, and R.J. ROBERTS, Cold Spring Harbor Laboratory, New York: Characterization of the mature transcripts from region E4 of Ad2.

M.G. KATZE, H. PERSSON, and L. PHILIPSON, Dept. of Microbiology, University of Uppsala, Sweden: A leftward reading transcript from the transforming region of adenovirus DNA encoding a low molecular weight polypeptide.

A.M. FRANCOEUR and M.B. MATHEWS, Cold Spring Harbor Laboratory, New York: Formation of ribonucleoprotein particle containing adenovirus VA RNAs and the lupus antigen, La.

J. MANLEY, D. GRASS, Y.-T. YU, and D. LEWIS, Dept. of Biological Sciences, Columbia University, New York, New York: Properties of some adenovirus-SV40 hybrids created in vitro.

SESSION 5 *Adenoviruses: Regulation of Gene Expression*

Chairperson: T. SHENK, State University of New York, Medical School, Stony Brook, New York

M.J. IMPERIALE, L.T. FELDMAN, H.-T. KAO, and J.R. NEVINS, Rockefeller University, New York, New York: Mechanism of induction of early adenovirus transcription by the E1A protein.

D.L. WEEKS and N.C. JONES, Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: The effect of the E1a gene product on early viral promotors.

J.L. BOS, A.-G. JOCHEMSEN, H. TEN WOLDE, H. VAN ORMONDT, and A.J. VAN DER EB, Dept. of Medical Biochemistry, State University of Leiden, The Netherlands: Expression of an Ad12 E1B-hsv tk fusion gene is controlled by adenovirus region E1a.

C. WEINBERGER, R. SCHNEIDER, B. THIMMAPPAYA, and T. SHENK, Dept. of Microbiology, State University of New York, Stony Brook: Adenovirus VAI RNA is required for efficient translation of viral late mRNAs.

R.A. BHAT and B. THIMMAPPAYA, Dept. of Microbiology and Immunology, Northwestern University Medical School, Chicago, Illinois: Construction and in vivo analysis of deletion and substitution mutations of VA RNA genes of Ad5.

C. THUMMEL,[1] R. TJIAN,[1] S.-L. HU,[2] and T. GRODZICKER,[2] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Cold Spring Harbor Laboratory, New York: Regulatory sequences required for efficient expression of SV40 T antigen from the adenovirus late promoter.

B.D. KARGER,[1] Y.-S. HO,[1] C.L. CASSTIGLIA,[2] S.J. FLINT,[2] and J. WILLIAMS,[1] [1]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania; [2]Dept. of Biochemical Sciences, Princeton University, New Jersey: An adenoviral E1B gene product is involved in control of the early-late switch in lytic infection of HeLa cells.

G. KETNER, Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: An adenovirus mutant defective in the accumulation of translatable late mRNA.

S. RICE, R. BOONE, and D.F. KLESSIG, Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: Genetic and biochemical characterization of the adenovirus 72Kd DNA-binding protein.

L.FELDMAN, A. BABICH, and J. DARNELL, Rockefeller University, New York, New York: Effects of adenovirus infection on host cell RNA metabolism.

SESSION 6A *Poster Session: Papovaviruses. I.*

A. BARKAN and J.E. MERTZ, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Mutations in the SV40 VP-1 gene can partially compensate for deletions in the late leader region.

S. BEECHER, V. BLASQUEZ, and M. BINA, Dept. of Chemistry, Purdue University, West Lafayette, Indiana: A topoisomerase activity copurifies with SV40 virions.

A. BEN-ZE'EV, R. ABULAFIA, and Y. ALONI, Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: SV40 virions and viral RNA metabolism are associated with cellular substructures.

S.S. CHEN, [1] and M.T. HSU. [2] [1]Shanghai Second Medical College, China; [2]Dept. of Molecular Cell Biology, Rockefeller University, New York, New York: Enrichment of SV40 active chromatin by differential precipitation with Mg^{+2}.

L.T. CHOW and T.R. BROKER, Cold Spring Harbor Laboratory, New York: Transcription of human papilloma virus-1 from the SV40 early and late promotors in hybrid shuttle vectors transfected into COS-1 cells.

C. CRÊMISI, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Undifferentiated murine teratocarcinoma cells (EC cells) treated by 5-azacytidine become sensitive to polyoma virus and SV40.

H.J. EDENBERG, Dept. of Biochemistry, Indiana University Medical School, Indianapolis: Ultraviolet light inhibits SV40 DNA replication.

M.S. FEATHERSTONE, M.A. NAUJOKAS, B.J. POMERANTZ, C.R. MUELLER, and J.A. HASSELL, Dept of Microbiology and immunology, McGill University, Montreal, Canada: Construction of a plasmid vehicle suitable for the molecular cloning and characterization of mammalian promoters.

M.M. FLUCK, Dept. of Microbiology, Michigan State University, East Lansing: Recombination between unintegrated viral genomes—A potentially essential step in transformation by polyoma virus.

A. FRADIN, T. MICHAELI, R. JOVE, D. GRASS, C. PRIVES, and J. MANLEY, Dept. of Biological Sciences, Columbia University, New York, New York: The Influence of DNA structure on SV40 gene expression in *Xenopus Laevis* oocytes.

T. GURNEY, D. SORENSON, E. GURNEY, and N. WILLS, Dept. of Biology, University of Utah, Salt Lake City: A new method for rapid isolation of rare SV40 RNA species.

G.Z. HERTZ, L.A. TRIMBLE, A. BARKAN, M. SOMASEKHAR, and J.E. MERTZ, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Sequences involved in the promotion and initiation of late strand transcription.

M. HOROWITZ, R.J. KAUFMAN, and P.A. SHARP, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Expression of the mouse dihydrofolate reductase cDNA cloned in the early region of SV40.

E.B. JAKOBOVITS, R. ABULAFIA, and Y. ALONI, Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Temperature sensitive B mutants of SV40 de-assemble intracellular encapsidation particles at elevated temperature.

S.R. JASKUNAS, S.R. KING, and S.L. MARVO, Dept. of Chemistry, Indiana University, Bloomington: The role of DNA gyrase in catalyzing illegitimate recombinations.

M. LANGE and E. MAY, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Study of DNA sequences required for transcription of SV40 late genes.

S.G. LAZAROWITZ and S. LEE, Dept of Molecular Biology and Genetics, Johns Hopkins University Medical School, Baltimore, Maryland: Mutations in the control region of SV40 that affect late gene expression.

D. LYCAN and K.J. DANNA, Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Characterization of the 5′ termini of purified nascent SV40 late transcripts.

G. MAGNUSSON and S.V. NILSSON, Dept. of Biochemistry, Karolinska Institute, Stockholm, Sweden: Polyoma splice point mutants produce a restricted set of T-antigens.

H. MANOR, N. BARAN, and A. NEER, Dept. of Biology, Technion-Israel Institute of Technology, Haifa: "Onion skin" replication of chromosome-associated polyoma virus DNA and flanking sequences in polyoma-transformed rat cells—Identification of a cellular termination site for replication.

B. MILAVETZ, and T. HOPKINS, Cancer Research Laboratory, University of Western Ontario, Canada: Identification of an early SV40 encapsidation intermediate.

C.R. MUELLER, A.-M. MES, B.J. POMERANTZ, M.A. NAUJOKAS, M. FEATHERSTONE, and J.L. HASSELL, Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: The structure of the polyoma virus early promoter—Sequences required for expression and T-antigen binding.

W.J. MULLER, M.A. NAUJOKAS, C.S. MUELLER, A.-M. MES, M. FEATHERSTONE, and J.A. HASSELL, Dept. of Microbiology and Immunology, McGill University, Quebec, Canada: *Cis*-acting sequences required for the replication of polyoma virus DNA.

S.-C. NG, S. BEECHER, and M. BINA, Dept. of Chemistry, Purdue University, West Lafayatte, Indiana: Chromatin structure of the SV40 assembly defective mutant, tsBC216.

F. O'NEILL, [1,2]E. MARYON, [1]and T. MILLER, [2] [1]Dept. of Cellular, Viral, and Molecular Biology, University of Utah; [2]Veterans Administration Medical Center, Salt Lake City, Utah: Production of latent infections in green monkey cells with SV40 containing a bipartite genome.

A. PATER, M. PATER, L.S. CHANG, and G. DI MAYORCA, Dept. of Microbiology, University of Medicine and Dentistry of New Jersey, Newark: The genome of RF consists of two complementary defective molecules.

B.J. POMERANTZ, W.J. MULLER, A.-M. MES, M.A. NAUJOKAS, and J.A. HASSELL, Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Homologous recombination between cotransfected polyoma virus-plasmid genomes.

M. RABIN,[1] O.C. UHLENBECK,[1] D.M. STEFFENSEN,[2] and W.F. MANGEL,[1] [1]Dept. of Biochemistry; [2]Dept. of Genetics and Development, University of Illinois, Urbana: Chromosomal sites of integration of SV40 DNA sequences mapped in in situ hybridization are identical in two independently transformed hybrid cell lines.

K. RUDOLPH and K. MANN, Dept. of Biology, University of Alaska, Anchorage: Effect of salt on the association of SV40 T antigen with SV40 nucleoprotein complexes.

P. SHAW, and W. KELLER, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Chimaeric mRNAs in SV40-transformed cells.

C.C. SIMONSEN, H.M. SHEPARD, A.D. LEVINSON, and D.V. GOEDDEL, Dept. of Molecular Biology, Genentech, Inc., South San Francisco, California: Synthesis of human immune interferon in cultured monkey cells using SV40-pBR322 hybrid plasmids.

J.R. STRINGER and Y. GLUZMAN, Cold Spring Harbor Laboratory, New York: Exision of SV40 from a specialized Ad-SV40 hybrid virus.

K.N. SUBRAMANIAN, Dept. of Microbiology and Immunology, University of Illinois Medical Center, Chicago: Effect of in vitro methylation at CpG sites on gene expression in a papovavirus genome functioning autonomously in a vertebrate host.

G. SWIMMER and T. SHENK, Dept. of Microbiology, State University of New York, Stony Brook: Mutational events which restore poly A addition sites to SV40 variants whose late transcription unit lacks the wild-type signal.

A. VARSHAVSKY,[1] E. OZKAYNAK,[1] and O. SUNDIN.[2] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Cold Spring Harbor Laboratory, New York: Multiply intertwined catenated SV40 dimers as segregation intermediates—Formation, processing, and chromatin structure.

M.A. WAGAR, B.M. SAHAI, L.R. DAVIS, D. KOWALSKI, and J.A. HUBERMAN, Dept. of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: Association of exonuclease and topoisomerase II activities with SV40 chromosomes.

M. ZANNIS–HADJOPOULOS, A.B. CHEPELINSKY, and R.G. MARTIN, NIADDK, National Institutes of Health, Bethesda, Maryland: Mapping of the 3'-ends of SV40 nascent strands.

SESSION 6B *Poster Session: Papovaviruses. II.*

S. BENCHIMOL and L. CRAWFORD, Imperial Cancer Research Fund Laboratories, London, England: A novel radioimmunoassay to measure p53 levels in SV40-transformed and other rodent cells.

G. BLANCK,[1] A. LEVITT,[1] S. CHEN,[1] R. POLLACK,[1] and S. WEISSMAN,[2] [1]Dept. of Biological Sciences, Columbia University, New York, New York; [2]Yale University Medical School, New Haven, Connecticut: Integration and loss of defective viral DNA in SV40 mouse transformants.

K. BLASHKA, A. PATER, M. PATER, and G. DI MAYORCA, Dept. of Microbiology, University of Medicine and Dentistry of New Jersey, Newark: Cloning of BKV DNA sequences from a carcinoma of kidney and a carcinoid tumor.

C. BURGER, A. DORN, D. BRAUER, R. KNIPPERS, and E. FANNING, Faculty for Biology, University of Konstanz, Federal Republic of Germany: DNA binding properties of subclasses of large T antigen from SV40-infected and -transformed cells.

B. CARROLL,[1] E.G. GURNEY,[2] and J.A. MELERO,[1] [1]Dept. of Pathology, New York University Medical School, New York; [2]Dept. of Biology, University of Utah, Salt Lake City: Studies of the time-dependent maturation of the SV40 large T Ag•p53 complex using monoclonal antibodies.

A.E. CAMPBELL and S.S. TEVETHIA, Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Evidence for multiple antigenic sites of the SV40 TSTA using cytotoxic T lymphocyte clones.

K. CHANDRASEKARAN,[1] D.T. SIMMONS,[2] and P.T. MORA,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]School of Life and Health Sciences, University of Delaware, Newark: Selection of SV40 T antigen negative "mutants" and the cellular p53; also embryogenesis, embryonal carcinoma cell differentiation and the cellular p53.

S. CHEN and R. POLLACK, Dept. of Biological Sciences, Columbia University, New York, New York: Linkage of expression of host 54K protein and susceptibility to SV40 transformation in inbred strains of mice.

R. CLARK,[1] K. PEDEN,[2] J.M. PIPAS,[3] D. NATHANS,[2] and R. TJIAN,[1] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Dept. of Molecular Biology and Genetics, Johns Hopkins Medical School, Baltimore, Maryland; [3]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Biochemical analysis of mutant SV40 T antigens.

P. CLERTANT and F. CUZIN, Centre de Biochimie du CNRS, Université de Nice, France: Characterization of the nucleotide binding site evidenced by covalent affinity labeling with oxidized-ATP on the large-T proteins of polyoma and SV40 viruses.

L. COVEY, A. SCHELLER, and C. PRIVES, Dept. of Biological Sciences, Columbia University, New York, New York: The different forms and functions of SV40 large T antigen.

J. FEUNTEUN, F. PECCEU, and B. GRIMA, Institut de Recherches Scientifique sur le Cancer, Villejuif, France: Analysis of immortalization by SV40.

L. FISCHER FANTUZZI[1] and C. VESCO,[2] [1]Centro Studi Acidi Nucleici del CNR; [2]Istituto di Biologia Cellulare del CNR, Roma, Italy: Cold-sensitive growth of SV40 in semipermissive variants of CV1 cells.

R.E. GILES, F. BOYCE, and W.W. BROCKMAN, Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Evaluation of the mutagenic effects of SV40 in mouse, hamster, human and mouse X human hybrid cells.

J. GOUDSMIT, J. TER SCHEGGET, A. VAN STRIEN, and J. VAN DER NOORDAA, Laboratorium voor de Gezondheidsleer, University of Amsterdam, The Netherlands: Primary BKV infections associated with acute respiratory disease and characterization of a BKV isolate (Dik) from a child with tonsillitis.

D. GRASS, S. CHEN, G. BLANCK, N. NICHOLSON, A. SCHELLER, C. PRIVES, R. POLLACK, and J. MANLEY, Dept. of Biological Sciences, Columbia University, New York, New York: Lytic and transforming properties of a novel origin-minus SV40 mutant.

A. GRAESSMANN and M. GRAESSMANN, Institut für Molekularbiologie und Biochemie der Freien Universitaet Berlin, Federal Republic of Germany: Transformation capacity of early SV40 DNA fragments.

Y. ITO,[1] E. APPELLA,[2] K. SEGAWA,[1] and Y. HAMAGISHI,[1] [1]NIAID; [2]NCI, National Institutes of Health, Bethesda, Maryland: Antiserum against a synthetic polypeptide representing a part of the unique region of polyoma virus middle T antigen which inhibits middle T antigen phosphorylating activity.

D. KALDERON, B. OOSTRA, and A.E. SMITH, Biochemistry Division, National Institute for Medical Research, London, England: Site-specific mutagenesis to produce deletion and point mutants in a putative DNA domain of SV40 large-T.

D.P. LANE and J. GANNON, Dept. of Biochemistry, Imperial College of Science and Technology, London, England: Radioimmunometric assay of human p53—Application to the quantitation, characterization, and purification of human p53 proteins.

E.O. MAJOR,[1] [2] and T. KOKJOHN,[2] [1]NINCDS, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Microbiology, Loyola University Medical School, Maywood, Illinois: Expression of human papovaviruses in experimental cell substrates.

L.H. MALKAS,[1] E. BARIL,[2] and H.L. OZER,[1] [1]Hunter College, City University of New York, New York; [2]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Induction of enzymes of nucleotide metabolism by SV40 in quiescent mouse cells temperature sensitive for DNA synthesis.

N. MILLER, W. WALLEN, and W. LONDON, NIAID, National Institutes of Health, Bethesda, Maryland: Brain tumors produced in owl monkeys by JC virus contain integrated JCV DNA.

M. MONTENARH and R. HENNING, Dept. of Biochemistry, University of Ulm, Federal Republic of Germany: SV40 large T antigen is oligomerized in vivo via divalent cations.

M. OREN[1] and A.J. LEVINE,[2] [1]Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel; [2]Dept. of Microbiology, State University of New York, Stony Brook: Molecular cloning of cDNA specific for the murine p53 cellular tumor antigen.

N.C. REICH, R. THOMAS, and A.J. LEVINE, Dept. of Microbiology, State University of New York, Stony Brook: The regulation of a cellular protein, P53, in nontransformed and transformed cells.

K. RUNDELL, Dept. of Microbiology and Immunology, Northwestern University, Chicago, Illinois: Effects of MGBG and some mitochondrial ionophores on SV40 small t-antigen functions.

A.H. SAMAD,[1] W.N. SCHMIDT,[2] L.S. HNILICA,[2] J.E. HARPER,[1] A.S. BLUM,[1] R.S. MANN,[1] S.I. KENNETT,[1] and R.B. CARROLL,[1] [1]Dept. of Pathology, New York University Medical School, New York; [2]Dept. of Biochemistry, Vanderbilt University Medical School, Nashville, Tennessee: SV40 T antigen, the host p53, Novikoff hepatoma p39, p49, and p56 antigens, and cytokeratins cross-react with purified lamins (M, 60K, 67K, and 70K, nuclear matrix proteins).

B. SCHAFFHAUSEN,[1] G. CARMICHAEL,[2] T. ROBERTS,[3] H. DORAI,[1] and T. BENJAMIN,[1] [1]Dept. of Pathology, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Microbiology, University of Connecticut, Farmington; [3]Sidney Farber Cancer Institute, Boston, Massachusetts: Studies on polyoma virus middle T antigen.

K.H. SCHEIDTMANN, B. ECHLE, and G. WALTER, Institut für Immunbiologie, Freiburg, Federal Republic of Germany: Mapping of phosphorylation sites of SV40 large T antigen.

M.K. SCHMITT and K. MANN, Dept. of Biology, University of Alaska, Anchorage: Modification of SV40 T antigen by glycosylation.

K. SEGAWA,[1] D. COSMAN,[2] T. KANDA,[1] G. KHOURY,[2] and K.K. TAKEMOTO,[1] [1]NIAID; [2]NCI, National Institutes of Health, Bethesda, Maryland: Identification of B-lymphotropic papovavirus-coded proteins.

R. SEIF, Laboratoire d'Enzymologie du CRNS, Gif-sur-Yvette, France: Rat cells transformed by SV40 give rise to tumor cells that contain no viral proteins and often no detectable viral DNA.

A.E. SMITH, M. GALLEGO, E. PAUCHA, R. HARVEY, N. LINDSAY, R. FAULKES, and P. GILLETT, Biochemistry Division, National Institute for Medical Research, London, England: Conventional and monoclonal antibodies against peptides from SV40 large-T and small-t and polyoma virus middle-T.

E. SOEDA, Y. MAKI, Y. NAKANO, and H. TASHIRO, National Institute of Genetics, Misima, Japan: Transformation with the DNA fragments encompassing the polyoma virus promoter.

M. STAL'FENBIEL and W. DEPPERT, University of Ulm, Federal Republic of Germany: Nuclear subclasses of SV40 T-antigen.

D. STEDMAN and R. HAND, McGill Cancer Centre, Montreal, Canada: Enzymatic activities of the multiple forms of D2 T antigen.

V. STEINBERG and L. NORKIN, Dept. of Microbiology, University of Massachusetts, Amherst: State of the viral genomes in Rhesus monkey kidney, human neuroblastoma, and human glioblastoma cell lines persistently infected with SV40 or BKV.

J. TER SCHEGGET, C.J.A. SOL, E. WOUTERS, and J. VAN DER NOORDAA, Laboratorium voor de Gezondheidsleer, University of Amsterdam, The Netherlands: Comparison of the genomes of two BKV variants BKV (JL) and BKV (Dik) with prototype BKV.

J. WALSH,[1] J. OELTGEN,[1] S. ZIMMER,[2] and M. PERDUE,[3] [1]Dept. of Neurosurgery, Lexington VA Hospital, University of Kentucky; [2]Dept. of Pathology and [3]Dept. of Medical Microbiology and Immunology, University of Kentucky, Lexington: Determinants of histological type and degree of invasiveness in experimentally produced primary intracranial tumors.

G. WALTER, M. HARDUNG, and K.-H. SCHEIDTMANN, Institute for Immunebiology, University of Freiburg, Federal Republic of Germany: Relationship between DNA-binding, age, oligomerization, and phosphorylation state of SV40 large T antigen.

SESSION 7 *SV40, Polyoma, and Adenovirus: Transformation*

Chairperson: P. RIGBY, Imperial College, London, England

C. GÉLINAS, C. ASSELIN, and M. BASTIN, Dept. of Microbiology, University of Sherbrooke, Canada: Mutant of polyoma virus with normal transforming ability but impaired tumorigenic potential.

H. Ozer, B. Stillman, P. Sharp, E. Winocour

M. Horowitz, A. Levine

D. DORSKY and T. BENJAMIN, Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Host cell alterations induced by polyoma small t-antigen.

M. RASSOULZADEGAN,[1] A. COWIE,[2] A. CARR,[2] Z. NAGHASHFAR,[1] N. GLAICHENHAUS,[1] R. TREISMAN,[2] J. FAVALORO,[2] R. KAMEN,[2] and F. CUZIN,[1] [1]Centre de Biochimie du CNRS, Université de Nice, France; [2]Transcription Laboratory, Imperial Cancer Research Fund Laboratories, London, England: Separate and complementary roles for polyoma virus early proteins in the alteration of cell growth requirements leading to transformation.

L.-S. CHANG, M.M. PATER, and G. DI MAYORCA, Dept. of Microbiology, University of Medicine and Dentistry of New Jersey, Newark: SV40 small t can enhance large T to transform BHK cells to anchorage independence.

O. SUNDIN and Y. GLUZMAN, Cold Spring Harbor Laboratory, New York: Stable transformation of monkey cells by genes expressing temperature sensitive SV40 T antigen.

K.-H. CHOI,[1] S. TEVETHIA,[2] and S. SHIN,[1] [1]Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York; [2]Dept. of Microbiology, Pennsylvania University College of Medicine: Tumor rejection of SV40- transformed human cells by nude mice.

E.H. BROWN, F. LABELLA, and C. BASILICO, Dept. of Pathology, New York University School of Medicine, New York: Induction of amplification of the DHFR gene in polyoma transformed rat cells.

M.R.D. SCOTT,[1] P.M. BRICKELL,[1] D.S. LATCHMAN,[1] D. MURPHY,[1] K.-H. WESTPHAL,[1] K. WILLISON,[2] and P.W.J. RIGBY,[1] [1]Dept. of Biochemistry, Imperial College of Science and Technology; [2]Chester Beatty Research Institute, London, England: The activation of cellular genes in SV40-transformed cells.

T. SCHUTZBANK, R. ROBINSON, and A.J. LEVINE, Dept. of Microbiology, State University of New York, Stony Brook: Cells transformed by a wide variety of agents express higher abundance levels of some cellular RNA species.

R. BERNARDS, M.J. VAESSEN, P.I. SCHRIER, and A.J. VAN DER EB, Dept. of Medical Biochemistry, State University of Leiden, The Netherlands: Localization of the oncogenic potential of human adenoviruses.

P.J. BYRD, J.L. WHITTAKER, K.W. BROWN, and P.H. GALLIMORE, Dept. of Cancer Studies, University of Birmingham Medical School, England: Transformation of human cells by cloned Ad12 E1.

SESSION 8 *SV40 and Polyoma: T Antigens. I.*

Chairperson: M. FRIED, Imperial Cancer Research Fund Laboratories, London, England

R. TJIAN, D. RIO, and M. LEARNED, Dept. of Biochemistry, University of California, Berkeley: Regulation of viral and host transcription by SV40 T antigen.

R.F. MARGOLSKEE, R.A.F. DIXON, and D. NATHANS, Dept. of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, Maryland: The binding of SV40 T antigen to DNA of viral regulatory mutants.

A. DELUCIA, B. LEWTON, K. RYDER, V. WILSON, and P. TEGTMEYER, Dept. of Microbiology, State University of New York, Stony Brook: The arrangement of SV40 A protein bound to the SV40 origin of replication.

D.G. TENEN,[1] R.G. MARTIN,[2] L.L. HAINES,[1] and T.S. TAYLOR,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massacusetts; [2]National Institutes of Health, Bethesda, Maryland: Origin specific DNA binding activity of SV40 T antigen (T) from lytically infected cells and comparison of its behavior with D2T antigen.

A. COWIE,[1] S. DILWORTH,[1] F. CHAUDRY,[2] R. KAMEN,[1] S. LUPTON,[1] C. TYNDALL,[2] G. VELDMAN,[1] and Z. ZHU,[1] [1]Imperial Cancer Research Fund; [2]National Institute for Medical Research, London, England: Two independent binding sites for the large-T protein in polyoma virus DNA.

A. SCHELLER and C. PRIVES, Dept. of Biological Sciences, Columbia University, New York, New York: Polyoma and SV40 T antigens bind to two discrete sites at both viral origins.

P. GAUDRAY, L. TREJO-AVILA, C. GALUP, and F. CUZIN, Centre de Biochimie du CNRS, Université de Nice, France: A high affinity binding site for polyoma virus large-T protein in the mouse genome.

J. PIPAS,[1] K. PEDEN,[2] K. SOPRANO,[3] N. GALANTI,[3] G. JONAK,[3] R. BASERGA,[3] and D. NATHANS,[2] [1]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; [2]Dept. of Molecular Biology and Genetics, Johns Hopkins University Medical School, Baltimore, Maryland; [3]Dept. of Pathology, Temple University Medical School, Philadelphia, Pennsylvania: Characterization of mutants with deletions in the gene for SV40 T antigen.

J. TORNOW and C.N. COLE, Dept. of Human Genetics, Yale University, New Haven, Connecticut: Intracistronic complementation in the A gene of SV40.

H. TÜRLER and C. SALOMON, Dept. of Molecular Biology, University of Geneva, Switzerland: Role of small and middle T-antigens in lytic and abortive infections by polyoma virus.

D. TEMPLETON[1] and W. ECKHART,[2] [1]University of California, San Diego; [2]Salk Institute, La Jolla, California: Cold-sensitive non-transforming mutants of polyoma virus.

SESSION 9 *SV40, Polyoma, and Adenoviruses: T Antigens. II.*

Chairperson: C. PRIVES, Columbia University, New York, New York

K. SEGAWA and Y. ITO, NIAID, National Institutes of Health, Bethesda, Maryland: Differential subcellular localization of in vivo phosphorylated and non-phosphorylated middle T antigen of polyoma virus and its relationship to middle T antigen phosphorylating activity in vitro.

B. ELY and A.E. SMITH, Biochemistry Division, National Institute for Medical Research, London, England: Temperature-sensitive mutants in the polyoma virus middle-T associated protein kinase.

G. WALTER,[1] M.A. HUTCHINSON,[2] T. HUNTER,[2] and W. ECKHART,[2] [1]Institut für Immunbiologie der Albert-Ludwigs Universität, Freiburg, Federal Republic of Germany; [2]Salk Institute, San Diego, California: Purification of the polyoma virus middle size t antigen by immune affinity chromatography.

S.M. DILWORTH,[1] M. GINSBURG,[1] B.E. GRIFFIN,[1] H.A. HANSON,[2] and G. BJURSELL,[3] [1]Imperial Cancer Research Fund, London, England; [2]Dept. of Histology; [3]Dept. of Medical Biochemistry, University of Gothenberg, Sweden: Investigation of the properties of polyoma T-antigens by monoclonal antibodies.

K. LEPPARD and L.V. CRAWFORD, Imperial Cancer Research Fund Laboratories, London, England: A new series of monoclonal antibodies specific for primate p53.

I. BIKEL, T. ROBERTS, R. GREEN, M.L. BLADON, and D. LIVINGSTON, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Extensive purification of biologically active SV40 small t antigen from an overproducing clone of *E. coli*

R. SEIF, Laboratoire d'Enzymologie du CNRS, Gif-sur-Yvette, France: SV40 large T releases a C-terminal peptide, binds to protease inhibitors and is similar to Rec A protein of *E. coli*.

D.T. ROWE and F.L. GRAHAM, Depts. of Biology and Pathology, McMaster University, Hamilton, Canada: Kinetics of synthesis of early viral proteins in KB cells infected with WT and HR mutants of human Ad5.

R. GAYNOR,[1] K. SPINDLER,[2] A. TSUKAMOTO,[2] F. PONTICELLI,[2] and A. BERK,[2] [1]Dept. of Medicine, School of Medicine; [2]Molecular Biology Institute, University of California, Los Angeles: Enhanced expression and analysis of phosphorylation of adenovirus E1A proteins.

P. SARNOW,[1] C.W. ANDERSON,[2] N.C. REICH,[1] and A.J. LEVINE,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Dept. of Biology, Brookhaven National Laboratory, Upton, New York; Identification and characterization of two adenovirus type 5 early region 4 polypeptides.

SESSION 10 *SV40 and Polyoma: Chromatin Replication, Organization and Virion Assembly*

Chairperson: M. DEPAMPHILIS, Harvard Medical School, Boston, Massachusetts

W. WALDECK, Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: SV40 chromatin associated topoisomerase recombines DNA in a homologous reaction.

K. MANN, Dept. of Biology, University of Alaska, Anchorage: Association of topoisomerase activity with SV40 nucleoprotein complexes.

M.E. CUSICK, M.L. DEPAMPHILIS, and P.M. WASSARMAN, Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Segregation and maturation of nucleosomes on replicating SV40 chromosomes

G.W. ROBINSON,[1] S.K. KONDOLEON,[2] and L.M. HALLICK,[2] [1]Dept. of Biochemistry; [2]Dept. of Microbiology and Immunology, Oregon Health Sciences University, Portland: Psoralen photoaddition to SV40—Virion "nucleosomes" differ from intracellular nucleosomes.

S.A. NEDOSPASOV,[1] A.N. SHAKHOV,[1] S.V. RASIN,[1] S.A. CHUVPILLO,[2] V.G. KOROBKO,[2] and G.P. GEORGIEV,[1] [1]Institute of Molecular Biology; [1]Shemyakin Institute of Bioorganic Chemistry, Moscow, USSR: Specific structure of SV40 minichromosomes.

E.B. JAKOBOVITS, S. BRATOSIN, and Y. ALONI, Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Formation of a nucleosome-free region in SV40 minichromosomes is dependent upon a restricted segment of DNA.

R.D. GERARD,[1] M. WOODWORTH-GUTAI,[2] and W.A. SCOTT,[1] [1]Dept. of Biochemistry, University of Miami

Medical School, Florida; [2]Dept. of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: Deletion mutants which affect the nuclease-sensitive site in SV40 chromatin.

W. Lin, M. Shyamala, and H. Kasamatsu, Dept. of Biology, University of California, Los Angeles: Biosynthesis of SV40 structural polypeptides. I. Amount of viral polypeptides synthesized and the identification of complexes of VP1, VP2, and VP3.

V. Blasquez, S. Beecher, and M. Bina, Dept. of Chemistry, Perdue University, West Lafayette, Indiana: SV40 morphogenesis—An analysis of assembly defective tsB and tsC mutants.

R. Garcea,[1,2] and T. Benjamin,[2] [1]Sidney Farber Cancer Institute; [2]Harvard Medical School, Boston, Massachusetts: Intermediate steps in polyoma virion assembly are controlled by the host-range transforming gene products.

Bacteriophage

August 24–August 29

Arranged by **Ahmad Bukhari**, Cold Spring Harbor Laboratory

139 participants

The bacteriophage meeting has been a tradition at Cold Spring Harbor since 1950. The meeting has been the focal point for discussions on the regulatory mechanisms in bacteriophages lambda and P22 and on the replication of various bacteriophages, particularly T4. Integrative recombination has also been featured prominently at the meeting, and in the last few years the integration of transposable genetic elements has been a major part of this discussion. Phage morphogenesis is another important topic that is discussed regularly at the meeting.

Work on bacterial control mechanisms has increasingly been a topic of discussion at the phage meeting. For example, at this year's meeting the Gross and Godson groups presented their work on the organization of genes encoding *rpsU* (the ribosomal protein S21 that seems to interact with the Shine-Dalgarno sequence), dnaG (the protein that primes Okazaki fragments), and rpoD (the sigma factor required for initiation of transcription). The three "initiation" genes apparently constitute one operon, and their regulation is connected with the cell cycle.

Many bacterial control mechanisms are now being studied in great detail. In view of this expanding domain of prokaryotic molecular biology, it was decided at the phage meeting this year that the future meetings will formally include work on bacterial systems. The name of the meeting in 1983 will be Phage and Bacterial Regulatory Mechanisms.

This year's meeting was supported in part by a grant from the National Cancer Institute, National Institutes of Health.

SESSION 1 *DNA Modification and Metabolism*

S. HATTMAN, Dept. of Biology, University of Rochester, New York: DNA- methylase-dependent transcription of the phage Mu *mom* gene.

M. GORADIA and A.I. BUKHARI, Cold Spring Harbor Laboratory, New York: How is the *mom* gene expression controlled?

W.P. FITZMAURICE, R.C. BENJAMIN, P.C. HUANG, and J.J. SCOCCA, Dept. of Biochemistry, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland: Phage DNA sequences that interact with the DNA receptor of competent *Haemophilus influenzae*.

K. CARLSON[1,2] and J. WIBERG,[1] [1]Dept. of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, New York; [2]Institute of Pharmaceutical Microbiology, University of Uppsala Biomedical Center, Sweden: In vivo degradation of cytosine-containing bacteriophage T4 DNA to genetically distinct, discrete-size fragments.

J.R. ALLEN, J.W. BOOTH, D.A. GOLDMAN, G.W. LASSER, R.G. SARGENT, and C.K. MATHEWS, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Regulation of the branched pyrimidine phosphate pathway in the dNTP-synthesizing multienzyme complex of T4.

K. Ebisuzaki

A. Bukhari, E. Ljunquist

R. Weisberg

SESSION 2 *Morphogenesis*

C. Escarmís, R.P. Mellado, and M. Salas, Centro de Biología Molecular, Universidad Autónoma, Madrid, Spain: Sequencing and cloning of the gene coding for the protein covalently bound to the 5′ends of the DNA of bacteriophage φ29.

H. Drexler, Dept. of Microbiology and Immunology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: A phage T1 preferred packaging site is located between genes *P* and *Q* in phage λ.

J. Kochan and H. Murialdo, Dept. of Medical Genetics, University of Toronto, Canada: Bacteriophage λ preconnector—An intermediate in λ prohead assembly.

E. Wyckoff and S. Casjens, Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City: Phage P22 scaffolding protein is autoregulatory.

R.-Z. Jiang, J. Geisselsoder, C. Diana, J. Sedivy, and R. Goldstein, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: In vitro construction of new P4 *sid*-type mutants based on analysis of the reiterated genome of P4 *sid1*.

P.B. Berget and M. Chidambaram, University of Texas Medical School, and Graduate School of Biomedical Sciences, Houston: Alignment of the genetic and physical maps of the phage P22 tail protein gene.

C. Roessner, D. Struck, and G. Ihler, Dept. of Medical Biochemistry, Texas A & M University, College Station: Binding of bacteriophage λ to liposomes containing λ receptor protein.

K.D. Young,[1] E.C. Altman,[2] J.M. Garrett,[1] and R. Young,[1] [1]Dept. of Medical Biochemistry, Texas A & M University College of Medicine, College Station; [2]Division of Biology, California Institute of Technology, Pasadena: Lysis gene function of λ and φX174.

SESSION 3 *DNA Replication. I.*

T. Formosa, R.L. Burke, and B.M. Alberts, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Affinity chromatography on columns containing immobilized T4 DNA replication proteins.

B. Kemper, F. Jensch, H.-J. Fritz, and M.V. Depka, Institut für Genetik der Universität zu Köln, Federal Republic of Germany: Cleavage of hairpins by T4-induced endonuclease VII.

G. Mosig and M. Levin, Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Phage T4 topoisomerase mutations differentially influence expression of late T4 genes in a *ts gyrB* host.

K. Horiuchi, G.P. Dotto, and K.S. Jakes, Rockefeller University, New York, New York: Initiation and termination signals at the origin of DNA replication of bacteriophage f1.

K. Horiuchi, Rockefeller University, New York, New York: Evolution of bacteriophage f1 during the mass transfer.

J. Lupski, B. Smiley, and G.N. Godson, Dept. of Biochemistry, New York University Medical Center, New York: Is antitermination involved in initiating DNA replication?—The cloning, transposon (Tn*5*) mapping, and nucleotide sequence of the *dnaG* chromosomal region of *E. coli* K-12.

SESSION 4 *DNA Replication. II.*

G. Dehó, D. Ghisotti, S. Zangrossi, M.G. Borrello, P. Alano, and G. Sironi, Institute of Genetics, University of Milano, Italy: Satellite phage P4 in the plasmid state—Induction and maintenance.

R. Lagos and R. Goldstein, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Characterization of the plasmid state of satellite phage P4.

A. Abeles, S. Austin, D. Chattoraj, and M. Yarmolinsky, NCI, Frederick Cancer Research Facility, Frederick, Maryland: Plasmid replication and incompatibility functions of the phage P1.

V.N. Krylov, E.N. Eremenko, and M.A. Bychkova, All-Union Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow, USSR: R plasmids' interference with Mu-like phage D3112 growth in *Pseudomonas aeruginosa* PAO1.

N.P. Higgins,[1] D. Moncecchi,[1] P. Manlapaz-Ramos,[2] and B.M. Olivera,[2] [1]Dept. of Biochemistry, University of Wyoming, Laramie; [2]Dept. of Biology, University of Utah, Salt Lake City: Mu DNA replication in vitro.

M. Pato,[1] C. Reich,[1] B. Waggoner,[1] and A. Toussaint,[2] [1]National Jewish Hospital and Research Center, Denver, Colorado; [2]Free University of Brussels, Belgium: Replication of bacteriophage Mu DNA.

A. RÉSIBOIS, M. COLET, and A. TOUSSAINT, Genetic Laboratory, Free University of Brussels, Belgium: Replication intermediates of mini-MuΔ26.

SESSION 5 *Poster Session*

B.A. CASTILHO,[1] P. OLFSON,[2] and M.J. CASADABAN,[1,3] [1]Committee on Genetics, [2]Dept. of Microbiology, and [3]Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: Mini-Mu–*lac* fusion elements—Novel constructions and uses.

R. CALENDAR and G.E. CHRISTIE, Dept. of Molecular Biology, University of California, Berkeley: Bacteriophage P2 late gene expression requires the *E. coli* RNA polymerase sigma factor.

N. HANNETT, M. COSTANZO, J. JOLLY, G. LEE, and J. PERO, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Bacteriophage SPO1 late gene promoters are different from early and middle gene promoters.

I. TESSMAN, J.S. FASSLER, and D.C. BENNETT, Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Mapping of the *rep* gene of *E. coli* between *rho* and *ilvC*.

J. HOSODA and H. MOISE, Lawrence Berkeley Laboratory, University of California, Berkeley: The role of T4 gene-32 protein terminal domains on protein-protein interactions, as examined by affinity chromatography.

M.A. JABBAR and L. SNYDER, Dept. of Microbiology and Public Health, Michigan State University, East Lansing: Studies on the host locus responsible for the restriction of polynucleotide kinase and RNA ligase mutants of T4.

T. ELLIOT, G. KASSAVETIS, and E.P. GEIDUSCHEK, Dept. of Biology, University of California, San Diego, La Jolla: A newly detected class of T4 late transcripts—Are mutually interacting transcription events important for late RNA synthesis?

S.L. SCHLAGMAN and S.M. HATTMAN, University of Rochester, New York: Cloning and expression of the phage T4 DNA adenine methylase gene (*dam*$^+$).

M. TROJANOWSKA, C. ALFORD, M. DAWSON, and J. KARAM, Dept. of Biochemistry, Medical University of South Carolina, Charleston: Structure and function of the T4 *regA* gene and its protein product.

K.-C. LUK and W. SZYBALSKI, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Cloning and characterization of the transcription termination sites in the major leftward operon of bacteriophage λ.

I. RASCHED, P. BROSS, K. BUSSMANN, and B. PELZER, Fakultat für Biologie, Universität KonstanZ, Federal Republic of Germany: Identification of a 60 kd protein associated with purified bacteriophage fd, necessary for infection, but apparently not coded in its DNA.

K.-C. LUK and W. SZYBALSKI, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Cloning and characterization of the leftward promoter *pbL* in the *b2* region of bacteriophage λ DNA.

A.B. CHEPELINSKY, T. VOGEL, D. COURT, and M. ROSENBERG, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of a bacterial promoter signal carried on the SV40 genome.

SESSION 6 *Transposition*

A.H. PUSPURS and J.N. REEVE, Dept. of Microbiology, Ohio State University, Columbus: Infecting Mu DNA forms circles.

R.H. HARSHEY, R. MCKAY, and A.I. BUKHARI, Cold Spring Harbor Laboratory, New York: DNA intermediates in transposition of phage Mu.

R.M. HARSHEY, Cold Spring Harbor Laboratory, New York: Protein-mediated switch in the transposition products of Mu DNA—Cointegrates vs. simple insertions.

M. MIZUUCHI and K. MIZUUCHI, NIADDKD, National Institutes of Health, Bethesda, Maryland: Effect of distance between the ends of bacteriophage Mu on the transposition reaction.

V.N. KRYLOV, T.G. PLOTNIKOVA, and A.S. YANENKO, All-Union Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow, USSR: Transposition of D3112 phage specific for *Pseudomonas aeruginosa* PAO1 in *E. coli* cells.

E.A. RALEIGH and N. KLECKNER, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Multiple- linked Tn*10*-promoted rearrangements occur at high frequency.

R.W. SIMONS and N. KLECKNER, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Translation of the IS*10* transposase gene is regulated by a *trans*-acting IS*10*-specified RNA.

J. WAY and N. KLECKNER, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Tn*10* mutants that fail to transpose.

SESSION 7 *Recombination and Repair*

M. BETTER,[1,2] C. LU,[1] R.C. WILLIAMS,[1] and H. ECHOLS,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Site-specific DNA condensation and synapsis mediated by the *int* protein of bacteriophage λ.

A.M. SEGALL,[1] M. GELLERT,[2] D.L. CARVER,[1] and D.I. FRIEDMAN,[1] [1]Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor; [2]NIAMDD, National Institutes of Health, Bethesda, Maryland: Synergistic effect of *himA* and *gyrB* mutations.

T.A.G. SMITH and J.B. HAYS, Dept. of Chemistry, University of Maryland Baltimore County, Catonsville: Excision repair of pyrimidine dimers stimulates recombination of nonreplicating phage λ DNA.

D. LEE and P. SADOWSKI, Dept. of Medical Genetics, University of Toronto, Canada: Genetic recombination of phage T7 DNA studied by a simple in vitro assay.

J. DUL and H. DREXLER, Dept. of Microbiology and Immunology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: The effect of transcription on recombination—Transduction of markers stimulated and repressed in transcription.

B.E. WINDLE and J.B. HAYS, Dept. of Chemistry, University of Maryland Baltimore County, Catonsville: A P1 prophage function that stimulates certain *recA*-dependent recombination events.

N.E. MELECHEN and G. GO, Dept. of Microbiology, St. Louis University Medical School, Missouri: Dissection of the SOS responses.

E.M. PHIZICKY and J.W. ROBERTS, Dept. of Biochemistry, Cornell University, Ithaca, New York: The interaction of *recA* protein with NTP and ssDNA.

D. BURBEE and J.W. ROBERTS, Dept. of Biochemistry, Cornell University, Ithaca, New York: Cleavage of *lexA* repressor by *recA430* protein.

A.R. POTEETE and A.C. FENTON, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Structure and activities of the P22 *erf* protein.

SESSION 8 *Gene Expression. I.*

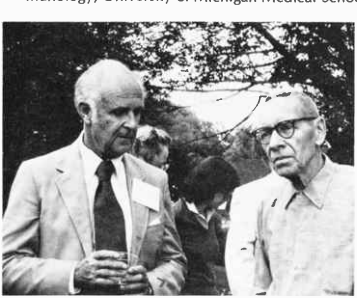
K. FIEN, J. PLACE, S. KIELTY, M. MAHONEY, and D.L. WULFF, Dept. of Biological Sciences, State University of New York, Albany: *N* protein is required for efficient translation of the λ cII gene.

E.R. OLSON,[1] E.L. FLAMM,[2] and D.I. FRIEDMAN,[1] [1]Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor; [2]NICHHD, National Institutes of Health, Bethesda, Maryland: Analysis of *nutR*, a region required for the action of the λ pN antitranscription termination function.

G. SOMASEKHAR, J.S. SALSTROM, D. DRAHOS, and W. SZYBALSKI, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Sequencing of phage λ *nutL* mutations, gene-*N* deletions, and modified *N* products.

D. DRAHOS and W. SZYBALSKI, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Importance of sequences flanking the 17-bp *nutL* core in the λ *N*-mediated antitermination of transcription.

A.T. SCHAUER, D.L. CARVER, E.J. MASHNI, B. SHELL, and D.I. FRIEDMAN, Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: What's new with *nus*.



W. Szybalski, A. Hershey

H. Murialdo, H. Drexler

L. PERITZ and J. GREENBLATT, Dept. of Medical Genetics and Banting and Best Dept. of Medical Research, University of Toronto, Canada: The *nusA* protein of *E. coli* is required for transcription termination in vivo.

R. HABER,[1] S. ADHYA,[1] S. GARGES,[1] D. FRIEDMAN,[2] and L. BARON,[3] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Microbiology, University of Michigan, Ann Arbor; [3]Walter Reed Army Institute of Research, Washington, DC: Structural and functional analysis of the *nusA* gene of *E. coli*.

J. GREENBLATT,[1] J. LI,[1] and D. FRIEDMAN,[2] [1]Banting and Best Dept. of Medical Research, University of Toronto, Canada; [2]Dept. of Microbiology, University of Michigan, Ann Arbor: Regulation by the *nusB* gene of *E. coli* gene expression in vitro.

D. WARD, A. DELONG, and M. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: The role of *nusB* in the specificity of action of the λ *N* gene product.

SESSION 9 *Gene Expression. II.*

E.J. GRAYHACK and J.W. ROBERTS, Dept. of Biochemistry, Cornell University, Ithaca, New York: Antitermination of λ 6S RNA transcription by λ Q protein in vitro.

L.F. LAU, J.W. ROBERTS, and R. WU, Dept. of Biochemistry, Cell and Molecular Biology, Cornell University, Ithaca, New York: Transcription termination at λ t_{R1}.

G.N. GUSSIN, K. MATZ, and M. SCHMANDT, Zoology Dept., University of Iowa, Iowa City: *rex* redux—Two λ *rex* genes.

M.A. HOYT, D. KNIGHT, and H. ECHOLS, Dept. of Molecular Biology, University of California, Berkeley: Post transcriptional control of cII function—The role of cIII, HflA, HimA, and HimD.

A. HOCHSCHILD, N. IRWIN, and M. PTASHNE, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: The characterization of positive control mutations in the λ cI gene and in the P22 c2 gene.

P. YOUDERIAN,[1] M.M. SUSSKIND,[1] D. HAWLEY,[2] and W. MCCLURE,[2] [1]Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester; [2]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Dual repressor control of P22 *ant* gene expression.

D.K. HAWLEY,[1] W.R. MCCLURE,[1] P. YOUDERIAN,[2] and M.M. SUSSKIND,[2] [1]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania; [2]Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: DNA sequence determinants of promoter strength.

W. RICE, R. GREEN, and L. SNYDER, Dept. of Microbiology and Public Health, Michigan State University, East Lansing: Studies on the shutoff of λ late transcription by the T4 *unf/alc* function.

SESSION 10 *Gene Expression. III.*

E. LJUNGQUIST and K. KOCKUM, Dept. of Microbial Genetics, Karolinska Institutet, Stockholm, Sweden: The DNA sequence of the phage P2 repressor gene, the early operator, and the coxII gene.

K. Mizuuchi, B. Kemper

G. Mosig, N. Godson

A. SPADARO,[1] E. LJUNGQUIST,[2] and R. GOLDSTEIN,[1] [1]Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Microbial Genetics, Karolinska Institutet, Stockholm, Sweden: Sequencing analyses of the regulatory region of phage P4.

G.E. CHRISTIE and R. CALENDAR, Dept. of Molecular Biology, University of California, Berkeley: Identification of P2 late gene promoters by sequence analysis and S1 mapping.

H. PRIESS,[1] D. KAMP,[1] R. KAHMANN,[1] B. BRÄUER,[1] and H. DELIUS,[2] [1]Max Planck Institute of Biochemistry, Martinsreid; [2]EMBO Laboratory, Heidelberg, Federal Republic of Germany: Nucleotide sequence of the immunity and the early region of phage Mu.

C.F. MARRS and M.M. HOWE, Dept. of Bacteriology, University of Wisconsin, Madison: Kinetics and regulation of transcription of bacteriophage Mu.

S.F. STODDARD and M.M. HOWE, Dept. of Bacteriology, University of Wisconsin, Madison: Cloning and mapping of bacteriophage Mu DNA fragments with promoter activity.

M. COSTANZO and J. PERO, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Cloning the regulatory genes of bacteriophage SPO1.

H. AIBA, Radioisotope Laboratory, Kyoto University, Japan: Autogenous regulation of the *E. coli crp* gene—Transcriptional repression by CRP- cAMP.

A. GOLDFARB[1,2] and T. BARKAY,[3] [1]Dept. of Microbiology, Columbia University, New York, New York: [2]Max Planck Institute of Biochemistry, Martinsreid, Federal Republic of Germany; [3]Dept. of Biochemistry, Weizmann Institute, Rehovot, Israel: Processing of phage T4 transcripts with RNase III.

M.L. ROMANTSCHUK and U.R. MULLER, Dept. of Microbiology, East Carolina University, School of Medicine, Greenville, North Carolina: Loss of a hairpin structure in the J-F intercistronic region of bacteriophage ϕX174 causes overexpression of downstream genes.

SESSION 11 *Gene Mapping and Cloning*

A.D. GROSSMAN, W. WALTER, R. BURGESS, and C. GROSS, Dept. of Bacteriology, University of Wisconsin, Madison: Mutations in the *lon* gene of *E. coli* K12 phenotypically supress mutations in the sigma subunit of RNA polymerase.

J. HU and C. GROSS, Dept. of Bacteriology, University of Wisconsin, Madison: Fine-structure mapping of *ts* mutations in *rpoD*, the structural gene for the sigma subunit of *E. coli* RNA polymerase.

A.R. SHATZMAN, C. DEBOUCK, and M. ROSENBERG, NCI, National Institutes of Health, Bethesda, Maryland: A plasmid cloning vector using λ regulatory sequences for inducible overproduction of proteins in *E. coli*.

J. TEIFEL, Institut für Genetik und Mikrobiologie der Universität Munchen, Federal Republic of Germany: Bacteriophage-Mu-mediated transduction of multicopy plasmids.

C. WAGHORNE, Dept. of Medical Genetics, University of Toronto, Canada: Analysis of the cloned *E. coli mat* locus.

R. Christiansen, T. Broker, J. Wiberg

A. Bukhari, A. Toussaint

Herpesviruses

August 31–September 5

Arranged by **George Miller**, Yale University School of Medicine, **Mark Stinski**, University of Iowa College of Medicine

387 participants

This meeting, which covered most aspects of basic research on herpesviruses, was an effort to integrate findings on the biology of these complex viruses with molecular biology. Among the more striking findings presented were the identification of sequences involved in the initiation of herpes simplex DNA synthesis and in packaging and the identification of sequences responsible for inversion of the genome of herpes simplex. The construction and assay of tRNAs which would suppress mutations in the herpes simplex thymidine kinase gene was reported, and preliminary data on the complete nucleotide sequence of EB viral DNA were presented. The location of a gene in the EB viral genome responsible for synthesis or induction of the EB nuclear antigen was described. Two groups reported that lymphocyte transformation by EBV called forth the expression of a novel B-cell surface antigen that could be recognized by a monoclonal antibody.

The meeting was supported in part by a small grant from the National Science Foundation and by contributions from Abbott Laboratories; American Cyanamid Company-Lederle Laboratories; Beckman Instruments, Inc.; Boehringer Mannheim Biochemicals; Bristol-Myers Co.; Burroughs Wellcome Co.; Cetus Corp.; CIBA-Geigy Corp.; Genetics Systems Corp.; Hoffmann-LaRoche, Inc.; Merck Sharp and Dohme Research Laboratories; National Science Foundation; New England Biolabs, Inc.; New England Nuclear Corp.; Ortho Pharmaceutical Corporation; Schering Coroporation; Searle Laboratories; Showa University Research Institute for Biomedicine in Florida; Upjohn Co.; and Viratek, Inc. These funds were used as partial stipends to assist graduate students and postdoctoral fellows in covering expenses for the meeting.

SESSION 1 *Latency and Persistent Infection*

Chairperson: J. STEVENS, University of California, Los Angeles, California

A. ADAMS, M. BYWATER, and E. GUSSANDER, Wallenberg Laboratory, University of Uppsala, Sweden: New intracellular forms of latent EBV DNA in leukemic lymphocytes.

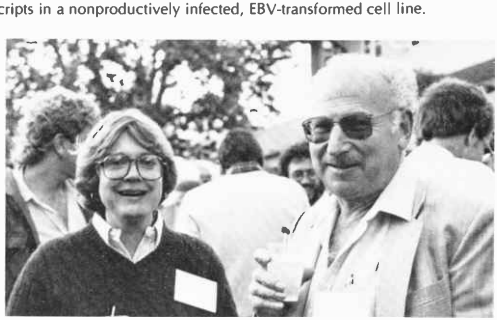
A.K. SAEMUNDSEN, C. PERLMAN, and G. KLEIN, Dept. of Tumor Biology, Karolinska Institutet, Stockholm, Sweden: Methylation in EBV DNA.

H. COLLANDRE, D. GUÉTARD, and L. MONTAGNIER, Unité d'Oncologie Virale, Institut Pasteur, Paris, France: Methylation state of EBV DNA in Daudi cells.

R. WEIGEL and G. MILLER, Yale University Medical School, New Haven, Connecticut: Major EBV-specific cytoplasmic transcripts in a Burkitt lymphoma line during latency and after induction of viral replicative cycle by phorbol esters.

V. VAN SANTEN and E. KIEFF, Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Detailed mapping of EBV-specific transcripts in a nonproductively infected, EBV-transformed cell line.

D. Galloway



P. Schaffer, J. Subak-Sharpe

B. L. WIGDAHL,[1] A.C. SCHECK,[1] E. DE CLERCQ,[2] and F. RAPP,[1] [1]Pennsylvania State University College of Medicine, Hershey; [2]Katholieke Universiteit Leuven, Belgium: Analysis of latent HSV-1 genome in human cells.

D. L. ROCK and N. W. FRASER, Wistar Institute, Philadelphia, Pennsylvania: Detection of the HSV-1 genome in the central nervous system of persistently infected mice.

R. TENSER and J. JONES, Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Acute and latent trigeminal ganglion infection by thymidine kinase mutants of HSV-2.

M. COOK and J. STEVENS, Dept. of Microbiology and Immunology, University of California Medical School, Los Angeles: A basis for differing susceptibilities of mouse strains to HSV.

S. AL-SAADI, G. CLEMENTS, and J. H. SUBAK-SHARPE, Institute of Virology, Glasgow, Scotland: HSV-2 wt and ts mutants can be recovered from the footpad and dorsal root ganglia of latently infected mice.

SESSION 2 *Genome Structure, Replication and Recombination*

Chairperson: G. S. HAYWARD, Johns Hopkins University, Baltimore, Maryland

Y. BECKER, Y. SHTRAM, and Y. ASHER, Dept. of Molecular Virology, Faculty of Medicine, Hebrew University, Jerusalem, Israel: Cloning and properties of HSV-1 genes present in the *Bgl*II fragment of HSV-1 DNA that carries one of the origins of replication of the viral genome.

N. D. STOW and E. C. MCMONAGLE, Institute of Virology, Glasgow, Scotland: Localization of sequences involved in the initiation of HSV-1 DNA synthesis and in the packaging of replicated viral DNA.

E. MOCARSKI and B. ROIZMAN, Kovler Viral Oncology Laboratories, University of Chicago, Illinois: HSV genome inversion is mediated by viral gene products and the viral a sequence.

S. K. AMUNDSEN and D. S. PARRIS, Dept. of Medical Microbiology and Immunology and Comprehensive Cancer Center, Ohio State University, Columbus: Early recombination during HSV productive replication.

R. L. LAFEMINA and G. S. HAYWARD, Johns Hopkins University Medical School, Baltimore, Maryland: Replicative forms of the human cytomegalovirus genome are found in permissive but not in non-permissive cells.

J. TAMASHIRO,[1] D. FILPULA,[2] T. FRIEDMANN,[2] and D. SPECTOR,[1] [1]Dept. of Biology; [2]Pediatrics, University of California San Diego, La Jolla: Characterization of the heterogeneous joint region of human cytomegalovirus (strain AD169).

B. E. HENRY, N. J. RAAB-TRAUB, and J. S. PAGANO, Cancer Research Center, University of North Carolina, Chapel Hill: Determination of the number and location of autonomous replicating sequences present in the genome of EBV.

M. HELLER,[1] V. VAN SANTEN,[1] M. HUMMEL,[1] E. KIEFF,[1] and A. HENDERSON,[2] [1]University of Chicago, Illinois; [2]Columbia University, New York, New York: A simple sequence repeat array in EBV DNA has homology to cell DNA and is transcribed.

M.-S. CHO, G. W. BORNKAMM, and H. ZUR HAUSEN, Institut für Virologie, Zentrum für Hygiene University of Freiburg, Federal Republic of Germany: Inter- and intramolecular recombination in EBV DNA.

J. HAY, T. A. CASEY, W. T. RUYECHAN, and S. E. STRAUS, Depts. of Microbiology and Biochemistry, NIAID, National Institutes of Health, Bethesda, Maryland: Insertion and deletion events are the basis for some stable strain differences in VZV DNAs.

K. PEDEN, P. MOUNTS, K.-T. JEANG, E. GAVIS, and G. S. HAYWARD, Johns Hopkins Medical School, Baltimore, Maryland: Further studies on cell-virus homology sequences in HSV, EBV and CMV.

WORKSHOP 1 *Herpesvirus Nuclear Proteins*

Chairperson: R. J. COURTNEY, University of Tennessee, Knoxville, Tennessee

V. BIBOR-HARDY and R. SIMARD, Institut du Cancer de Montréal, Canada: Modifications of the nuclear matrix during HSV infection.

B. J. DILLE and R. J. COURTNEY, Dept. of Microbiology, University of Tennessee, Knoxville: Synthesis of HSV-1 polypeptides and subsequent association with the nuclear fraction.

C. K. LEE, M. P. QUINLAN, and D. M. KNIPE, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: HSV mutations that alter the nuclear localization or DNA-binding function of an early viral protein.

C. M. PRESTON, M. E. M. CAMPBELL, and E. L. NOTARIANNI, Institute of Virology, Glasgow, Scotland: Studies on the function and modification of the HSV-1 immediate early polypeptide Vmw 175.

S. FABER and K. WILCOX, Dept. of Microbiology, Medical College of Wisconsin, Milwaukee: Synthesis and properties of an immediate early HSV polypeptide produced by tsLB2.

E. L. NOTARIANNI and C. M. PRESTON, Institute of Virology, Glasgow, Scotland: HSV immediate early polypeptides induce cellular heat-shock proteins.

P. F. PIGNATTI,[1] F. SAMMARTANO,[1] G. RAPPAZZO,[1] C. PARISI,[1] M. TOGNON,[2] R. MANSERVIGI,[2] and E. CASSAI,[2] [1]Dept. of Animal Biology, University of Catania; [2]Institute of Microbiology, University of Ferrara, Italy: Proteins bound to HSV DNA during infection, and their possible role in the expression of the viral genetic program.

E.-C. MAR and E.-S. HUANG, Cancer Research Center, University of North Carolina, Chapel Hill: A novel human cytomegalovirus-specific 150K phosphorylated DNA binding protein in purified virion and virus-infected cell.

J. LUKA,[1] H. JORNVALL,[2] and G. KLEIN,[1] [1]Dept. of Tumor Biology; [2]Dept. of Chemistry, Karolinska Institutet, Stockholm, Sweden: The 48 K component of the EBV determined nuclear antigen (EBNA) and higher molecular weight forms of the antigen are structurally related.

J.-C. LIN, M. C. SMITH, and J. S. PAGANO, Cancer Research Center, University of North Carolina, Chapel Hill: Selective stimulation of synthesis of chromosomal proteins associated with activation of latent EBV genomes by a tumor promoter.

C. C. SMITH and L. AURELIAN, Johns Hopkins University Medical School, Baltimore, Maryland: Further characterization of the transformation associated protein ICP-10.

WORKSHOP 2 *VZV*

Chairperson: S. E. STRAUS, National Institutes of Health, Bethesda, Maryland

C. GROSE, D. P. EDWARDS, W. E. FRIEDRICHS, K. A. WEIGLE, and W. L. MCGUIRE, Depts. of Pediatrics, Microbiology and Medicine, University of Texas Health and Science Center, San Antonio: Glycoproteins specified by VZV.

E. KINNEY-THOMAS, K. SHRIVER, L. GOLDSTEIN, T. CROY, and K. BEACH, Genetic Systems Corporation, Seattle, Washington: Monoclonal antibodies specific for varicella zoster-infected cell proteins.

C. M. EDSON,[1] B. HOSLER,[2] D. WATERS,[2] and D. A. THORLEY-LAWSON,[1] [1]Depts. of Pathology and Geographic Medicine, Tufts University Medical School; [2]Dept. of Public Health, Boston, Massachusetts: Functional and biochemical analysis VZV proteins with monoclonal antibodies.

Z. WROBLEWSKA[1] and D. H. GILDEN,[1,2] [1]Multiple Sclerosis Research Center, Wistar Institute; [2]Dept. of Neurology, University of Pennsylvania Medical School, Philadelphia: Monoclonal antibodies against VZV.

S. E. STRAUS,[1] J. HAY,[2] H. A. SMITH,[1] J. OWENS,[1] and P. BRUNNELL,[3] [1]National Institutes of Health; [2]Uniformed Services University of the Health Sciences, Bethesda, Maryland; [3]University of Texas, San Antonio: Molecular epidemiologic studies of VZV infections.

M. SEIDLIN,[1] H. E. TAKIFF,[1] S. E. STRAUS,[1] T. CASEY,[2] and J. HAY,[2] [1]National Institutes of Health; [2]Uniformed Services University of the Health Sciences, Bethesda, Maryland: Dot-blot hybridization assay for rapid diagnosis of VZV infection.

A. BUCHAN,[1] A. FULLER,[1] C. R. ROBERTS,[2] J. HAY,[2] M. BARTOSKI,[2] and G. S. HAYWARD,[3] [1]Dept. of Medical Microbiology, University of Birmingham Medical School, England; [2]Dept. of Microbiology, Uniformed Services University of Health Services, Bethesda, Maryland; [3]Dept. of Pharmacology, Johns Hopkins University, Baltimore, Maryland: The immunological cross reactions between HSV and VZV.

WORKSHOP 3 *DNA of Lymphotropic Viruses*

Chairperson: A. ADAMS, University of Uppsala, Uppsala, Sweden

R. BAER, A. BANKIER, B. BARRELL, M. BIGGIN, P. DEININGER, P. FARRELL, T. GIBSON, G. HUDSON, S. SATCHWELL, and C. SEGUIN, MRC Laboratory of Molecular Biology, Cambridge, England: Nucleotide sequences of EBV DNA.

J. SKARE, J. FARLEY, and J. STROMINGER, Sidney Farber Cancer Institute, Boston, Massachusetts: Small tandem repeats in EBV DNA.

T. DAMBAUGH, W. KING, and E. KIEFF, University of Chicago, Illinois: The P3HR-1 deletion includes one member of a pair of related tandem direct repeats in EBV DNA.

M. D. JONES, J. R. ARRAND, T. SHEEDY, L. KARRAN, A. HARRIS, and B. E. GRIFFIN, Imperial Cancer Research Fund Laboratories, London, England: Analysis of the large interval repeat unit of EBV and adjacent DNA sequences.

S. YANO, T. AYA, K. TAKADA, and T. OSATO, Dept. of Virology, Cancer Institute, Hokkaido University Medical School, Sapporo, Japan: Identification of incomplete B95-8 EBV—Evidence indicating its transforming ability.

M. ANVRET and K. KARLSSON , Dept. of Medical Biochemistry, University of Göteborg, Sweden: Detection and characterization of a new *Eco*RI EBV DNA fragment in Raji cellular DNA.

R. DESROSIERS, A. BAKKER, R. BURGHOFF, and L. FALK, Harvard Medical School, New England Regional Research Center, Southborough, Massachusetts: The nononcogenic variant of *Herpesvirus saimiri* strain 11.

A. BANKIER,[1] R. BAER,[1] B. G. BARRELL,[1] F. COLBÈRE-GARAPIN,[2] W. DIETRICH,[3] E. KNUST,[3] and B. FLECKENSTEIN,[3] [1]Laboratory of Molecular Biology, MRC, Cambridge, England; [2]Institut Pasteur, Paris, France; [3]Institut für Klinische Virologie, University of Erlangen-Nürnberg, Federal Republic of Germany: Nucleotide sequence of *Herpesvirus saimiri* H-DNA.

W. BODEMER and E. KNUST, Institut für Klinische Virologie, University of Erlangen-Nürnberg, Federal Republic of Germany: *Herpesvirus saimiri* and *Herpesvirus ateles* transcription in lytically infected and transformed cells.

S. SCHIRM,[1] C. KASCHKA-DIERICH,[1] R. C. DESROSIERS,[2] D. ABLASHI,[3] H. RABIN,[3] and B. FLECKENSTEIN,[1] [1]Institut für Klinische Virologie, University of Erlangen-Nürnberg, Federal Republic of Germany; [2]New England Primate Research Center, Harvard Medical School, Southborough, Massachusetts; [3]NCI, National Institutes of Health, Bethesda, Maryland: Deletions in the circular, nonintegrated viral DNA of *herpesvirus saimiri*-transformed lymphoid cell lines.

SESSION 3 *Transformation of Cell Growth*

Chairperson: M. NONOYAMA, Showa University Research Institute for Biomedicine, Clearwater, Florida

J. A. NELSON,[1] J. K. MCDOUGALL,[1] D. A. GALLOWAY,[1] R. GELINAS,[1] L. GOLDSTEIN,[1] A. GRASSMANN,[2] H. SCHMULLA,[3] T. SARRE,[3] G. JAHN,[3] and B. FLECKENSTEIN,[3] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Institut für Physiologische Chemie der Freien Universität Berlin; [3]Institut für Klinische Virologie, University of Erlangen-Nürnberg, Federal Republic of Germany: Analysis of the transforming sequences of human cytomegalovirus strain AD169.

D. A. GALLOWAY, S. P. WEINHEIMER, M. A. SWAIN, J. B. LEWIS, K. SHRIVER, and J. K. MCDOUGALL, Fred Hutchinson Cancer Research Center, Seattle, Washington: An unusual mechanism of transformation by a fragment of HSV-2 DNA.

R. J. JARIWALLA,[1] L. AURELIAN,[1,2] and P. O. P. TS'O,[1] [1]Dept. of Biophysics and [2]Dept. of Comparative Medicine, Johns Hopkins University, Baltimore, Maryland: Neoplastic transformation by various fragments of HSV-2 DNA.

B. A. BLAZAR, L. SUTTON, and M. STROME, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts: Autostimulatory growth factor production by B lymphocyte lines from tumors and EBV transformed cells—A mechanism for perpetual growth.

J. W. SIXBEY and J. S. PAGANO, Cancer Research Center, University of North Carolina, Chapel Hill: EBV transformation of human lymphocytes in the presence of the viral DNA polymerase inhibitor acyclovir.

B. E. GRIFFIN, Imperial Cancer Research Fund, London, England: Mediation of epithelial cell transformation by cloned fragments of EBV DNA.

R. H. NEUBAUER, R. L. BROWN, and H. RABIN, NCI, Frederick Cancer Research Facility, Frederick, Maryland: *Herpesvirus saimiri*-induced lymphoma—Tumor phenotype defined by monoclonal antibodies and T cell growth factor responses.

M. RABSON and G. MILLER, Dept. of Epidemiology and Public Health, Yale University, New Haven, Connecticut: Nonimmortalizing P3J-HR-1 EBV—A deletion mutant of its transforming partent, Jijoye.

J. STOERKER and R. GLASER, College of Medicine and Comprehensive Cancer Center, Ohio State University, Columbus: Rescue of transforming EBV from IUdr induced D98/HR-1 epithelial hybrid cells after transfection with cloned subgenomic fragments of B95-8 virus.

I. M. SHAPIRO and D. J. VOLSKY, Dept. of Pathology and Laboratory Medicine, University of Nebraska

Medical Center, Omaha: Infection of normal human nasopharyngeal epithelial cells by EBV following EBV receptor transplantation.

SESSION 4 *Proteins and Glycoproteins*

Chairperson: G. H. COHEN, University of Pennsylvania, Philadelphia, Pennsylvania

P. J. GODOWSKI and D. M. KNIPE, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Role of the HSV DNA binding protein in regulation of HSV gene expression.

S. S. LEINBACH, Dept. of Microbiology, University of South Alabama, College of Medicine, Mobile: Studies on DNP complexes containing a HSV-1 protein of molecular weight 133,000.

L. COHEN,[1] R. IMHOF,[1] B. ROBERTS,[2] and J. STROMINGER,[1] [1]Sidney Farber Cancer Institute; [2]Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Identification and genomic mapping of EBV DNA binding proteins.

T. COMPTON and R. J. COURTNEY, Dept. of Microbiology, University of Tennessee, Knoxville: Virus-specific glycoproteins associated with the nuclear fraction of HSV-infected cells.

L. PEREIRA,[1] D. DONDERO,[1] and B. ROIZMAN,[2] [1]California Dept. of Public Health, Berkeley; [2]Kovler Viral Oncology Laboratories, University of Chicago, Illinois: HSV glycoprotein gA/B—Evidence that the infected Vero cell products co-map and arise by proteolysis.

A. M. ARVIN,[1] C. M. KOROPCHAK,[1] A. S. YEAGER,[1] and L. PEREIRA,[2] [1]Stanford University Medical School, California; [2]California Dept. of Health Services, Berkeley: The detection of type specific antibody to HSV-1 by radioimmunoassay using HSV-1 glycoprotein C purified with monoclonal antibody.

J. T. MATTHEWS,[1] G. H. COHEN,[1] and R. J. EISENBERG,[2] [1]Center for Oral Health Research, School of Dental Medicine and [2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia: In vitro translation and processing of glycoprotein gD of HSV-1 and HSV-2.

H. HAMPL and T. B. PORAT, Dept. of Microbiology, Vanderbilt University Medical School, Nashville, Tennessee: Arrangement of the glycoproteins in the envelope of pseudorabies virus.

J. ZAIA, B. CLARK, R. EBERLE, and Y.-P. TING, Divisions of Pediatrics and Immunology, City of Hope Medical Center, Duarte, California: Isolation and characterization of a 64,000 dalton polypeptide of human cytomegalovirus (CMV) using high performance liquid chromatography (HPLC).

T. J. STINCHCOMBE and W. G. CLOUGH, Dept. of Molecular Biology, University of Southern California, Los Angeles: EBV-producing cell lines contain a unique pyrimidine deoxynucleoside kinase activity.

D. C. JOHNSON AND P. G. SPEAR, Dept. of Microbiology, University of Chicago, Illinois: Maturation of HSV-1 glycoproteins.

J. L. M.C . GEELEN, W. R. BOOM, C. BUURMAN, and J. VAN DER NOORDAA, Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, The Netherlands: Protein kinase activity associated with an immediate-early CMV-induced protein.

SESSION 5 *Poster Session*

R. H. COSTA, L. M. HALL, K. G. DRAPER, and E. K. WAGNER, Dept. of Molecular Biology and Biochemistry, University of California, Irvine: High resolution mapping of HSV-1 transcripts encoded by the left third of the U_L region.

D. YAGER, E. HARRIS, and S. BACHENHEIMER, Dept. of Bacteriology and Immunology, University of North Carolina, Chapel Hill: Physical mapping of transcripts in the 0.0 to 0.3 region of the HSV-1 genome.

V. C. BOND and S. PERSON, Molecular and Cell Biology Program, Pennsylvania State University, University Park: Fine-structure mapping of loci that affect cell fusion in HSV-1 (KOS).

A. EPSTEIN and B. JACQUEMONT, Unité de Virologie Fondamentale et Appliquée, INSERM, CNRS, Lyon, France: Expression of different HSV variants in nonpermissive rat XC cells—Localization of implicated virus functions.

H. J. FIELD, Dept. of Pathology, University of Cambridge, England: The role of latent TK-defective viruses in the emergence of clinical resistance to acyclovir.

B. SVENNERHOLM, S. OLOFSSON, R. LUNDÉN, A. VAHLNE, and E. LYCKE, Dept. of Virology, University of Göteborg, Sweden: Adsorption and penetration of HSV enveloped particles modified by tunicamycin and 2-deoxy-D-glucose.

Y. SAITO and R. W. PRICE, Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer

Center, New York, New York: Prospects for in vivo diagnosis of HSV encephalitis using radiolabeled antivirals—Autoradiographic studies using an animal model.

A. LARSSON, M. BERG, C-E. HAGBERG, N-G. JOHANSSON, and B. ÖBERG, Astra Läkemedel AB, Research and Development Laboratories, Dept. of Antiviral Chemotherapy, Södertälje, Sweden: A 36434—A new nucleoside analog with selective antiherpes activity.

E. THIRY, P.-P. PASTORET, H. VINDEVOGEL, A. SCHWERS, B. BROCHIER, and P. LEROY, Faculty of Veterinary Medicine, University of Liège, Bruxelles, Belgium: In vitro and in vivo effect of acyclovir on infectious bovine rhinotracheitis virus, pseudorabies virus, and pigeon herpesvirus.

N. SCHEK, G. SHERMAN, E. HARRIS, and S. BACHENHEIMER, Dept. of Bacteriology and Immunology, University of North Carolina, Chapel Hill: Effects of amino acid analogs on the regulation of HSV gene expression.

D. M. MEREDITH, Research Division, G. D. Searle and Co., High Wyncombe, England: Phosphorylation and subsequent incorporation of E-5-(2-bromovinyl)2'-deoxyuridine (BVDU) into DNA in cells infected with HSV-1 and 2.

M. D. TROUSDALE, A. B. NESBURN, and D. E. WILLEY, Estelle Doheny Eye Foundation, Los Angeles, California: Effect of intensive ACV therapy during stimulation to induce reactivation of latent HSV in rabbits.

S. HARADA, R. ANDERSON, T. BECHTOLD, and D. PURTILO, Dept. of Pathology and Laboratory Medicine, University of Nebraska Medical Center, Omaha: Comparison of EBV-specific antibody, memory T cells in regression assays, and natural killer-cell activity in X-linked lymphoproliferative syndrome.

B. VOLSKY and D. J. VOLSKY, Dept of Pathology and Laboratory Medicine, University of Nebraska Medical Center, Omaha: Anti-EBV immune response in mice inoculated with autologous EBV-infected lymphocytes.

C. KUSZYNSKI and D. J. VOLSKY, Dept. of Pathology and Laboratory Medicine, University of Nebraska Medical Center, Omaha: Cytofluorographic studies on the distribution and relationship between EBV receptors and complement c3 receptors.

J. JONCAS, F. GHIBU, L. ROBILLARD, S. MONTPLAISIR, and M. BLAGDEN, Pediatric Research Center, University of Montreal, Ste-Justine Hospital, Canada: EBV target cell defect and other immunological defects in ataxia telangiectasia (AT).

R. W. ANDERSON, B. R. GREENBERG, S. J. KNOX, T. BECHTOLD, C. KUSZYNSKI, S. HARADA, D. T. PURTILLO, and D. J. VOLSKY, Dept. of Pathology and Laboratory Medicine, University of Nebraska Medical Center, Omaha: Lymphocyte abnormalities in preleukemia include lack of EBV receptors.

D. CASAREALE, T. SAIRENJI, W. JONES, and R. E. HUMPHREYS, University of Massachusetts Medical School, Worcester: p105—An EBV-induced, phosphonoacetic acid-insensitive, glycoprotein target of the anti-EBV immune response.

G. AHRONHEIM,[1] F. AUGER,[1] L. ROBILLARD,[1] F. GHIBU,[1] J. JONCAS,[1] J. DEMERS,[2] and N. TRAUB,[3] [1]Research Institute and [2]Dept. of Hematology, Hopital Ste-Justine, University of Montreal, Canada; [3]Cancer Research Center, University of North Carolina, Chapel Hill: Aplastic anemia and primary EBV infection.

T. OOKA, D. DECAUSSIN, F. ANGEL, A. CHEVALLIER, E. MANET, and J. DAILLIE, Laboratoire d'Immunovirologie, Faculté de Médecine Alexis Carrel, Lyon, France: Immunological characterization of EBV-associated early antigens induced by TPA in combination with N-butyrate.

G. BAUER, Institut für Virologie, Zentrum für Hygiene, University of Freiburg, Federal Republic of Germany: Quantitative analysis of the cooperative effect between inducers of EBV antigen synthesis.

E. FOWLER,[1] L. M. HUTT-FLETCHER,[2] and R. J. FEIGHNY,[1] [1]Cancer Research Center, University of North Carolina, Chapel Hill; [2]Dept. of Experimental and Comparative Pathology, University of Florida, Gainesville: Nonionic density gradient purification of EBV.

M. J. REDDEHASE, G. M. KEIL, and U. H. KOSZINOWSKI, Federal Research Institute for Animal Virus Diseases, Tübingen, Federal Republic of Germany: Estimation of frequency, specificity, and state of activation of cytolytic T-lymphocyte precursors generated in vivo during MCMV infection.

J. G. NEDRUD,[1] R. WU,[2] S. L. JOHNSON,[1] and J. S. PAGANO,[1] [1]Cancer Research Center, University of North Carolina, Chapel Hill; [2]Laboratory of Pulmonary Function and Toxicology, Research Triangle Park, North Carolina: MCMV infection of isolated tracheal epithelial cells—Differences and similarities to infection of tracheal organ cultures.

J. F. BASKER, S. C. STANAT, and E.-S. HUANG, Cancer Research Center, University of North Carolina, Chapel Hill: Interaction of MCMV with the reproductive system.

M. J. REDDEHASE, R. L. LORENZ, M. REDDEHASE, and U. H. KOSZINOWSKI, Federal Research Institute for Animal Virus Diseases, Tübingen, Federal Republic of Germany: Poisson distribution analysis of the in vivo expressed cytolytic T-lymphocyte repertoire during MCMV infection.

J. R. MARKS, J. A. MERCER, and D. H. SPECTOR, Dept. of Biology, University of California San Diego, La Jolla: Molecular cloning and restriction mapping of the MCMV genome.

P. C. PATEL, J.-L. LIU, E.-C. MAR, and E.-S. HUANG, Cancer Research Center, University of North Carolina Medical School, Chapel Hill: Monoclonal antibodies against the 54K and 94K proteins of human cytomegalovirus.

S. CHOU and T. C. MERIGAN, Stanford University Medical School, California: Preliminary observations on the effect of human leukocyte interferon on viral excretion in CMV retinitis.

A. S. TYMS, Dept. of Medical Microbiology, St. Mary's Hospital Medical School, London, England: Strain characterization of fresh isolates of HCMV.

U. HEEG,[1] G. HILLER,[2] V. HÄRLE-GRUPP,[1] and D. FALKE,[1] [1]Division of Experimental Virology, Institute for Medicine Microbiology, Mainz; [2]Max-Planck-Institute of Biophysical Chemistry, Göttingen, Federal Republic of Germany: The cytoskeleton of HSV-infected cells.

S. L. VARMUZA and J. R. SMILEY, Dept. of Pathology, McMaster University, Hamilton, Canada: Intramolecular sequence heterozygosity within the inverted repeats flanking the S segment of HSV DNA.

S. K. WELLER, B. A. PANCAKE, W. SACKS, D. COEN, and P. A. SCHAFFER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Genetic analysis of HSV-1, strain KOS—An update.

C. H. SCHRÖDER and B. HENNES-STEGMANN, Institute of Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Intra- and intertypic interference by HSV DNA in transfection assays.

C. P. GRAY and H. C. KAERNER, Institute of Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Sequence arrangement of defective HSV-1 DNA—About 500 bp of the "c"-sequence are multiply amplified in the repeat unit of a class II defective HSV-ANG DNA.

S. JEANSSON and E. NILHEDEN, Institute of Medical Microbiology, University of Göteborg, Sweden: Typing of HSV strains with monoclonal antibodies.

P.-P. PASTORET, B. BROCHIER, E. THIRY, and G. DERBOVEN, Dept. of Virology, University of Liège, Bruxelles, Belgium: Evolution of specific and nonspecific blastogenesis following experimental infection of cattle with *Bovid herpesvirus 1*.

H.-J. RZIHA, H.-J. THIEL, F. WEILAND, N. LUKACS, and G. WITTMAN, Federal Research Institute for Animal Virus Diseases, Tübingen, Federal Republic of Germany: Pseudorabiesvirus DNA in continuous cell lines derived from tissues of latent-infected pigs.

V. MISRA and L. A. BABIUK, Western College of Veterinary Medicine, University of Saskatchewan, Canada: Characterization of a herpesvirus induced 'early' glycoprotein.

J. JANIK,[1] J. A. ROSE,[1] R. M. L. BULLER,[1] and J. HAY,[1,2] [1]NIAID; [2] USUHS, National Institutes of Health, Bethesda, Maryland: An HSV function(s) exerts translational control over AAV mRNA.

N. H. PARK,[1,2] and D. PAVAN-LANGSTON[2,3] [1]Dept. of Oral Biology, [2]Eye Research Institute of Retina Foundation, and [3]Dept. of Ophthalmology, Harvard Medical School, Boston, Massachusetts: Therapeutic efficacy of E-5-(2-bromovinyl)-2'-deoxyuridine on herpes simplex and virus encephalitis in mice.

S. MODROW, H. SCHMIDT, and H. WOLF, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: Characterization of proteins induced by *Herpesvirus saimiri*.

L. L. WILLIAMS and G. R. PENN, Dept. of Pediatrics, Ohio State University, Columbus: CMV-specific suppressor cells identified in Charcot-Marie-tooth disease peripheral blood.

WORKSHOP 4 *Herpesvirus Pathogenesis*

Chairperson: R. TENSOR, Pennsylvania State University, Hershey, Pennsylvania

G. DARAI and J. SCHOLZ, Institut für Medizinische Virologie, University of Heidelberg, Federal Republic of Germany: Generation of intertypic recombinants of HSV in vivo for the study of viral latency and tropism.

Y. BECKER,[1] Y. SHTRAM,[1] Y. ASHER,[1] E. TABOR,[1] Y. GORDON,[2] T. BEN-HUR,[1] J. HADAR,[1] and D. GILDEN,[3] [1]Dept. of Molecular Virology; [2]Dept. of Opthalmology, Hebrew University-Hadassah Medical Center, Jerusalem, Israel; [3]Wistar Institute, University of Pennsylvania Medical School, Philadelphia: Role of HSV-1 *tk* gene in viral neurovirulence in weanling and adult mice.

Z.-Y. FANG, R. TENSER, and F. RAPP, Dept. of Microbiology, Pennsylvania State University, Hershey: HSV replication in regenerating liver cells.

J. M. HILL, Y. SHIMOMURA, G. L. COLBORN, and L. P. GANGAROSA, Medical College of Georgia, Augusta: In-

duced reactivation of HSV-1—Infectious virus in neural tissue homogenates from latently infected rabbits.

L. Pulliam, R. D. Dix, and J. R. Baringer, Veterans Administration Medical Center, University of California, San Francisco: Use of aggregating brain cultures to study the replication of HSV-1 and HSV-2 within central nervous system tissue.

R. Braun, H. Teute, M. Dawson, K. Munk, and H. Kirchner, Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Replication of HSV in human lymphocytes—Identification of the target cell of virus replication.

A. L. Epstein,[1] J. Bertoglio,[2] and B. Jacquemont,[1] [1]Unité de Virologie Fondamentale et Appliquée (INSERM U 51) CNRS; [2]Unité de Recherches d'Immunologie et de Cancerologie Expérimentale (INSERM U 218), Centre Leon Berard, Lyon, France: Differential replication of HSV-1 in human lymphoid cell lines representing various steps of differentiation.

R. D. Dix and J. R. Baringer, Veterans Administration Medical Center, University of California, San Francisco: Passive transfer studies using monoclonal antibodies to glycoprotein gA/gB of HSV-1 or 2.

G. R. B. Skinner,[1] A. Buchan,[1] C. Woodman,[1] C. E. Hartley,[1] A. Fuller,[1] J. Melling,[2] J. Wiblin,[2] and G. Wilkins,[2] [1]Dept. of Medical Microbiology, University of Birmingham Medical School; [2]Centre for Applied Microbiological Research, Salisbury, England: Prevention of herpes genitalis in subjects at risk by immunization with antigenoid vaccine Ac $NFU_1(S^-)$ MRC.

J. Rector, R. Lausch, and J. Oakes, Dept. of Microbiology, University of South Alabama Medical College, Mobile: Neutralizing and nonneutralizing monoclonal antibodies specific for HSV-1 glycoproteins mediate protection against acute herpetic encephalitis.

J. F. Sheridan,[1] T. Iwasaka,[2] E. Chaikof,[1] and L. Aurelian,[1,2] [1]Dept. of Comparative Medicine; [2]Dept. of Biophysics, Johns Hopkins University Medical School, Baltimore, Maryland: HSV-specific supressor cells in exogenous reinfection.

R. J. Ziegler[1] and R. S. Pozos,[2] [1]Dept. of Medical Microbiology; [2]Dept. of Physiology, University of Minnesota Medical School, Duluth: Protective effects of various immune mechanisms on rat sensory neurons infected by HSV-1.

WORKSHOP 5 *Viral Enzymes*

Chairperson: W. Summers, Yale University, New Haven, Connecticut

R. M. Flügel[1] and G. Darai,[2] [1]Institute of Virus Research, German Cancer Research Center; [2]Institute of Medical Virology, University of Heidelberg, Federal Republic of Germany: Specific phosphorylation of viral proteins by the protein kinase activity associated with the tree shrew herpesvirus.

R. M. Flügel[1] and G. Darai,[2] [1]Institute of Virus Research, German Cancer Research Center; [2]Institute of Medical Virology, University of Heidelberg, Federal Republic of Germany: Thymidine kinase induced by Tupaia herpesvirus.

J. S. Gronowitz and C. Källander, Dept. of Medical Virology, Biomedical Center, University of Uppsala, Sweden: dTk isozyme expression as the result of herpes virus infection.

L. M. Hutt-Fletcher,[1] R. J. Feighny,[2] E. Fowler,[2] and J. S. Pagano,[2] [1]Dept. of Comparative and Experimental Pathology, University of Florida, Gainesville; [2]Cancer Research Center, University of North Carolina, Chapel Hill: Phosphorylation of extracts from EBV receptor positive cells by an EBV-associated protein kinase.

K. Leary, B. Garrett, and B. Francke, Dept. of Human Genetics, Yale University, New Haven, Connecticut: Growth of HSV on hydroxyurea-resistant cells.

E. A. Cohen, J. Perret, and Y. Langelier, Institut du Cancer, Montreal, Canada: Ribonucleotide reductase induced by HSV.

D. Huszar and S. Bacchetti, Dept. of Pathology, McMaster University, Hamilton, Canada: HSV-induced ribonucleotide reductase—Development of antibodies specific for the enzyme.

J. D. Hall,[1] D. M. Coen,[2] and P. A. Schaffer,[2] [1]Dept. of Cellular and Developmental Biology, University of Arizona, Tucson; [2]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: An apparent antimutator phenotype in HSV maps in the DNA polymerase locus.

C. Knopf[1] and P. A. Schaffer,[2] [1]Institute of Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany; [2]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: HSV DNA polymerase associated 3′ exonuclease provides an editing function.

F. Wohlrab and B. Francke, Dept. of Human Genetics, Yale University, New Haven, Connecticut: Ther-

molability of the deoxypyrimidine triphosphatase induced by a *ts* mutant in complementation group 2–4.

WORKSHOP 6 *Cytomegaloviruses*

Chairperson: D. SPECTOR, University of California, San Diego, California

S. MCDONOUGH and D. SPECTOR, Dept. of Biology, University of California San Diego, La Jolla: Transcription of HCMV strain AD169 during the permissive infection.

S. STAPRANS and D. H. SPECTOR, University of California San Diego, La Jolla: Transcription from a region of the HCMV genome which contains sequences related to the avian retrovirus oncogene *myc*.

K.-T. JEANG and G. S. HAYWARD, Dept. of Pharmacology, Johns Hopkins University Medical School, Baltimore, Maryland: Regulation of CMV(Colburn) IE94 gene expression after infection of permissive and nonpermissive cells.

A. IRMIERE and W. GIBSON, Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins University Medical School, Baltimore, Maryland: Characterization of a novel virionlike particle from CMV-infected cell medium.

J. EPSTEIN, W. TURNER, and G. QUINNAN, Food and Drug Administration, Bethesda, Maryland: Immunogenic and surface glycoproteins of HCMV, strain AD169, identified by 1-D and 2-D polyacrylamide gel electrophoresis (PAGE).

M. G. DAVIS and E.-S. HUANG, Cancer Research Center, University of North Carolina, Chapel Hill: Distribution of repetitive sequences at the L-S repeats and L-S junction of HCMV DNA.

K. M. SCHEGG and S. C. ST. JEOR, Dept. of Microbiology, University of Nevada Medical School, Reno: Replicative intermediates of HCMV DNA.

D. H. SPECTOR, J. P. VACQUIER, S. STAPRANS, and S. MCDONOUGH, University of California San Diego, La Jolla: HCMV strain AD69 contains sequences related to the avian retrovirus onogene *myc*.

R. RÜGER and B. FLECKENSTEIN, Institut für Klinische Virologie, University of Erlangen-Nürnberg, Federal Republic of Germany: Repetitive cellular DNA in the HCMV genome.

L. EINHORN, H. GADLER, and B. WAHREN, Dept. of Virology, National Bacteriological Laboratory, Stockholm, Sweden: Adsorption of purified HCMV and induction of early antigens in different cells.

J. M. MIDDELDORP, L. DE LEIJ, and T. H. THE, Dept. of Clinical Immunology, University Hospital Groningen, The Netherlands: Monoclonal antibodies against HCMV-specific membrane antigens (CMV-MA).

SESSION 6 *Transcription*

Chairperson: C. M. PRESTON, Institute of Virology, Glasgow, Scotland

M. G. CORDINGLEY, M. E. M. CAMPBELL, and C. M. PRESTON, Institute of Virology, Glasgow, Scotland: Analysis of HSV-1 immediate early promoter sequences.

J. B. CLEMENTS, J. L. WHITTON, J. MCLAUCHLAN, and F. J. RIXON, Institute of Virology, University of Glasgow, Scotland: Signals involved in transcriptional regulation of HSV immediate-early and early genes.

K. G. DRAPER, R. J. FRINK, R. H. COSTA, and E. K. WAGNER, Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Comparative analysis of putative class-specific HSV-1 promoters.

R. J. FRINK and E. K. WAGNER, Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Detailed analysis of a family of HSV-1 mRNAs apparently related by splicing.

T. BECK, K. ENGSTROM, and R. MILLETTE, Dept. of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan: Transcription of HSV-1 genes in vitro by RNA polymerase II prepared from infected and uninfected HEp-2 cells.

J. BETZ,[1] T. HILL,[1] M. PEAKE,[2] J. SADLER,[1] and L. PIZER,[2] [1]Dept. of Biochemistry, Biophysics, and Genetics and [2]Dept. of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver: Transcripts from the short unique region of HSV-1.

M .F. STINSKI,[1] D. R. THOMSEN,[1] and L. C. GOLDSTEIN,[2] [1]Dept. of Microbiology, University of Iowa, Iowa City; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Organization and expression of the immediate early genes of HCMV.

R. M. STENBERG and M. F. STINSKI, Dept. of Microbiology, University of Iowa, Iowa City: Structural analysis of the major immediate early mRNA in HCMV infected cells.

K.-T. JEANG and G. S. HAYWARD, Dept. of Pharmacology, Johns Hopkins University Medical School,

Baltimore, Maryland: Characterization of the CMV(Colburn) IE94 gene and expression of the IE94 protein in transfected L-cells.

K.-T. JEANG and S. D. HAYWARD, Johns Hopkins University Medical School, Baltimore, Maryland: The *Not*I repeats in the EBV DNA molecule form part of an abundant transcript synthesized in the B95–8 cell line but are deleted from the genome of HR-1.

SESSION 7 *Gene Structure and Function*

Chairperson: W. SUMMERS, Yale University, New Haven, Connecticut

S. MACKEM and R. ROIZMAN, Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Functional and structural characterization of the promoter-regulatory regions of α genes of HSV-1.

G. S. READ,[1] J. R. SMILEY,[2] and W. C. SUMMERS,[1] [1]Yale University Medical School, New Haven, Connecticut; [2]McMaster University Medical Center, Hamilton, Canada: Analysis of the DNA sequences required for in vitro transcription of the tk gene of HSV.

J. R. SMILEY and M. HALPERN, Dept. of Pathology, McMaster University, Hamilton, Canada: Upstream DNA sequences required for positive control of HSV tk expression.

L. HAARR,[1] C. M. PRESTON,[2] and H. S. MARSDEN,[2] [1]Dept. of Biochemistry, University of Bergen, Norway; [2]MRC Institute of Virology, Glasgow, Scotland: Translation of tk mRNA is initiated at three separate AUG codons.

W. C. SUMMERS,[1] W. P. SUMMERS,[1] F. A. LASKI,[2] U. L. RAJBHANDARY,[2] P. A. SHARP,[2] and R. BELAGAJE,[3] [1]Yale University Medical School, New Haven, Connecticut; [2]Massachusetts Institute of Technology, Cambridge; [3]Lilly Research Laboratories, Indianapolis, Indiana: Suppression in mammalian cells of HSV tk nonsense mutations by translational suppressors contructed in vitro.

F. JENKINS, M. K. HOWETT, and F. RAPP, Dept. of Microbiology, Pennsylvania State University Medical College, Hershey: Detection by RNA blot hybridization of RNA sequences homologous to the *Bgl*II-N fragment of HSV-2 DNA.

L. E. HOLLAND, R. M. SANDRI-GOLDIN, M. LEVINE, and J. C. GLORIOSO, University of Michigan, Ann Arbor: Correlation of transcription and complementation group mapping for a region of the HSV-1 genome.

M. HUMMEL and E. KIEFF, University of Chicago, Illinois: Mapping of polypeptides encoded by EBV in productive infection.

W. P. SUMMERS,[1] E. A. GROGAN,[2] D. SHEDD,[2] M. ROBERT,[3] C.-R. LIU,[2] and G. MILLER,[2,3] [1]Dept. of Therapeutic Radiology; [2]Dept. of Epidemiology and Public Health; [3]Dept. of Pediatrics, Yale University School of Medicine, New Haven, Connecticut: Stable expression in mouse cells of nuclear neoantigen following transfer of a 3.4 megadalton cloned fragment of EBV DNA.

WORKSHOP 7 *HSV Glycoproteins*

Chairperson: L. PEREIRA, University of California, Berkeley, California

K. KOUSOULAS, D. DONDERO, P. BOEHME, and L. PEREIRA, California Dept. of Public Health, Berkeley: HSV glycoprotein gA/B—Purification and studies on two antigenic domains.

P. R. KINCHINGTON and. I. W. HALLIBURTON, Dept. of Microbiology, University of Leeds, England: Biochemical and antigenic comparisons of glycoprotein B of HSV-1 and -2.

D. J. BZIK, B. A. FOX, N. DeLUCA, and S. PERSON, Molecular and Cell Biology Program, Pennsylvania State University, University Park: Nucleotide sequence of a region of HSV-1 (KOS) DNA encoding phenotypes associated with glycoprotein B.

J. W. PALFREYMAN,[1] L. HAARR,[2] A. CROSS,[1] R. G. HOPE,[1] and H. S. MARSDEN,[1] [1]MRC Institute of Virology, Glasgow, Scotland; [2]Dept. of Biochemistry, University of Bergen, Norway: Processing of glycoproteins induced by HSV-1 and identification of a new and major glycosylated species.

B. NORRILD,[1] I. VIRTANEN,[2] B. PEDERSEN,[1] and L. PEREIRA,[3] [1]Institute of Medical Microbiology, University of Copenhagen, Denmark; [2]Dept. of Pathology, University of Helsinki, Finland; [3]Dept. of Health Services, University of California, Berkeley: Processing of the HSV-1 glycoproteins in infected cells.

V. MISRA and L. A. BABIUK, Western College of Veterinary Medicine, University of Saskatchewan, Canada: Bromovinyl deoxyuridine (BVdU) inhibits the glycosylation of herpesvirus glycoproteins.

R. KUMARASAMY and H. A. BLOUGH, Scheie Eye Institute, University of Pennsylvania, Philadelphia: Oligosaccharides of envelope glycoprotein gC of HSV-1.

S. OLOFSSON, S. JEANSSON, and E. LYCKE, Dept. of Virology, University of Göteborg, Sweden: The heterogeneity of O-linked carbohydrates associated with the HSV-1 glycoprotein gC.

R. MANSERVIGI,[1] M. TOGNON,[1] A. ROTOLA, [1] P. F. PIGNATTI,[2] and E. CASSAI,[1] [1]Institute of Microbiology, University of Ferrara; [2]Dept. of Animal Biology, Catania, Italy: Characterization of a syn mutant of HSV-1 (HFEM) defective in gA/gB dimer and gC accumulation.

R. A. RESPESS, B. A. PANCAKE, and P. A. SCHAFFER, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Lentil lectin-binding characteristics and immunologic specificity of HSV-1(KOS)- and HSV-2(186)-specified glycoproteins.

I. HIRSCH, A. SUCHÁNKOVÁ, and V. VONKA, Dept. of Experimental Virology, Institute of Sera and Vaccines, Prague, Czechoslavakia: A novel assay of type-specific antibodies in HSV.

M. ZWEIG, S. D. SHOWALTER, C. J. HEILMAN, JR., S. V. BLADEN, and B. HAMPAR, NCI, Frederick Cancer Research Facility, Frederick, Maryland: A monoclonal antibody against an HSV-2 75K MW glycoprotein that also reacts with HSV-1 glycoprotein gC.

WORKSHOP 8 *Genome Structure and Cloning*

Chairperson: D. J. O'CALLAGHAN, University of Mississippi, Jackson, Mississippi

A. J. DAVISON[1] and N. M. WILKIE,[2] [1]Institute of Virology; [2]Beatson Institute for Cancer Research, Glasgow, Scotland: Localization of homologous regions in the genomes of five members of the alphaherpesvirinae.

M. WATHEN,[1] W. T. RUYECHAN,[1] T. CASEY,[1] S. E. STRAUS,[1] D. J. O'CALLAGHAN,[2] and J. HAY,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Microbiology, University of Mississippi Medical School, Jackson: Sequence relationships between VZV DNA and the DNAs of other human and nonhuman herpesviruses.

J. C. RUIZ, J. B. DODGSON, and R. K. MAES, Dept. of Microbiology and Public Health, Michigan State University, East Lansing: Cloning and characterization of pseudorabies virus DNA sequences.

A. GIELKENS,[1] J. VAN OIRSCHOT,[1] and A. BERNS,[2] [1]Dept. of Virology, Central Veterinary Institute, Lelystad; [2]Laboratory of Biochemistry, University of Nijmegen, The Netherlands: Aujeszky's disease virus—Genetic variation of viral genomes and differentiation of field isolates and modified live virus vaccines.

D. SULLIVAN,[1] S. ATHERTON,[1] W. RUYECHAN,[2] S. DAUENHAUER,[1] and D. O'CALLAGHAN,[1] [1]Dept. of Microbiology, University of Mississippi Medical Center, Jackson; [2]USUHS, National Institutes of Health, Bethesda, Maryland: Physical structure and properties of the equine herpesvirus type 3(EHV-3) genome.

H.-G. KOCH,[1] H. DELIUS,[2] and G. DARAI,[1] [1]Institut für Medizinische Virologie, University of Heidelberg; [2]European Molecular Biology Laboratory, Heidelberg; Federal Republic of Germany: Molecular cloning and characterization of Tupaia herpesvirus DNA.

H. ROUHANDEH, R. COHRS, and A. VAFAI, Dept. of Microbiology, Southern Illinois University, Carbondale: Molecular biology of herpesvirus sylvilagus.

S. ST. JEOR,[1] B. SEAL,[1] and R. TAYLOR,[2] [1]School of Medicine; [2]School of Veterinary Medicine, University of Nevada, Reno: Characterization of infectious bovine rhinotracheitis virus isolates.

J. SKARE,[1] N. SULLIVAN,[2] and I. B. SKARE,[2] [1]Sidney Farber Cancer Institute, Boston; [2]Dept. of Biological Sciences, University of Lowell, Massachusetts: Cloning of bovine herpesvirus-1 DNA and localization of sequences in defective genomes.

S. T. PORTEOUS,[1] J. SKARE,[2] and I. B. SKARE,[1] [1]Dept. of Biological Sciences, University of Lowell; [2]Sidney Farber Cancer Institute, Boston, Massachusetts: Analysis of defective interfering particles of bovine herpesvirus-1.

K. FUKUCHI, M. SUTO, A. TANAKA, and M. NONOYAMA, Dept. of Virology, Showa University Research Institute for Biomedicine, Clearwater, Florida: Marek's disease virus DNA and expression of the viral genome.

WORKSHOP 9 *EBV Cellular Biology and Immunology*

Chairperson: D. THORLEY-LAWSON, Tufts University, Boston, Massachusetts

R. GLASER,[1] A. BOYD,[2] J. STOERKER,[1] and B. HAMPAR,[2] [1]Ohio State University, College of Medicine, Col-

umbus; [2]Frederick Cancer Research Facility, Frederick, Maryland: Microinjection of EBV DNA and cloned fragments into EBV-genome negative epithelial tumor cells and EBV-genome positive epithelial hybrid cells.

R. J. FEIGHNY,[1] L. M. HUTT-FLETCHER,[2] E. FOWLER,[1] and J. S. PAGANO,[1] [1]Cancer Research Center, University of North Carolina, Chapel Hill; [2]Dept. of Comparative and Experimental Pathology, University of Florida, Gainesville: Structural polypeptides of Epstein-Barr virions.

H. RABIN,[1] B. C. STRNAD,[1] R. F. HOPKINS, III,[1] M. NONOYAMA,[2] A. TANAKA,[2] and R. H. NEUBAUER,[1] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Showa University Research Institute for Biomedicine, St. Petersburg, Florida: Studies on EBV in cell lines established from P3HR-1 virus superinfected Raji cells.

A. J. MORGAN, J. R. NORTH, and M. A. EPSTEIN, Dept. of Pathology, University of Bristol Medical School, England: Purification, properties, and immunogenicity of the gp340 component of EBV membrane antigen (MA).

G. BAUER and H. ZUR HAUSEN, Institut für Virologie, Zentrum für Hygiene, University of Freiburg, Federal Republic of Germany: Induction of EBV antigens by a serum factor.

A. TANAKA, D. CASAREALE, S. SHIN, M. SMITH, and M. NONOYAMA, Dept. of Virology, Showa University Research Institute, Clearwater, Florida: Early events during blastogenic transformation of B-lymphocytes with EBV.

D. CASAREALE,[1] R. E. HUMPHREYS,[2] and M. NONOYAMA,[1] [1]Showa University Research Institute for Biomedicine, Clearwater, Florida; [2]University of Massachusetts Medical School, Worcester: Effect of lytic infection by EBV on HLA-DR synthesis.

R. SZIGETI,[1] G. KLEIN,[1] G. MASUCCI,[1] K. BENDTZEN,[2] B. EHLIN-HENRIKSEN,[1] and A. K. SAEMUNDSEN,[1] [1]Dept. of Tumor Biology, Karolinska Institutet, Stockholm, Sweden; [2]Laboratory of Clinical Immunology, Rigshospital, Copenhagen, Denmark: Studies of EBNA-induced leukocyte migration inhibition.

D. J. VOLSKY, Dept. of Pathology and Laboratory Medicine, University of Nebraska Medical Center, Omaha: New EBV-producer line established from EBV receptor-negative human lymphocytes.

R. KHELIFA and J. MENEZES, Pediatric Research Center, University of Montreal, Ste-Justine Hospital, Canada: A new approach for infecting EBV receptor-negative cells with EBV.

C. DESGRANGES,[1] J. M. SEIGNEURIN,[2] and J. PAIRE,[1] [1]Laboratory of Epidemiology and Immunovirology of Tumors, Faculty of Medicine, Alexis Carrel, Lyon; [2]Virology Laboratory, Grenoble, France: Production of human monoclonal antiviral antibodies by "EBV-immortalized" lymphoblastoid lines.

B. A. BLAZAR,[1] L. SUTTON,[1] M. STROME,[1] D. ARBIT,[2] and R. SCHOOLEY,[2] [1]Brigham and Women's Hospital; [2]Massachusetts General Hospital, Harvard Medical School, Boston: Interferon dependent and independent natural killing of human lymphoid lines following induction of Epstein-Barr viral cycle by P3HR-1 superinfection.

L. STERNÅS,[1] J. LUKA,[1] B. KALLIN,[1] A. ROSEN,[1] W. HENLE,[2] G. HENLE,[2] and G. KLEIN,[1] [1]Dept. of Tumor Virology, Karolinska Institutet, Stockholm, Sweden; [2]Joseph Stokes, Jr. Research Institute, Philadelphia, Pennsylvania: Enzyme linked immunosorbent assay (ELISA) for the detection of EBV-induced antigens.

B. KALLIN,[1] J. LUKA,[1] A. K. SAEMUNDSEN,[1] L. STERNÅS,[1] H. JÖRNVALL,[2] and G. KLEIN,[1] [1]Dept. of Tumor Biology; [2]Dept. of Medical Chemistry, Karolinska Institutet, Stockholm, Sweden: Isolation of EBV-binding polypeptides and characterization of associated enzymatic activities.

G. H. PI,[1] C. DESGRANGES,[2] G. BORNKAMM,[3] Y. ZENG,[1] and G. DE-THÉ,[2] [1]Institute of Virology, Chinese Academy of Science, Peking; [2]Faculty of Medicine, Alexis Carrel, Lyon, France; [3]Institut für Virologie, Zentrum für Hygiene, Freiburg, Federal Republic of Germany: Pre-NCP conditions—IgA/VAC antibodies do not relate to increase presence of EBV/DNA in NP mucosa.

G. J. BAYLISS and H. WOLF, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: Multiplicity dependence of EBV expression in P3HR1 superinfected Raji cells and infected BJA cells.

M. LOSTROM, Genetic Systems Corp., Seattle, Washington: Cell-driven viral transformation—An efficient method to prepare human monoclonal antibody secreting cell lines.

WORKSHOP 10 *Herpesvirus Transformation*

Chairperson: D. GALLOWAY, Fred Hutchinson Cancer Research Center, Seattle, Washington

M. PARK, H. C. KITCHENER, R. P. EGLIN, J. B. CLEMENTS, and J. C. M. MACNAB, Institute of Virology, Glasgow,

Scotland: Analysis of HSV DNA in oncogenically transformed rodent cells and cervical carcinoma tissue.

Y. EIZURU,[1] R. HYMAN,[1] J. KREIDER,[2] and F. RAPP,[1] [1]Dept. of Microbiology and [2]Dept. of Pathology, Pennsylvania State University College of Medicine, Hershey: Map positions of HSV RNA in an HSV-1-transformed hamster cell line.

F. M. VAN DEN BERG,[1] A. J. VAN OOYEN,[2] and J. M. M. WALBOOMERS,[1] [1]Section for Experimental Oncology, University of Amsterdam; [2]Dutch Cancer Institute, Section for Virology, Amsterdam, The Netherlands: Structural and biological analysis of molecularly cloned HSV-2 DNA fragments covering the entire virus genome.

M. SUH,[1] C. CHAUVIN,[2] M. FILION,[1] G. SHORE,[3] and E. FROST,[1] [1]Institut du Cancer de Montréal, Centre Hospitalier Notre-Dame, Canada; [2]Centre d'Etude et de Recherches sur les Macromolécules Organisées, Grenoble, France; [3]Dept. of Biochemistry, McGill University, Montreal, Canada: Localization of 35K polypeptide in HSV-2 infected cells with the *Bgl*II "N" fragment.

J. K. MCDOUGALL,[1] H. K. TAMIMI,[2] P. SMITH,[1] E. TOLENTINO,[1] and D. A. GALLOWAY,[1] [1]Fred Hutchinson Cancer Research Center; [2]Dept. of Obstetrics and Gynecology, University of Washington, Seattle: HSV DNA in genital neoplasia.

J. R. SCHLEHOFER and H. ZUR HAUSEN, Institut für Virologie, University of Freiburg, Federal Republic of Germany: Host cell genome mutations induced by infection with inactivated HSV-1.

M. MIZELL,[1] M. A. DIBERARDINO,[2] P. W. SIMONEAUX, D. G. FRIESENDORF,[1] and N. J. HOFFNER,[2] [1]Laboratory of Tumor Cell Biology, Tulane University, New Orleans, Louisiana; [2]Dept. of Physiology and Biochemistry, Medical College of Pennsylvania, Philadelphia: Sequential transformation in frog embryos using the Lucke' tumor herpesvirus—Frog larvae from cloned tumor nuclei.

J. STACZEK and D. J. O'CALLAGHAN, University of Mississippi Medical Center, Jackson: Detection of ECMV DNA and antigens in transformed and tumor cell lines.

SESSION 8 *Immunology*

Chairperson: B. SUGDEN, University of Wisconsin, Madison, Wisconsin

A. A. NASH, Dept. of Pathology, University of Cambridge, England: Lyt phenotype of effector and suppressor T cells involved in delayed type hypersensitivity and immunity to HSV.

S. R. JENNINGS, P. L. RICE, R. W. ANDERSON, and S. S. TEVETHIA, Pennsylvania State University College of Medicine, Hershey: Reduction of class I major histocompatability antigens (H-2) on mouse cells infected with HSV and its consequences on H-2 restricted lysis by cytotoxic T lymphocytes.

B. PEDERSEN, H. J. KREBS, and B. NORRILD, Institute of Medical Microbiology, University of Copenhagen, Denmark: The effect of Monensin on target and effector cells participating in antibody-dependent cell-mediated immunecytolysis of HSV-1 infected cells.

G. A. BISHOP,[1] S. A. SCHWARTZ,[2] and J. C. GLORIOSO,[3] [1]Graduate Program in Cellular and Molecular Biology; [2]Depts. of Epidemiology and Pediatrics; and [3]Unit for Laboratory Animal Medicine, Universi-

J. McDougall

Coffee break

ty of Michigan, Ann Arbor: Characterization of human natural killer cell activity against target cells infected with HSV.

Y. GORDON,[1] B. JOHNSON,[2] P. SIMON,[1] J. ARMSTRONG,[3] and S. BROWN,[1] [1]Dept. of Opthalmology, [2]Dept. of Pathology, and [3]Dept. of Microbiology, Graduate School of Public Health, University of Pittsburgh, Pennsylvania: The effect of immunosupression on HSV-1 TK$^-$ CNS avirulence.

A. B. RICKINSON, I. HART, M. ROWE, and M. A. EPSTEIN, Dept. of Pathology, University of Bristol Medical School, England: T cell control of EBV infection—Studies with cyclosporin A.

S. F. SLOVIN,[1] R. SCHOOLEY,[2] and D. A. THORLEY-LAWSON,[1] [1]Dept. of Pathology, Tufts University Medical School; [2]Division of Infectious Disease, Massachusetts General Hospital, Boston, Massachusetts: Cloned cytotoxic T-cell lines against EBV LYDMA-complexity of the response and HLA restriction.

B. SUGDEN and S. METZENBERG, McArdle Laboratory, University of Wisconsin, Madison: An analysis of EBV-cell surface (EBVCS) determinants.

D. THORLEY-LAWSON,[1] R. SCHOOLEY,[2] and L. NADLER,[3] [1]Dept. of Pathology and Medicine, Tufts University Medical School; [2]Division of Infectious Diseases, Massachusetts General Hospital; [3]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: EBV superinduces a new human B cell differentiation antigen (B-LAST 1) expressed on transformed lymphoblasts.

W. FREDERICK,[1] H. MASUR,[2] A. ROOK,[1] K. MITTAL,[1] J. MANISCHEWITZ,[1] L. JACKSON,[1] S. STRAUS,[2] and G. QUINNAN, JR.,[1] [1]FDA and [2]NIAID, National Institutes of Health, Bethesda, Maryland: Immune functions during CMV infection in immunodeficient male homosexuals.

SESSION 9 *Gene Transfer and Expression*

Chairperson: M. LEVINE, University of Michigan, Ann Arbor, Michigan

S. L. BACHENHEIMER and S. J. SILVERSTEIN, Dept. of Microbiology, Columbia University College of Physicians and Surgeons, New York, New York: Transfer and expression of HSV immediate-early genes to mammalian cells.

J. C. LANG[1] and N. M. WILKIE,[2] [1]Institute of Virology; [2]Beatson Institute for Cancer Research, Glasgow, Scotland: The use of hybrid tk genes as an assay for eukaryote promoters and enhancer sequences.

Y. BECKER, Y. SHTRAM, Y. ASHER, and E. TABOR, Faculty of Medicine, Hebrew University, Jerusalem, Israel: Role of bacterial, viral, and cellular promoters in the expression of HSV-1 tk gene in bacterial and mammalian host cells.

J. R. ARRAND and E. HARLOW, Imperial Cancer Research Fund Laboratories, London, England: Expression in *E. coli* of fusion proteins containing herpesvirus specified peptides.

A. M. COLBERG-POLEY,[1] C. MARCUS-SEKURA,[1] M. WEST,[1] B. CARTER,[1] and L. W. ENQUIST,[2] [1]NIADDK, National Institutes of Health, Bethesda, Maryland; [2]Molecular Genetics, Inc., Minnetonka, Minnesota: Expression of the HSV-1 gD gene in *Xenopus* oocytes.

R. J. WATSON, J. H. WEIS, and L. W. ENQUIST, Molecular Genetics, Inc., Minnetonka, Minnesota: Genome localization, DNA sequence and expression in *E. coli* of the HSV-1 glycoprotein D gene.

R. R. SPAETE, L. P. DEISS, and N. FRENKEL, Dept. of Biology, University of Chicago, Illinois: Construction of eukaryotic cloning vectors containing *cis* replication functions of HSV.

D. COEN,[1] M. MANOS,[2,4] R. MULLIGAN,[3] L. MCREYNOLDS,[4] and P. A. SCHAFFER,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]Cold Spring Harbor Laboratory, New York; [3]Cancer Research Center, Massachusetts Institute of Technology, Cambridge; [4]Dept. of Biochemistry, University of Arizona, Tuscon: Introduction of an intron and other DNA modifications into HSV using a cloned mutant tk gene.

L. RYMO, Dept. of Clinical Chemistry, University of Gothenburg, Sahlgran's Hospital, Sweden: Transformation of lymphoid cells with vectors that contain the gene for the *E. coli* enzyme xanthine-guanine phosphoribosyltransferase and defined segments of the EBV genome.

G. R. REYES, E. R. GAVIS, D. CIUFO, P. PITHA, and G. S. HAYWARD, Johns Hopkins Medical School, Baltimore Maryland: Studies of HSV gene regulation using a hybrid tk-interferon gene and microinjection into *Xenopus* oocytes.

R. M. SANDRI-GOLDIN, A. L. GOLDIN, L. E. HOLLAND, M. LEVINE, and J. C. GLORIOSO, University of Michigan, Ann Arbor: Expression of nonselected HSV-1 sequences in cell lines transformed by protoplast fusion gene transfer.

SESSION 10 *Antivirals*

Chairperson: C. SHIPMAN, Yale University, New Haven, Connecticut

M. J. OTTO and W. H. PRUSOFF, Yale University Medical School, New Haven, Connecticut: Production of interfering particles during infection of Vero cells by HSV-1 in the presence of AIdUrd (5-IODO-5'-AMINO-2',5'-dideoxyuridine) or IdUrd (5-IODO-2'-deoxyuridine).

D. M. COEN, D. P. ASCHMAN, P. T. GELEP, S. K. WELLER, and P. A. SCHAFFER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Cloning and fine structural mapping of mutations in or near the HSV DNA polymerase locus which confer altered sensitivity to antiviral drugs, temperature and aphidicolin.

C. CRUMPACKER,[1] H. S. ALLAUDEEN,[2] and L. E. SCHNIPPER,[1] [1]Dept. of Medicine, Beth Israel Hospital, Boston, Massachusetts; [2]Dept. of Pharmacology, Yale School of Medicine, New Haven, Connecticut: Physical mapping of mutations conferring resistance of purified herpes DNA polymerase to nucleoside triphosphate analogues.

L. SCHNIPPER,[1] C. CRUMPACKER,[1] H. S. ALLAUDEEN,[2] and D. KUFE,[1] [1]Harvard Medical School, Boston, Massachusetts; [2]Yale School of Medicine, New Haven, Connecticut: Mechanism of resistance to arabinofuranosyl derivatives in a mutant herpesvirus.

A. K. DATTA and J. S. PAGANO, Cancer Research Center, University of North Carolina, Chapel Hill: Phosphorylation of acyclovir [9-(2-hydroxyethoxymethyl) guanine] in vitro in activated Burkitt somatic cell.

P. C. PATEL, E.-C. MAR, Y.-C. CHENG, and E.-S. HUANG, Cancer Research Center, University of North Carolina, Chapel Hill: Antiviral activities of deoxyuridine and deoxycytidine analogues on HCMV.

B. ERIKSSON AND B. ÖBERG, Astra Läkemedel AB, Dept. of Antiviral Chemotherapy, Södertälje, Sweden: Different susceptibilities of herpesvirus DNA polymerases to pyrophosphate analogs.

C. SHIPMAN, JR., S. H. SMITH, S. R. TURK, C. M. REINKE, and J. C. DRACH, University of Michigan, Ann Arbor: 2-acetylpyridine thiosemicarbazones—A new class of compounds active against HSV-1 and 2.

W. GIBSON, R. VANBREEMEN, A. FIELDS, and R. LAFEMINA, Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins Medical School, Baltimore, Maryland: Alpha-difluoromethylornithine inhibits CMV replication.

H. GADLER, Dept. of Virology, National Bacteriology Laboratory, Stockholm, Sweden: Use of nucleic acid hybridization for studies on the efficacy of antiviral compounds.

SESSION 11 *Genetics (Gene Location)*

Chairperson: P. A. SCHAFFER, Harvard University Medical School, Boston, Massachusetts

N. DELUCA, D. J. BZIK, V. C. BOND, W. SNIPES, and S. PERSON, Molecular and Cell Biology Program, Pennsylvania State University, University Park: Genetic domains of the HSV-1 (KOS) genome affecting virus entry, cell fusion and the production of gB.

W.-C LEUNG, Dept. of Medicine, University of Alberta, Canada: In vitro mutagenesis of a HSV regulatory protein ICP4.

S. K. WELLER,[1] R. A. F. DIXON,[2] K. J. LEE,[1] D. J. SABOURIN,[1] and P. A. SCHAFFER,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]Johns Hopkins University Medical School, Baltimore, Maryland: Genetic analysis of *ts* mutants which define the gene for the major HSV DNA-binding protein.

B. A. PANCAKE, D. P. ASCHMAN, and P. A. SCHAFFER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Genetic and phenotypic analysis of HSV-1 *ts* mutants resistant to immune cytolysis—Identification of four new HSV-1 complementation groups.

S. PERSON, N. DELUCA, V. C. BOND, D. J. BZIK, and W. SNIPES, Molecular and Cell Biology Program, Pennsylvania State University, University Park: Regions of the HSV-1 (KOS) genome affecting cell fusion and the production of gB and gC.

S. D. MARLIN,[1] T. C. HOLLAND,[2] M. LEVINE,[2] and J. GLORIOSO,[3] [1]Graduate Program in Cellular and Molecular Biology, [2]Dept. of Human Genetics, and [3]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor: Antigenic variants of HSV-1 selected with glycoprotein specific monoclonal antibodies.

V. G. PRESTON, B. MATZ, and F. J. RIXON, Institute of Virology, Glasgow, Scotland: Identification and

characterization of an HSV-1 gene which is required for packaging virus DNA into empty nucleocapsids.

B. M. DUTIA, MRC Virology Unit, Institute of Virology, Glasgow, Scotland: The genetic control of the ribonucleotide reductase activity induced by HSV-1 and HSV-2.

B. FRANCKE and B. GARRETT, Dept. of Human Genetics, Yale University, New Haven, Connecticut: In vitro complementation of *ts* DNA mutants of HSV-1.

10th Cold Spring Harbor Conference on Cell Proliferation
Teratocarcinoma Stem Cells

September 8–September 12

Arranged by **Lee Silver**, Cold Spring Harbor Laboratory, **Gail Martin**, University of California, San Francisco, **Sidney Strickland**, The Rockefeller University

131 participants

This meeting represented the first time in seven years that an open international forum had been devoted entirely to the teratocarcinoma system. The last such meeting was held at the Roche Institute of Molecular Biology in Nutley, New Jersey, and much progress in this field has been made since that time. We were pleased to have in attendance representatives of almost every laboratory in the world in which teratocarcinoma stem cell biology is being studied. This field has its roots in classical embryology and has long been of interest to those concerned with the problem of tumorigenesis. The program of this meeting, however, reveals the extend to which embryonal/embryonic stem cells have become experimental material for investigators with a diversity of primary scientific interests Embryology, tumorigenesis, virology, carbohydrate chemistry, DNA methylation, and eukaryotic vectors were among the topics discussed. It was gratifying to find that there was a free flow of ideas, catalyzed by the beautiful weather and relaxed atmosphere, among scientists using techniques as diverse as descriptive morphology and molecular cloning of DNA. The proceedings of this meeting will be published in 1983 as volume 10 in *Cold Spring Harbor Conferences on Cell Proliferation: Teratocarcinoma Stem Cells.*

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

SESSION 1 *Introduction*

S. GLUECKSOHN-WAELSCH, Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Genetic control of differentiation.

G. B. PIERCE and R. WELLS, Dept. of Pathology, University of Colorado Health Sciences Center, Denver: Malignancy and differentiation—The role of trophectoderm in control of colony formation.

L. C. STEVENS, Jackson Laboratory, Bar Harbor, Maine: Origin and development of testicular, ovarian, and embryo-derived teratomas.

SESSION 2 *In Vitro Differentiation*

Chairperson: G. MARTIN, University of California, San Francisco, California

R. KEMLER, Friedrich-Miescher-Laboratorium, Max-Planck-Gesellschaft, Tübingen, Federal Republic of Germany: Differentiation induction of embryonal carcinoma cells—Cell type analysis with monoclonal antibodies.

H. Fox, M. Krangel, G. Martin, L. Silver, N. Sarvetnick

J. Rossant, V. Papaioannou, H. Axelrod, C. Webb, R. Kemler, I. Damjanov

R. G. OSHIMA,[1] J. M. TABOR,[2] W. H. HOWE,[1] and E. ADAMSON,[1] [1]La Jolla Cancer Research Foundation, California; [2]Bristol-Myers Company, Syracuse, New York: Identification of extra-embryonic endodermal cytoskeletal proteins and mRNAs in retinoic acid-treated embryonal carcinoma cells and blastocyst stage embryos.

D. TALMAGE and S. STRICKLAND, Rockefeller University, New York, New York: Disappearance of an HMG-like protein following retinoic acid treatment of F9 cells.

E. D. ADAMSON and A. GROVER, La Jolla Cancer Research Foundation, California: The production and maintenance of a functioning epithelial layer from embryonal carcinoma cells.

A. RIZZINO, Laboratory of Viral Carcinogenesis, NCI, Frederick, Maryland: Differentiation of three multipotent embryonal carcinoma cell lines into a single cell type—The use of defined media and the role of fibronectin and laminin.

M. DARMON[1] and G. SERRERO,[2] [1]Institut Pasteur, Paris, France; [2]Dept. of Biology, University of California, San Diego, La Jolla: Isolation of two different fibroblastic cell types from the embryonal carcinoma cell line 1003—Study of tumorigenic properties, surface antigens, and differentiation responses to 5-azacytidine and dexamethasone.

M. W. MCBURNEY,[1] E. M. V. JONES-VILLENEUVE,[2] and M. K. S. EDWARDS,[2] Depts. of [1]Medicine and [2]Biology, University of Ottawa, Ontario, Canada: Controlled differentiation of an embryonal carcinoma cell line.

W. B. ANDERSON and A. S. KRAFT, NCI, National Institutes of Health, Bethesda, Maryland: Effect of retinoic acid and phorbol ester treatment of embryonal carcinoma cells on calcium, phospholipid-dependent protein kinase activity.

M.J. SHERMAN, M.L. PATERNOSTER, M.A. EGLITIS, and P.A. MCCUP, Roche Institute of Molecular Biology, Nutley, New Jersey: Studies on the mechanism by which chemical inducers promote differentiation of embryonal carcinoma cells.

SESSION 3 *Poster Session*

S. AIZAWA,[1,3] L. A. LOEB,[2] and G. M. MARTIN,[1] [1]Division of Genetic Pathology; [2]Joseph Gottstein Memorial Cancer Research Laboratory, Dept. of Pathology, University of Washington, Seattle; [3]Dept. of Biochemistry, Tokyo Metropolitan Institute of Gerontology, Japan: Isolation and preliminary characterization of mouse teratocarcinoma cells resistant to aphidicolin and arabinofuranosyl cytosine.

S.-Y. WANG[1,2] and L. J. GUDAS,[1,2] [1]Sidney Farber Cancer Institute; [2]Dept. of Pharmacology, Harvard Medical School, Boston, Massachusetts: Selection of F9 stem cell mutants with altered responses to retinoic acid.

S. KARTHA and J. S. FELIX, Developmental Genetics Laboratory, Dept. of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Cell surface interactions of H6 teratocarcinoma cells.

G. D. PATERNO and M. M. MCBURNEY, Depts. of Biology and Medicine, University of Ottawa, Ontario, Canada: X chromosome inactivation in embryonal carcinoma cells.

E. GOOTWINE and C. G. WEBB, Dept. of Hormone Research, Weizmann Institute of Science, Rehovot, Israel: Embryo-derived teratomas in Wistar rats—Characterization of transplantable tumors and cell lines.

A. GIALLONGO, K. B. TAN, C. GREEN, and C. M. CROCE, Wistar Institute, Philadelphia, Pennsylvania: Monoclonal antibodies to chromosomal proteins and teratocarcinoma stem cell surface antigens.

J. F. HARRIS,[1,2] J. CHIN,[1] N. KARTNER,[1] and M. A. S. JEWETT,[2] Depts. of [1]Medical Biophysics and [2]Surgery, University of Toronto, Ontario, Canada: Characterization of two monoclonal antibodies against mouse embryonal carcinoma cells.

B. FENDERSON, A. HAHNEL, and E. M. EDDY, Dept. of Biological Structure, University of Washington, Seattle: Patterns of cell surface carbohydrate expressions in early mouse embryos detected with monoclonal antibodies.

F. FUJIMURA, La Jolla Cancer Research Foundation, California: Polyoma mutant DNA replication in F9 embryonal carcinoma cells.

K. SEKIKAWA, Dept. of Molecular Biology, Cancer Institute, Sapporo Medical College, Japan: *cis*-acting regulatory mutation for early and late gene expression of polyoma virus on nullipotential embryonal carcinoma cells.

J. CAMPIONE-PICCARDO and M. W. MCBURNEY, Dept. of Medicine, University of Ottawa, Canada: Experimental and theoretical kinetics of the response of embryonal cells to retinoic acid.

W. DeWolf and P. Carroll, Divisions of Immunogenetics, Sidney Farber Cancer Institute and Cellular Genetics, Charles A. Dana Research Institute, Boston, Massachusetts: An immunological profile characterizing human teratocarcinoma cell differentiation.

T. Ducibella,[1] J. Aalberg,[1] D. Anderson,[2] and W. C. DeWolf,[2] [1]Dept. of Anatomy, Tufts University School of Medicine; [2]Division of Immunogenetics, Sidney Farber Cancer Institute, Boston, Massachusetts: Human teratocarcinoma embryoid bodies are characterized by cell surface polarization, tight junctions, and eccentric inner cells.

P. Narayan,[1] C. Millette,[2] and W. DeWolf,[1] [1]Dept. of Cellular Genetics, Beth Israel Hospital; [2]Dept. of Anatomy and Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts: Biochemical characterization of the stage specific proteins in normal human germ cells and human teratocarcinoma.

C. L. Stewart, D. Jähner, H. Stuhlmann, and R. Jaenisch, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Universität Hamburg, Federal Republic of Germany: De novo methylation, expression, and infectivity of retroviral genomes introduced into early mouse embryonic cells.

C. M. Isacke and J. K. Heath, CRC Developmental Tumour Group, Dept. of Zoology, University of Oxford, England: Secretion of growth regulatory molecules by PC13 embryonal carcinoma cells.

SESSION 4 *Cell Surface Molecules*

Chairperson: F. Jacob, Institut Pasteur, Paris, France

L. B. Grabel, M. S. Singer, S. D. Rosen, and G. R. Martin, Dept. of Anatomy, University of California, San Francisco: The role of carbohydrates in the intercellular adhesion and differentiation of teratocarcinoma stem cells.

M. Takeichi,[1] C. Yoshida-Noro,[1] S. Ogou,[1] Y. Shirayoshi,[1] and J. Wartiovaara,[2] [1]Dept. of Biophysics, Kyoto University, Japan; [2]Dept. of Electron Microscopy, University of Helsinki, Finland: A teratocarcinoma cell-cell adhesion molecule and its roles in embryonic cellular interactions.

T. Muramatsu, Dept. of Biochemistry, Kagoshima University School of Medicine, Japan: The high-molecular-weight carbohydrates found in teratocarcinoma stem cells and early embryos.

B. D. Shur, Dept. of Anatomy, University of Connecticut Health Center, Farmington: The role of cell surface galactosyltransferase in embryonal carcinoma cell adhesion.

C. Howe, B. Dietzschold, and D. Solter, Wistar Institute, Philadelphia, Pennsylvania: Laminin as a marker for differentiation in teratocarcinoma cell system.

K. Willison,[1] A. Suzuki,[2] R. Karol,[3] S. Kundu,[3] and D. Marcus,[3] [1]Institute of Cancer Research, Chester Beatty Research Institute, London, England; [2]Tokyo Metropolitan Institute of Medical Science, Japan; [3]Baylor College of Medicine, Houston, Texas: Neutral glycolipid antigens of mouse teratocarcinoma and early embryos.

L. J. Gudas,[1,2] J. P. Singh,[1,3] and C. Stiles,[1,3] [1]Sidney Farber Cancer Institute; Depts. of [2]Pharmacology and [3]Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Growth response and binding of PDGF to mouse teratocarcinoma cells.

S. C. Chua and M. F. Maylie-Pfenninger, Dept. of Anatomy and Cell Biology, Columbia University, New York, New York: A 105-kD cell surface component is critical for mouse preimplantation development.

SESSION 5 *DNA Viruses*

Chairperson: A. Levine, State University of New York, Stony Brook, New York

M. Vasseur and C. Marle, Unité de Génétique Cellulaire du Collège de France et de l'Institut Pasteur, Paris: Properties of F9 cells chronically infected with polyoma EC virus.

E. Linney, S. Donerly, B. Olinger, M. Bender, and F. Fujimura, La Jolla Cancer Research Foundation, Cancer Research Center, California: Expression of genes transferred into teratocarcinoma cells.

P. Herbomel, B. de Crombrugghe, and M. Yaniv, Institut Pasteur, Paris, France: Recombinant vectors to study controlling elements of polyoma virus expression in EC cells.

K. Tanaka,[1] K. Chowdhury,[2] K. S. S. Chang,[1] M. Israel,[2] and Y. Ito,[2] [1]NCI; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Differentiation-stage-dependent polarity of the growth of polyoma virus mutants capable of growing in embryonal carcinoma and trophoblast cells.

F. KELLY,[1] C. BABINET,[1] and K. WILLISON,[2] [1]Institut Pasteur, Paris France; [2]Institute of Cancer Research, London, England: Studies on interactions between EC cells and early mouse embryo cells with SV40.

M. TAKETO, J. J. KOPCHICK, P. A. MCCUE, D. STACEY, and M. I. SHERMAN, Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: Studies on SV40 T antigen expression in wild-type and differentiation-defective murine embryonal carcinoma cell lines.

J. M. LEHMAN, K. TREVOR, M. ROSENBERG, and K. HELD, Dept. of Pathology, University of Colorado School of Medicine, Denver: Teratocarcinoma—A model for virus-cell interactions.

SESSION 6 *Retroviruses*

Chairperson: R. JAENISCH, Heinrich-Pette Institut Universität Hamburg, Federal Republic of Germany

K. HUEBNER,[1] A. LINNENBACH,[2] P. GHOSH,[2] H. ROMANCZUK,[1] A. AR-RUSHDI,[1] N. TSUCHIDA,[3] and C.CROCE,[1] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Dept. of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut; [3]Yakult Central Institute for Microbiological Research, Tokyo, Japan: Regulation of expression of oncogenic viral genomes in DNA-transformed F9 cells.

J. W. GAUTSCH and M. C. WILSON, Research Institute of Scripps Clinic, La Jolla, California: Restriction of M-MuLV growth in teratocarcinoma—Involvement of factors other than DNA methylation.

C. HAMMOND,[1] J. M. BISHOP,[1] and G. R. MARTIN,[2] Depts. of [1]Microbiology and Immunology and [2]Anatomy, University of California, San Francisco: Moloney murine leukemia viral expression in undifferentiated and differentiated teratocarcinoma cells.

R. JAENISCH, K. HARBERS, D. JÄHNER, J. LÖHLER, C. STEWART, and H. STUHLMANN, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Universitat Hamburg, Federal Republic of Germany: Expression of retroviral genomes introduced into early mouse embryos.

SESSION 7 *Differentiation Studies with Cloned DNA Probes*

Chairperson: S. STRICKLAND, The Rockefeller University. New York, New York

K. R. MAROTTI, D. A. TALMAGE, and S. STRICKLAND, Rockefeller University, New York, New York: Specific induction of gene expression during differentiation.

B. HOGAN, M. KURKINEN, A. COOPER, and D. BARLOW, Imperial Cancer Research Fund, Mill Hill Laboratories, London, England: Gene expression during endoderm differentiation.

P. BRÛLET, Unité de Génétique Cellulaire du Collège de France et de l'Institut Pasteur, Paris: Molecular probes to study the first differentiation in the mouse embryo.

M. H. BUC-CARON,[1] J. GAILLARD,[1] M. DARMON,[1] D. PAULIN,[1] C. SELLEM,[2] and J. SALA TREPAT,[2] [1]Génétique Cellulaire, Institut Pasteur, Paris; [2]Laboratoire d'Enzymologie du CNRS, France: Analysis of AFP and albumine gene expression in teratocarcinoma cells.

D. MORELLA, G. GACHELIN, and P. KOURILSKY, Unité de Biologie Moléculaire du Gène, CNRS and INSERM, Institut Pasteur, Paris, France: The expression of H-2 and $\beta 2$ microglobulin genes is controlled at the level of transcription.

P. N. GOODFELLOW,[1] P. W. ANDREWS,[2] M. V. WILES,[1] M. QUINTERO,[1] and F. BENHAM,[1] [1]Imperial Cancer Research Fund, London, England; [2]Wistar Institute, Philadelphia, Pennsylvania: Developmental genetics of MHC expression using human-mouse hybrid cell lines.

R. MÜLLER,[1] D. J. SLAMON,[2] E. ADAMSON,[3] J. M. TREMBLAY,[1] M. J. CLINE,[2] and I. M. VERMA,[1] [1]Tumor Virology Laboratory, Salk Institute, San Diego; [2]Dept. of Medicine, University of California, Los Angeles; [3]La Jolla Cancer Research Foundation, California: Expression of cellular oncogenes during pre- and postnatal development of the mouse and in embryonal carcinoma cell lines.

J. F. NICOLAS and P. BERG, Dept. of Biochemistry, Stanford University Medical Center, California: Expression of genes introduced into embryonal carcinoma cells (EC) by cotransformation.

J. JAMI, D. BUCCHINI, C. LASSERRE, R. LOVELL-BADGE, J. THILLET, F. KUNST, and R. PICTET, Institut de Recherche en Biologie Moléculaire, Paris, France: Stable transformation of embryonal carcinoma cells with the dominant marker *Eco.gpt* does not impair their developmental potentialities.

SESSION 8 *Genetics*

Chairperson: L. SILVER, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

I. DAMJANOV,[1] O. BAGASRA,[1] and D. SOLTER,[2] [1]Dept. of Pathology, Hahnemann University; [2]Wistar In-

stitute, University of Pennsylvania, Philadelphia: Genetic and epigenetic factors regulate the malignancy of embryo-derived teratomas.

S. OSTRAND-ROSENBERG, A. L. COHN, J. W. SANDOZ, M. L. SCHWARTZMAN, and C. MCCARTHY, Dept. of Biological Sciences, University of Maryland, Catonsville: Host regulation of major histocompatibility complex (MHC) antigens on 402AX teratocarcinoma cells during tumor rejection.

L. L. JOHNSON, A. SHEDLOVSKY, L. CLIPSON, and W. F. DOVE, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The genetics of allograft rejection of teratocarcinoma cell lines.

J. FOREJT, Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Czechoslovakia: Expression of T-t and H-2 genes in teratocarcinoma cells.

T. BOON, A. VAN PEL, and F. VESSIÈRE, Ludwig Institute for Cancer Research, Brussels, Belgium: Tumor cell variants with increased immunogenicity obtained by mutagen treatment.

M. J. ROSENSTRAUS, Dept. of Biological Sciences and Bureau of Biological Research, Rutgers, State University, New Brunswick, New Jersey: Isolation and characterization of mutant embryonal carcinoma cells lacking stage specific embryonic antigen 1.

L. NAGARAJAN, A. M. JETTEN, and W. B. ANDERSON, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of a new endodermal cell line (DIF 5) derived from retinoic acid-treated F9 teratocarcinoma cells.

SESSION 9 *Human Teratocarcinoma Workshop*

Chairperson: P. W. ANDREWS, The Wistar Institute, Philadelphia, Pennsylvania

P. W. ANDREWS,[1] P. N. GOODFELLOW,[2] and D. L. BRONSON,[3] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Imperial Cancer Research Fund, London, England; [3]University of Minnesota, Minneapolis: Cell surface characteristics and other markers of differentiation of human teratocarcinomas in culture.

Y. FRADET,[1] A. HOUGHTON,[1] G. BOSL,[1] D. BRONSON,[2] W. WHITMORE, JR.,[1] and L. J. OLD,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]University of Minnesota, Minneapolis: Cell surface antigens of human teratocarcinoma cell lines analyzed by monoclonal antibodies.

D. L. BRONSON,[1] P. W. ANDREWS,[2] R. L. VESSELLA,[1] and E. E. FRALEY,[1] [1]University of Minnesota Medical School, Minneapolis; [2]Wistar Institute, Philadelphia, Pennsylvania: In vitro differentiation of human embryonal carcinoma cells.

N. J. VOGELZANG,[1] D. L. BRONSON,[2] D. SAVINO,[2] and E. E. FRALEY,[2] [1]University of Chicago, Illinois; [2]University of Minnesota, Minneapolis: An in vivo and in vitro model of human embryonal and yolk sac carcinoma.

P. CARROLL,[1] W. DEWOLF,[2] and G. SCHWARTING,[3] [1]Tufts University, Boston; [2]Beth Israel Hospital, Boston; [3]Eunice Kennedy Shriver Center, Waltham, Massachusetts: Glycosphingolipids of human teratocarcinoma.

R. Jaenisch

F. Jacob, B. Pierce

S. Strickland

SESSION 10 *Embryos and Embryonic Stem Cell Lines*

Chairperson: V. E. PAPAIOANNOU, Tufts University, Boston, Massachusetts

J. ROSSANT[1] and M. W. MCBURNEY,[2] [1]Dept. of Biological Sciences, Brock University, St. Catharines; [2]Dept. of Medicine, University of Ottawa, Ontario, Canada: Euploid teratocarcinoma cell lines differ in their ability to differentiate normally after blastocyst injection.

G. R. MARTIN and L. F. LOCK, Dept. of Anatomy, University of California, San Francisco: Pluripotent cell lines derived from early mouse embryos.

M. J. EVANS, A. BRADLEY, E. J. ROBERTSON, and M. H. KAUFMAN. Depts. of Genetics and Anatomy, University of Cambridge, England: Isolation, properties, and karyotypes of pluripotential (EK) cells from normal and parthenogenetic mouse embryos.

H. R. AXELROD[1] and D. BENNETT,[2] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Sloan-Kettering Institute for Cancer Research, New York, New York: A simplified method for obtaining embryonic stem cell lines from blastocysts.

T. MAGNUSON,[1] G. R. MARTIN,[2] L. M. SILVER,[3] and C. J. EPSTEIN,[1] Depts. of [1]Pediatrics and [2]Anatomy, University of California, San Francisco; [3]Cold Spring Harbor Laboratory, New York: Studies of the viability of t^{w5}/t^{w5} embryonic cells in vitro and in vivo.

H.S. FOX[1,3], A. FRISCHAUT,[2] H. LEHRACH,[2] G.P. MARTIN[1] and L.M. SILVER,[3] [1]University of California, San Francisco; [2]EMBL, Heidelberg, Germany; [3]Cold Spring Harbor Laboratory, New York: *t* haplotype specific genomic clones derived from a homozygous t^{w5} embryonic stem cell line.

R. L. BRINSTER,[1] and R. D. PALMITER,[2] [1]Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia; [2]Howard Hughes Medical Institute Laboratory, Dept. of Biochemistry, University of Washington, Seattle: Introduction of new genes into mice.

Summary: F. JACOB, Institut Pasteur, Paris, France

Papilloma Viruses

September 14–September 18

Arranged by **Thomas R. Broker**, Cold Spring Harbor Laboratory, **Peter M. Howley**, National Cancer Institute

117 participants

A spin-off from the DNA Tumor Virus Meeting, this meeting was a new "specialty" conference at Cold Spring Harbor Laboratory. That such a meeting would be desirable became evident as interest in and research on these wart viruses, their interaction with epithelial cells, and their clinical and veterinary manifestations increased dramatically over the past few years. The meeting attracted over three times as many participants as any previous conference on the subject.

Until recently, few basic researchers studied the papilloma viruses (PVs) because they could not be propagated in cell culture. However, most of the 16 known types of human papilloma viruses (HPVs), the five bovine types, Shope (cottontail rabbit) papilloma virus, and the deer, European elk, and chaffinch papilloma viruses have now been cloned in bacterial plasmid vectors. This has permitted the preparation of homogeneous quantities of material for detailed molecular characterization, development of hybridization probes for clinical diagnosis, and use of papilloma virus genomes themselves as cloning vectors for the expression of bacterial, viral, and eukaryotic genes in eukaryotic cells.

The most notable clinical development reported was the increasing incidence (now 10%) of HPV type 6 in venereal disease and its correlation with cervical dysplasias and carcinomas. Several other HPV types have also been clearly implicated in carcinogenesis of human epithelia, the analogs of cancers caused by certain animal papilloma viruses. The DNA sequences of HPV-1, HPV-6, bovine PV-1, and Shope papilloma virus were presented. Their genetic organizations are extremely similar, and the disposition of all significant open translation frames along only one of the DNA strands clearly distinguishes the papilloma viruses from SV40 and polyoma viruses, with which they were originally classified as "papovaviruses." Some RNA transcripts of BPV-1, HPV-1, and Shope papilloma virus have been mapped and correlated with encoded functions. Various host systems were described, but a fully permissive system for lytic infection has yet to be found. The unique qualities as well as some of the problems of papilloma virus-based episomal cloning vectors were illustrated. An informal open discussion considered suggestions for nomenclature, taxonomy, and sharing of cloned prototype strains.

The success of this meeting has been marked by the decision of the European Molecular Biology Organization to sponsor a similar conference in Sweden in July 1983 and by the probability of holding such a meeting again at Cold Spring Harbor Laboratory in alternate years.

Through the generosity of the National Cancer Institute, Fogarty International Center, National Science Foundation, Council for Tobacco Research-USA, Abbott Laboratories, Applied Molecular Genetics, Biogen, CIBA-Geigy, Cetus Corporation, Genentech, and Lilly Research Laboratories we were able to fund many of the participants.

Papilloma Viruses Meeting

SESSION 1 *Human Disease. I. Genital Papillomas*

Chairperson: L. G. KOSS, Montefiore Medical Center, Bronx, New York

L. G. KOSS, Dept. of Pathology, Montefiore Medical Center, Bronx, New York: Papilloma virus infections and cancer of the female genital tract and anus.

R. J. KURMAN, A. B. JENSON, and W. D. LANCASTER, Georgetown University School of Medicine, Washington, DC: Immunocytochemical localization of papilloma virus antigens in cervical dysplasia and carcinoma in situ.

R. REID, Sinai Hospital and Wayne State University Medical School, Detroit, Michigan: Can HPV infection trigger cervical carcinogenesis?

K. SYRJÄNEN,[1] M. VÄYRYNEN,[2] O. CASTRÉN,[2] R. MÄNTYJARVI,[3] S. PYRHÖNEN,[4] S. SYRJÄNEN,[1] and M. YLISKOSKI,[2] Depts. of [1]Pathology, [2]Gynecology, and [3]Clinical Microbiology, University of Kuopio; [4]Dept. of Virology, University of Helsinki, Finland: HPV-antigens in the dysplastic lesions of the uterine cervix.

B. WINKLER,[1] C. CRUM,[1] R. RICHART,[1] T. FUJII,[2] A. FERENCZY,[3] M. BOON,[4] L. BRAUN,[5] W. LANCASTER,[6] and Y. FU,[7] [1]Dept. of Obstetrics and Gynecological Pathology, Columbia University, New York, New York; [2]Dept. of Obstetrics and Gynecology, Hiroshima University, Japan; [3]Dept. of Pathology, Jewish General Hospital, Montreal, Quebec, Canada; [4]Leids Cytologisch Laboratorium, Holland; [5]Dept. of Pathobiology, Johns Hopkins School of Medicine, Baltimore, Maryland; [6]Dept. of Pathology, Georgetown University, Washington, DC; [7]Institute of Pathology, Case Western Reserve University, Cleveland, Ohio: Koilocytic lesions of the cervix—The relationship of mitotic abnormalities to the presence of papilloma virus antigens and nuclear DNA content.

T. OKAGAKI,[1,2] B. A. CLARK,[1] L. B. TWIGGS,[1] K. ZACHOW,[3] R. OSTROW,[3] S. WATTS,[3] and A. FARAS,[3] Depts. of [1]Obstetrics and Gynecology, [2]Laboratory Medicine and Pathology, and [3]Microbiology, University of Minnesota Medical School, Minneapolis: HPV DNA and ultrastructurally identifiable viruslike particles in cervico-vaginal intraepithelial and invasive squamous cell neoplasia.

L. GISSMANN, H. IKENBERG, and H. ZUR HAUSEN, Institut fur Virologie, Freiburg, Federal Republic of Germany: HPV infections of the genital tract and the larynx.

L. G. KOSS, Dept. of Pathology, Montefiore Medical Center, Bronx, New York: Human polyoma virus infection.

SESSION 2 *DNA Sequences and Genetic Organization*

Chairperson: M. YANIV, Institut Pasteur, Paris, France

Y. YABE, Dept. of Virology, Cancer Institute, Okayama University Medical School, Japan: Bridge connection and subunits of capsomeres in HPV.

O. DANOS, and M. YANIV, Unité des Virus oncogènes, Dépt. de Biologie Moléculaire, Institut Pasteur, Paris, France: DNA sequence and genetic organization of papilloma viruses.

E. CHEN,[1] P. HOWLEY,[2] A. LEVINSON,[1] and P. SEEBURG,[1] [1]Dept. of Molecular Biology, Genentech, Incorporated, South San Francisco, California; [2]NCI, National Institutes of Health, Bethesda, Maryland: The primary structure and genetic organization of BPV-1 genome.

U. PETTERSSON, Dept. of Medical Genetics, Biomedical Center, Uppsala, Sweden: DNA sequence of BPV-1.

E. SCHWARZ, M. DUERST, O. LATTERMANN, C. WOELFLE, E. WOLFSPERGER, R. ZECH, and H. ZUR HAUSEN, Institut für Virologie, Universität Freiburg, Federal Republic of Germany: DNA sequence and genome organization of human genital papilloma virus HPV6b.

I. GIRI, O. DANOS, and M. YANIV, Unité des Virus Oncogènes, Dépt. de Biologie Moléculaire, Institut Pasteur, Paris, France: DNA sequence studies on the Shope CRPV.

Y. NAKABAYASHI, S. K. CHATTOPADHYAY, and D. R. LOWY, NCI, National Institutes of Health, Bethesda, Maryland: The sequences required for morphologic transformation by BPV DNA are discontinuous.

N. SARVER, Y.-C. YANG, J.C. BYRNE, and P. M. HOWLEY, NCI, National Institutes of Health, Bethesda, Maryland: Analysis of BPV-1 genetic elements required for transformation and extrachromosomal plasmid replication.

G. SAUER, F. RÖSL, and W. WALDECK, Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Isolation of BPV chromatin and identification of a DNase sensitive region.

SESSION 3 *Poster Session*

H. AHOLA,[1] A. OSTERHAUS,[2] A. STENLUND,[1] J. MORENO-LOPEZ,[3] and U. PETTERSSON,[1] [1]Dept. of Medical Genetics, Biomedical Center, Uppsala; [2]Rijksinstituut voor de Volksgenzondheid, Bilhoven, The Netherlands; [3]National Veterinary Institute, Biomedical Center, Uppsala, Sweden: Characterization of the avian (Fringilla Coelebs) papillomavirus DNA (FPV).

C. BLANCHET-BARDON,[1] M. A. LUTZNER,[1,2] A. PUISSANT,[1] and G. ORTH,[2] [1]Clinique Dermatologique, Hôpital Saint-Louis; [2]Unité INSERM 190, Institut Pasteur, Paris, France: Interferon treatment of skin cancers of patients with epidermocysplasia verruciformis.

M. H. BUNNEY, Dept. of Dermatology, Royal Infirmary, Edinburgh, Scotland: Some clinical pictures illustrating the influence of host defects on HPV infections.

M. S. CAMPO and D. A. SPANDIDOS, Beatson Institute for Cancer Research, Glasgow, Scotland: In vitro transformation of mouse fibroblasts by molecularly cloned BPV genomes.

O. CROISSANT, N. BONNEAUD, and G. ORTH, Unité INSERM 190, Institut Pasteur, Paris, France: Detection of the vegetative viral DNA replication in papillomavirus-induced tumors by in situ molecular hybridization.

O. CROISSANT,[1] S. JABLONSKA,[2] G. PEHAU-ARNAUDET, and G. ORTH,[1] [1]Unité INSERM 190, Institut Pasteur, Paris, France; [2]Dept. of Dermatology, Warsaw School of Medicine, Poland: Heteroduplex mapping of conserved nucleotidic sequences between animal and human papillomaviruses.

M. FAVRE,[1] S. JABLONSKA,[2] O. CROISSANT,[1] S. OBALEK,[2] and G. ORTH,[1] [1]Unité INSERM 190, Institut Pasteur, Paris, France; [2]Dept. of Dermatology, Warsaw School of Medicine, Poland: Genetic heterogeneity of human papillomaviruses from skin warts—Analysis by restriction endonucleases and molecular hybridization.

G. JAUREGUIBERRY, M. FAVRE, F. BREITBURD, and G. ORTH, Unité INSERM 190, Institut Pasteur, Paris, France: Bovine papillomavirus genome in hamster sarcoma cells in vivo and in vitro—Variation in the level of expression.

D. KREMSDORF,[1] S. JABLONSKA,[2] M. FAVRE,[1] and G. ORTH,[1] [1]Unité INSERM 190, Institut Pasteur, Paris, France; [2]Dept. of Dermatology, Warsaw School of Medicine, Poland: Molecular cloning and biochemical characterization of the genomes of eight human papillomaviruses associated with epidermodysplasia verruciformis.

W. D. LANCASTER,[1] R. J. KURMAN,[1] L. E. SANZ,[1] S. PERRY,[2] and A. B. JENSON,[1] [1]Georgetown University Medical Center; [2]DC General Hospital, Washington, DC: Detection of HPV DNA sequences and evidence for molecular heterogeneity in premalignant lesions of the uterine cervix.

M. H. MOAR[1] and W. F. H. JARRETT,[2] [1]Dept. of Zoology, University of Edinburgh; [2]Dept. of Veterinary Pathology, University of Glasgow, Scotland: Tumor induction and cell transformation by BPV-4.

D. M. MORGAN,[1] W. MEINKE,[2] and V. DEFENDI,[1] [1]Dept. of Pathology, New York University Medical Center, New York; [2]Dept. of Molecular and Medical Microbiology, University of Arizona College of Medicine, Tucson: Susceptibility of the rabbit to BPV-1 tumor induction, and transfection of cells with cloned HPV DNA fragments.

C. MORIN, M. CASAS-CORDERO, A. MEISELS, and M. RABREAU, Dept. of Pathology, Saint-Sacrement Hospital and Laval University, Québec, Canada: HPV infection and associated premalignant lesions of the uterine cervix.

P. SCHNEIDER,[1] W. TOPP,[2] A. ABRAMSON,[1] B. KRUMHOLTZ,[1] S. WEINBERG,[1] D. CASPER,[1] R. STRAUSS,[1] E. BODIAN,[1] and B. STEINBERG,[1] [1]Long Island Jewish Medical Center, New Hyde Park, New York; [2]Cold Spring Harbor Laboratory, New York: Molecular heterogeneity of human genital wart (condylomata acuminata) papilloma viruses.

A. STENLUND,[1] H. AHOLA,[2] J. MORENO-LOPEZ,[2] and U. PETTERSSON,[1] [1]Dept. of Medical Genetics; [2]National Veterinary Institute, Biomedical Center, Uppsala, Sweden: Structure of viral transcripts in BPV-tranformed mouse cells.

S. L. WATTS, R. S. OSTROW, W. C. PHELPS, and A. J. FARAS, Dept. of Microbiology, University of Minnesota, Minneapolis: CRPV DNA in warts and carcinomas of cottontail and domestic rabbits.

SESSION 4 *Transformation*

Chairperson: G. ORTH, Institut Pasteur, Paris, France

B. BINÉTRUY,[1] G. MENEGUZZI,[1] R. BREATHNACH,[2] and F. CUZIN,[1] [1]Centre de Biochimie du CNRS, Université

de Nice; [2]Laboratoire de Génètique Moléculaire des Eucaryotes du CNRS, Strasbourg, France: BPV-1—pBR322 recombinant DNA molecules maintained in a plasmidial state both in rodent fibroblast and in bacterial cells.

G. M. GRAHAM,[1] L. E. BABISS,[1] J. BRYNE,[2] P. M. HOWLEY,[2] and P. B. FISHER,[1] [1]Institute for Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York; [2]NCI, National Institutes of Health, Bethesda, Maryland: Transformation of Fischer rat embryo (CREF) cells by BPV and plasmid cloned BPV DNA.

I. DVORETZKY,[1] S. K. CHATTOPADHYAY,[1] W. D. LANCASTER,[2] and D. R. LOWY,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Pathology, Georgetown University Medical Center, Washington, DC: The in vitro biological activity of DFV differs from that of BPV.

S. L. WATTS, W. C. PHELPS, R. S. OSTROW, and A. J. FARAS, Dept. of Microbiology, University of Minnesota, Minneapolis: Human and rabbit papilloma viruses transform C127 and NIH 3T3 cells in culture.

L. TUREK,[1] J. BYRNE,[1] D. LOWY,[1] I. DVORETZKY,[1] R. FRIEDMAN,[2] and P. HOWLEY,[1] [1]NCI, National Institutes of Health; [2]Uniformed Services University of the Health Sciences, Bethesda, Maryland: Interferon induces morphologic reversion with elimination of extrachromosomal viral genomes in BPV-transformed cells.

M. HENLEY and W. MEINKE, Dept. of Molecular and Medical Microbiology, University of Arizona, Tucson: Effects of retinoic acid on BPV transformed mouse cells.

G. GROSS,[1] A. GASSENMAIER,[2] M. LAMMEL,[3] and H. PFISTER,[3] [1]Dept. of Dermatology; [2]Institute of Virology, Freiburg; [3]Institute of Clinical Virology, Erlangen, Federal Republic of Germany: In vivo and in vitro effect of Ro 10-9359 on papilloma virus DNA persistence.

SESSION 5 *RNA Transcription*

Chairperson: T. R. BROKER, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

L. BERG, M. LUSKY, H. WEIHER, and M. BOTCHAN, University of California, Berkeley: Regulatory signals in BPV.

M. S. CAMPO, D. A. SPANDIDOS, and N. WILKIE, Beatson Institute for Cancer Research, Glasgow, Scotland: Transcription-promoter sequences in BPV-1 genome.

C. HEILMAN, L. ENGEL, and P. HOWLEY, NCI, National Institutes of Health, Bethesda, Maryland: Analysis of the virus-specific transcripts in BPV-1 infected bovine fibropapillomas.

R. F. LAPORTA, S. REILLY, and L. B. TAICHMAN, School of Dental Medicine, State University of New York, Stony Brook: Transcription of HPV-1 in cultures of human keratinocytes.

L. T. CHOW, D. CAMPANELLI, R. GALLI and T. R. BROKER, Cold Spring Harbor Laboratory, New York: Transcription of HPV-1 DNA from hybrid shuttle vectors transfected into COS-1 cells.

M. NASSERI and F. O. WETTSTEIN, University of California School of Medicine, Los Angeles: Colinear and spliced viral transcripts are present in nonvirus producing benign and malignant neoplasms induced by the Shope (rabbit) papilloma virus.

E. GEORGES, O. CROISSANT, N. BONNEAUD, and G. ORTH, Unité INSERM 190, Institut Pasteur, Paris, France: Transcription of the genome of the CRPV in domestic rabbit papillomas and in the transplantable VX2 carcinoma.

S. MITRANI-ROSENBAUM,[1] L.MAROTEAUX,[2] Y. MORY,[2] M. REVEL,[2] and P.M. HOWLEY[1] [1]NCI, National Institutes of Health, NIH, Bethesda, Maryland, [2]Dept. of Virology, Weizmann Institute for Science, Rehovot, Israel; Inducible expression of the human beta-interferon gene linked to a BPV DNA vector and maintained extrachromosomally in mouse cells.

SESSION 6 *Epidermal Cells*

Chairperson: H. GREEN, Harvard Medical School, Boston Massachusetts

H. GREEN, Dept. of Physiology and Biophysics, Harvard Medical School, Boston, Massachusetts: Epidermal cell differentiation in tissue culture.

S. BANKS-SCHLEGEL,[1] and P. HOWLEY,[2] [1]Laboratory of Human Carcinogenesis; [2]Laboratory of Pathology, NCI, National Institutes of Health, Bethesda, Maryland: Differentiation of human epidermal cells transformed by SV40.

Y. CHARDONNET, C. JAILLARD, and P. ELBAZE, INSERM and ERA CNRS, Pavillon R, Hôpital E. Herriot, Lyon, France: Cultures of keratinocytes derived from cutaneous warts.

L. B. TAICHMAN, S. REILLY, and R. F. LAPORTA, School of Dental Medicine, State University of New York, Stony Brook: Replication of HPV-1 in cultured human keratinocytes.

T. S. BURNETT and P. H. GALLIMORE, Dept. of Cancer Studies, University of Birmingham, England: HPV-1 transcription in rat fibroblast and human keratinocyte cell lines.

F. BREITBURD,[1] O. CROISSANT,[1] S. JABLONSKA,[2] and G. ORTH,[1] [1]Unité INSERM 190, Institut Pasteur, Paris, France; [2]Dept. of Dermatology, Warsaw School of Medicine, Poland: Two low molecular weight cytoplasmic polypeptides induced in infected cells of human skin warts caused by HPV-1.

SESSION 7 *Human Disease. II. Laryngeal, Genital, and Epidermal Papillomas*

Chairperson: S. JABLONSKA, Warsaw School of Medicine, Warsaw, Poland

S. SYRJÄNEN,[1] K. SYRJÄNEN,[2] M. LAMBERG,[1] and S. PYRHÖNEN,[3] [1]Institute of Dentistry, University of Kuopio; [2]Dept. of Pathology, University of Kuopio; [3]Dept. of Virology, University of Helsinki, Finland: HPV involvement in the squamous cell lesions of the oral cavity.

A. ABRAMSON and B. STEINBERG, Dept. of Otolaryngology, Long Island Jewish-Hillside Medical Center, New Hyde Park, New York: Clinical aspects of laryngeal papilloma.

B. STEINBERG,[1] A. ABRAMSON,[1] and W. TOPP,[2] [1]Long Island Jewish Medical Center, New Hyde Park; [2]Cold Spring Harbor Laboratory, New York: HPV presence in laryngeal papilloma.

P. MOUNTS,[1] L. METCALFE,[1] K. V. SHAH,[1] J. D. WOODRUFF,[2] A. FERENCZY,[3] R. REID,[4] and H. KASHIMA,[2] [1]Johns Hopkins University; [2]Johns Hopkins Hospital, Baltimore, Maryland; [3]Jewish General Hospital, Montreal, Canada; [4]Sinai Hospital, Detroit, Michigan: Papilloma virus etiology of genital tract lesions and laryngeal papillomas.

A. B. JENSON,[1] R. J. KURMAN,[1] E. E. LACK,[2] and W. D. LANCASTER,[1] [1]Georgetown University Medical Center, Washington, DC; [2]Children's Hospital Medical Center, Boston, Massachusetts: Papilloma virus etiology of proliferative squamous epithelial lesions of the skin and oral, tracheo-laryngeal and anogenital mucosa.

S. JABLONSKA,[1] O. CROISSANT,[2] S. OBALEK,[1] M. FAVRE,[2] D. KREMSDORF,[2] and G. ORTH,[2] [1]Dept. of Dermatology, Warsaw School of Medicine, Poland; [2]Unité INSERM 190, Institut Pasteur, Paris, France: Morphology of HPV-induced lesions and immunologic response as related to the virus type.

G. ORTH,[1] S. JABLONSKA,[2] S. OBALEK,[2] L. A. RUEDA,[3] C. BLANCHET-BARDON,[1] M. LUTZNER,[1] M. FAVRE,[1] D. KREMSDORF,[1] N. JIBARD,[1] and O. CROISSANT,[1] [1]Unité INSERM 190, Institut Pasteur, Paris, France; [2]Dept. of Dermatology, Warsaw School of Medicine, Poland; [3]Bogota, Columbia; Role of the virus in the pathogenesis of epidermodysplasia verruciformis.

H. PFISTER,[1] A. GASSENMAIER,[2] and P. G. FUCHS,[1] [1]Institut für Klinische Virologie, Erlangen; [2]Institut für Virologie, Freiburg, Federal Republic of Germany: Characterization of HPV from warts and carcinoma of an epidermodysplasia verruciformis patient.

M. A. LUTZNER, M. F. DUCASSE, V. DUTRONQUAY, and G. ORTH, Unité INSERM 190, Institut Pasteur, Paris, France: HPV-5 genome found in in situ cancers of an immunosuppressed renal transplant recipient.

K. ZACHOW,[1] R. OSTROW,[1] T. OKAGAKI,[1] L. TWIGGS,[1] B. CLARK,[1] M. NIIMURA,[2] M. BENDER,[1] F. PASS,[3] and A. FARAS,[1] [1]University of Minnesota Medical School, Minneapolis; [2]Jikei University School of Medicine, Tokyo, Japan; [3]Molecular Genetics, Inc., Minnetonka, Minnesota: Presence of HPV DNA in human benign and malignant epithelial tumors.

M. GREEN,[1] K. BRACKMANN,[1] P. LOEWENSTEIN,[1] M. CARTAS,[1] H. THORNTON,[1] J. SYMINGTON,[1] and M. EISINGER,[2] [1]Institute for Molecular Virology, St. Louis University Medical Center, Missouri; [2]Memorial Sloan-Kettering Institute for Cancer Research, New York, New York: HPV genomes—DNA homology, presence in malignant human tissues, and introduction into mammalian cells.

H. PFISTER, Institut für Klinische Virologie, Erlangen, Federal Republic of Germany: Isolation and characterization of HPV-13 from certain ethnic tribes.

R. REID, Sinai Hospital and Wayne State University Medical School, Detroit, Michigan: Coloscopic movie of laser cauterization of an HPV-induced cervical lesion.

SESSION 8 *Animal Papilloma Viruses and Carcinogenesis*

Chairperson: Y. ITO, Kyoto University, Kyoto, Japan

C. OLSON, Dept. of Veterinary Science, University of Wisconsin, Madison: Introductory review of animal papilloma viruses.

K. SUGAWARA,[1] K. FUJINAGA,[2] and Y. ITO,[1] [1]Dept. of Microbiology, Faculty of Medicine, Kyoto University; [2]Cancer Research Institute, Sapporo Medical College, Japan: "Masking" revisted—Persistence of SPV genome in Shope papilloma-carcinoma complex.

F. O. WETTSTEIN[1] and M. NASSERI[2] [1]Dept. of Microbiology and Immunology and [2]Molecular Biology Institute, University of California Medical School, Los Angeles: The unique state of CRPV DNA in malignant rabbit tumors and tumor derived cell lines.

E. AMTMANN,[1] K. WAYHS,[2] M. VOLM,[2] and G. SAUER,[1] [1]Institute for Virus Research; [2]Institute for Pathology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Tissue specificity of a latent papilloma virus in *Mastomys natalensis*.

S. SCHERNECK,[1] J. FEUNTEUN,[2] F. VOGEL,[1] and E. GESSLER,[1] [1]Central Institute of Molecular Biology, Academy of Sciences of the German Democratic Republic, Berlin; [2]Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Sequence homology between polyoma virus, SV40, and a papilloma producing virus from a Syrian hamster—Evidence for highly conserved sequences.

J. MORENO-LOPEZ,[1] A. STENLUND,[2] H. AHOLA,[1] and U. PETTERSSON,[2] Depts. of [1]Virology, Faculty of Veterinary Medicine and [2]Medical Genetics, Biomedical Center, Uppsala, Sweden: The European elk papilloma virus (EEPV)—Characterization of the genome, induction of tumors in animals, and transformation in vitro.

D. E. GROFF and W. D. LANCASTER, Georgetown University Medical Center, Washington, DC: Two subtypes of DFV show specific regions of rapid nucleotide divergence.

P. SPRADBROW, Dept. of Veterinary Pathology and Public Health, University of Queensland, Brisbane, Australia: Papilloma viruses, papillomas, and ocular and cutaneous carcinomas in ruminant animals.

G. H. THEILEN,[1] E. B. WHEELDON,[2] N. EAST,[1] B. R. MADEWELL,[1] and W. D. LANCASTER,[3] [1]University of California School of Veterinary Medicine, Davis; [2]Smith Kline & French Laboratories, Philadelphia, Pennsylvania; [3]Georgetown University, Washington, DC: Goat papillomatosis.

SESSION 9 *Vectors*

Chairperson: P. Howley, National Cancer Institute, Bethesda, Maryland

D. DIMAIO, R. TREISMAN, and T. MANIATIS, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Properties of BPV/human globin gene recombinant plasmids that efficiently transform mouse cells.

N. SARVER, J. C. BYRNE, and P. M. HOWLEY, NCI, National Institutes of Health, Bethesda, Maryland: Transformation and replication in mouse cells of a BPV/pML2 plasmid vector that can be rescued in bacteria.

C. STRATOWA, Y. WANG, M. SCHÄFER-RIDDER, and P. H. HOFSCHNEIDER, Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Transfer of a nonselectable gene, hepatitis-B-surface-antigen, into NIH 3T3 fibroblasts using BPV DNA as a vector.

A. STENLUND,[1] D. LAMY,[3] J. MORENO-LOPEZ,[2] E. SOBZACK,[3] H. AHOLA,[2] P. TIOLLAIS,[3] and U. PETTERSSON,[1] Depts. of [1]Medical Genetics and [2]Virology, Faculty of Veterinary Medicine, National Veterinary Institute, Biomedical Center, Uppsala, Sweden; [3]Institut Pasteur, Paris, France: The use of BPV-1 genome as a vector for cloning and expression of the hepatitis B surface antigen.

K. ZINN, P. MELLON, and T. MANIATIS, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Regulated expression of an extrachromosomal human β-interferon gene in mouse cells.

J. JOUANNEAU, I. GIRI, and M. YANIV, Unité des Virus Oncogènes, Dépt. de Biologie Moléculaire, Institut Pasteur, Paris, France: Cell transformation by hybrid plasmids containing BPV DNA and *Eco gpt* gene.

M.-F. LAW, B. H. HOWLEY, Y.-C. YANG, and P. M. HOWLEY, NCI, National Institutes of Health, Bethesda, Maryland: The use of dominant selective markers in the BPV-1 vector system.

G.N. PAVLAKIS and D.H. HAMER, NCI, National Institutes of Health, Bethesda, Maryland: Regulated expression of cloned genes in bovine papilloma virus vectors.

Pox-Iridovirus

September 20–September 23

Arranged by **John A. Holoczak**, Rutgers Medical School, Piscataway, New Jersey, **Riccardo Wittek**, University of Lausanne, Switzerland

69 participants

A poxvirus-iridovirus workshop is held approximately every second year. This was the fourth such workshop at Cold Spring Harbor Laboratory, and, so far, these workshops have been the only gatherings to focus specifically on these viruses. They therefore represent a unique opportunity for people working in this field to meet one another and to discuss ongoing research. Although the number of participants was small, they represented a significant fraction of the people working on these viruses and they had the opportunity to engage in the informal exchange of ideas which may be more difficult at larger meetings.

More than fifty abstracts were submitted for presentation and these were organized into sessions covering all aspects of current poxvirus and iridovirus research, including such topics as DNA structure, replication, protein synthesis, genetics, transcription, and gene mapping. One entire session was devoted to the vaccinia virus thymidine kinase gene which has been analyzed in detail by several groups. The presentations on the use of vaccinia virus as a eukaryotic cloning vector attracted much attention. Large pieces of foreign DNA have successfully been inserted into the vaccinia virus genome by in vivo recombination. Moreover, these foreign genes can be expressed during vaccinia virus infection. These recombinant viruses should provide a powerful tool for exploring the genetic regulation of vaccinia virus and may finally allow the development of vaccinia virus vectors for the production of live vaccines. The future will have to show whether our optimism is justified.

SESSION 1

Chairperson: J.A. Holoczak, Rutgers Medical School, Piscataway, New Jersey

G.D. Wallace, National Institues of Health, Bethesda, Maryland: Research on ectromelia at NIH.

J. Benavente and M. Esteban, Downstate Medical Center, Brooklyn, New York: Mechanism of inhibition of vaccinia virus protein synthesis by interferon.

A. Rice, T. Hunt, and I.M. Kerr, Imperial Cancer Research Fund Laboratories, London, England: Translational control in interferon-treated vaccinia virus-infected cells.

E. Paez and M. Esteban, Downstate Medical Center, Brooklyn, New York: Vaccinia virus can overcome the antiviral action of interferons in some cells.

M.G. Santoro, J. Bevevente, E. Paez, B. Jafe, and M. Esteban, Downstate Medical Center, Brooklyn, New York: Inhibition of vaccinia virus protein synthesis by prostaglandins of the A series.

SESSION 2

Chairpersons: R.W. Moyer, Vanderbilt University, Nashville, Tennessee and A. Granoff, St. Judes Children's Research Hospital, Memphis, Tennessee

S. Dales, E. Paoletti

R. Wittek, D. Pickup, A. Mahr

R.M. FLUGEL and G. DARAI, Institut Virusforschung, Heidelberg, Federal Republic of Germany: Identification of viral proteins and enzymatic activities of purified fish lymphocystic disease virus.

C. MONNIER, S. BARRAY, F. PETIT, and G. DEVAUCHILL, CNRS, Mont-St. Aignan, France: Chilo-iridescent virus replication Structural and non structural proteins in infected cells.

S. DALES and M.B.A. OLSTONE, University of Western Ontario, Ontario, Canada: Interrelationship at the cell surface between the vaccinia virus-induced hemagglutinin (HA) and the major histocompatibility complex.

V. RUSZALA and J.A. HOLOWCZAK, Rutgers Medical School, Piscataway, New Jersey: Membrane modifications in vaccinia virus infected cells.

R. GOORHA and K.G. MURTI, St. Judes Children's Research Hospital, Memphis, Tennessee: Role of DNA methylation in frog virus 3 (FV3) replication.

E.M. BERKOWITZ[1] and B.G.T. POGO,[2,1]New York, New York; [2]Mt. Sinai School of Medicine, New York, New York: Comparative studies with two stains of Shope fibroma virus.

R. GOORHA, St. Judes Children's Research Hospital, Memphis, Tennessee: A temperature-sensitive (TS) mutant of frog virus 3 (FV3) is defective in second-stage DNA replication.

B.G.T. POGO, Mt. Sinai School of Medicine, New York, New York: Mechanism of DNA replication of a temperature sensitive mutant of vaccinia virus.

T. CALANDRA and J.A. HOLOWCZAK, Rutgers Medical School, Piscataway, New Jersey: Vaccinia virus DNA replication in infected HeLa cells.

SESSION 3

Chairpersons: M.J. ENSINGER, Wistar Institute, Philadelphia, Pennsylvania, and R. DRILLEN, St. Mary's Hospital Medical School, London, England

M.J. ENSINGER and M. ROVINSKY, Wistar Institute, Philadelphia, Pennsylvania: Genetic and physical mapping of temperature-sensitive mutations of vaccinia virus WR.

V.G. CHINCHAR and A. GRANOFF, St. Jude Children's Hospital, Memphis, Tennessee: Isolation and characterization of frog virus 3 (FV3) temperature-sensitive mutants.

R. CONDIT, A. MOTYCZKA, and G. SPIZZ, State University of New York, Buffalo: Temperature sensitive mutants of vaccinia virus.

R.W. MOYER and G. DALON BROWN, Vanderbilt University, Nashville, Tennessee: In vitro translational analyses of two early RPUHR mutants of rabbit poxvirus.

P. SRIDHAR and R. CONDIT, State University of New York, Buffalo: Isolation and characterization of a PAA-resistant ts-mutant of vaccinia virus.

B. MOSS and E. JONES, National Institutes of Health, Bethesda, Maryland: Genetic evidence for vaccinia virus encoded DNA polymerase. Isolation of phosphonoacetate-resistant enzyme from the cytoplasm of cells infected with mutant virus.

E. KATZ, Hebrew University-Hadassah Medical, Jerusalem, Israel: Differences in restriction DNA fragment profiles between IBT resistant and dependent mutants of vaccinia virus and the wild type strain.

P. RACZYNSKI and R. CONDIT, State University of New York, Buffalo: Inhibition of vaccinia virus replication by 2'-0-methyladenosine-isolation of a drug resistant mutant.

R. DRILLEN[1] and K.R. DUMBELL,[2,1] St. Mary's Hospital Medical School, London, England; [2] St. Mary's Hospital Medical School, Berkhampstead, England: Physical mapping of a cowpox host-range gene(s) and a vaccinia virus TS-mutation.

M. MACKETT[1], C.K. SAM and K.R. DUMBELL[2], [1]National Institutes of Health, Bethesda, Maryland, [2]St. Mary's Hospital Medical School, Berkhampstead, England: Analysis of orthopoxvirus interspecies recombinants.

SESSION 4

Chairpersons: E. PAOLETTI, New York State Dept. of Health, Albany, New York and J. Esposito, CID Division of Viral Diseases, Atlanta, Georgia

M.L. SALAS, J.M. ALMENDRAL, A. TALAVERA, and E. VINUELS, Universidad Autonoma, Madrid, Spain: RNAs synthesized in vitro and in vivo by African swine fever virus-hybridization to DNA restriction fragments and cell-free translation.

F. GOLIN and J. KATZ, Hebrew University Hadassah Medical, Jerusalem, Israel: Transcription of an early region of the vaccinia genome characterized by overlapping RNAs.

A. MAHR and B. ROBERTS, Department of Biology, Harvard Medical School, Boston, Massachusetts: Mapping of early and late mRNAs to a 7.6 Kb DNA fragment.

J. MORGAN and B. ROBERTS, Harvard Medical School, Boston Massachusetts: RNA transcript organization within an early gene cluster.

S. VENKATESON, M. HAFFEY, B.M. BAROUDY, and B. MOSS, National Institutes of Health, Bethesda, Maryland: Nucleotide sequences of five vaccinia virus early genes.

J. MORGAN, A. MAHR, P. TRAKTMAN, and B. ROBERTS, Depts. of Biology and Chemistry, Harvard Medical School, Boston Massachusetts: Mapping of the DNA binding proteins of vaccinia virus.

R. WITTEK,[1] G. BAJSZAR,[2] J. WEIR,[2] and B. MOSS,[2] [1]Institute of Animal Biology, Lausanne, Switzerland; [2] National Institutes of Health, Bethesda, Maryland: Transcriptional mapping of the HindIII J fragment of vaccinia virus.

P. TRAKTMAN and B. ROBERTS, Harvard Medical School, Boston, Massachusetts: The role of DNA replication in the early-late switch of vaccinia virus.

M. ESTEBAN, C. BONI, and A. PELLICER, Downstate Medical Center, Brooklyn, New York: Phenotypic changes of cells transformed with vaccinia virus.

SESSION 5

Chairpersons: K.R. DUMBELL, St. Mary's Hospital Medical School, Berkhampstead England and B. MOSS, National Institutes of Health, Bethesda, Maryland.

J. ESPOSITO, CID Division of Viral Diseases, Atlanta, Georgia: A comparison of orthopoxvirus DNA maps.

A.A. DOLLERY,[1] D. KINCHINGTON,[2] P.J. GREENAWAY,[3], and K.R. DUMBELL,[3] PHLS Centre APP Micro, Salisbury, England; St. Mary's Hospital Medical School, Salisbury, England;[3] St. Mary's Hospital Medical School, Berkhampstead, England: Comparison of variola and monkeypox genomes by restriction endonuclease analysis.

A. TALAVERA, J.M. ALMENDRAL, V. LEY, R. BLASCO, J.M. SOGO, and E. VINUELA, Universidad Autonoma, Madrid, Spain: Structure of the African swine fever virus genome.

R.D. WESLEY, Plum Island Animal Disease Center, Greenport, New York: African swine fever virus DNA-differentiation of field isolates by restriction endonuclease analyses.

G. DARAI, H. DELIUS, and R.M. FLUGEL, Institute Virusforschung, Heidelberg, Germany: Analysis of the genome of fish lymphocystis disease virus.

D.J. PICKUP, H.O. STONE, D. BASTIA, and W.K. JOKLIK, Duke University Medical Center, Durham, North Carolina: The arrangement of repeated and unique sequences at the termini of cowpox virus DNA.

D. KINCHINGTON,[1] A.A. DROLLERY,[1] P.G. GREENAWAY,[2] and K.R. DUMBELL,[2] [1]St. Mary's Hospital Medical School, Salisbury, England; [2]St. Mary's Hospital Medical School, Berkhampstead, England: Electron microscopy of recombinant plasmid DNA containing fragments of variola or of monkeypox DNA.

D. Moyer, D. Hruby, S. Holoczak

A. Granoff, F. Fenner

B.M. BAROUDY and B. MOSS, National Institutes of Health, Bethesda, Maryland: Structure and replication of vaccinia virus telomeres.

R. GOORHA and G. KITCHINGMAN, St. Judes Children's Research Hospital, Memphis, Tennessee: A high degree of frog virus 3 (FV3) DNA methylation inhibits its cloning.

M. MACKETT, G.L. SMITH, and B. MOSS, National Institutes of Health, Bethesda, Maryland: Vaccinia virus-A selectable eukaryotic cloning and expression vector.

SESSION 6

Chairperson: R. WITTEK, Institute Animal Biology, Lausanne, Switzerland

D. PANICALI and E. PAOLETTI, New York State Dept. of Health, Albany, New York: Poxviruses as eukaryotic cloning vectors.

A.M. DELANGE, B. FUTCHER, A.R. MORGAN, and G. MCFADDEN, University of Alberta, Canada: Replication of cloned vaccinia virus DNA terminal restriction fragments containing the hairpin turnaround sequence in a yeast plasmid vector.

D.E. HRUBY[1] and L.A. BALL.[2] [2]University of Texas, Austin; [2]University of Wisconsin, Madison: Fine structure analysis of the vaccinia virus thymidine kinase gene.

A. VASSEL, F. BEN HAMIDA, F. KUNST, R. PICTET, and G. BEAUD, Institute I.R.B.M. -J. Monod, Paris, France: Mapping of a mutation site in the thymidine kinase gene of vaccinia virus by marker rescue with restricted fragments of a cloned DNA.

JEFFY W. WEIR, G. BAJSZAR, and B. MOSS, National Institutes of Health: Identification and nucleotide sequence of the thymidine kinase gene of wild-type vaccinia virus and nonsense mutants.

D.J. ALBRIGHT[1], L.A. BALL,[1] and D.E. HRUBY,[2] [1]University of Wisconsin, Madison; [2]University of Texas, Austin: Transcription of the vaccinia virus thymidine kinase gene during infection.

D.R. HRUBY,[1] D.B. MILLER,[2] and L.A. BALL.[2] [1]University of Texas, Austin; [2]University of Wisconsin, Madison: Synthesis of vaccinia virus thymidine kinase in microinjected *Xenopus* oocytes.

BANBURY CENTER
1982 ACTIVITIES

1982 represented the fifth year of Banbury Center programs. During the course of the year, the Center was the site of nine conferences as well as a full program of high-level summer courses. Four new books in the ongoing Banbury Report series were also published during the year, bringing the total in that series to thirteen.

The range of Banbury meetings held in 1982 reflected the widening sphere of social impact of biological sciences. Two of these emanated from rapidly developing areas of recombinant DNA applications, while a third addressed new insights, gained at least partly through recombinant DNA methods, into the molecular processes of carcinogenesis. Two other conferences looked at carcinogenesis from more immediate aspects—one through an assessment of one particular prevalent class of agents, the nitrosamines; the other by assembling a range of newly developed approaches for directly assessing the effects of genotoxic chemical exposure. The remaining 1982 conferences included a novel combination of clinicians and basic researchers in an exploration of biological aspects of Alzheimer's disease, a probing of one rapidly developing area of molecular genetic applications to industrial processes, and two retrospective meetings placing some current thrusts of molecular biology within historical perspectives.

The first meeting of the year was on a topic which, barely ten months later, would become the subject of newspaper editorials and congressional hearings. This conference, *Gene Therapy: Fact and Fiction*, was held early in February and was organized by W. French Anderson from the National Heart, Lung and Blood Institute, Paul Berg of Stanford University, and Theodore Friedmann from the University of California at San Diego. Supported by a grant from the Kaiser Family Foundation, the three-day meeting explored current capabilities, likely future developments, and the needs which might best be addressed through the at least theoretically possible direct alteration or replacement of aberrant disease-causing genes. This meeting transcript is currently being excerpted and edited for inclusion in a broadly accessible description of the nature and current state of this field.

The two Banbury spring conferences explored, respectively, two different aspects in assessing human carcinogenesis. The first of these, organized by Peter Magee of the Fels Research Institute, was a comprehensive assessment of the role of nitrosamines in the genesis of human cancers. Over one hundred nitrosamine compounds are currently known to be carcinogenic, several of these proving to be so in a wide range of animal species. These compounds may be found in the environment, or may be formed metabolically from precursors once inside the body. This Banbury conference specifically addressed human aspects of such metabolic transformations, detection of these compounds and their metabolites, and their biological effects. *Nitrosamines and Human Cancer* was published as Banbury Report 12 in December 1982.

Also published in December was Banbury Report 13, emanating from the other spring conference, which was held during the third week of April. This conference, *Indicators of Genotoxic Exposure*, was organized by Bryn Bridges of the University of Sussex in England, Byron Butterworth from the Chemical Industry Institute of Toxicology, and I. Bernard Weinstein from Columbia University. The meeting brought together the multiplicity of emerging procedures for directly assessing the effects of genotoxic agents in the very individuals placed at risk through such exposure. Approaches of this sort will play important roles in quantitating the effects of such exposure and in the further development of rational bases in the regulation of such agents.

In the summer of 1982 three events were added to the regular program of summer courses. Two of these were an unusual departure for Banbury and were very special meetings. The first was held in honor of the memory of Luigi Gorini and, appropriately enough, was a high-level scientific conference on gene expression in prokaryotic organisms with nearly all participants tracing the origin of their research to some aspect of Gorini's seminal work in this area. The second conference was on the use of the simple fungus *Phycomyces* as a model organism in which to begin to understand the molecular mechanisms involved in the transduction of sensory input into behavioral responses. These participants were the disciples of Max Delbrück. After playing a major role in the forties and fifties in establishing bacterial viruses as simple model organisms with which to probe the molecu-

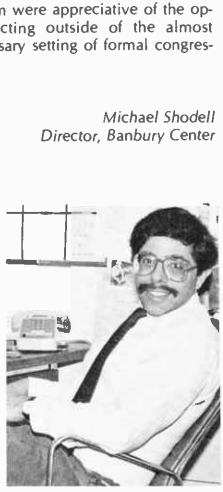
lar basis of heredity, Delbrück turned to the question of the mechanisms by which cells or organisms respond to their environment. His choice simple organism for probing this area was *Phycomyces*, whose rather rudimentary cellular organization belied a prodigious capacity for responding to an array of environmental stimuli. Again, the tracing of these scientific lines of investigation to an individual whose intellect and personality had such an impact upon both the origins of the field and current workers in it gave great insight both into the development of science and into the nature of this extraordinary individual. The Delbrück and Gorini meetings' proceedings will both be appearing as books from the Cold Spring Harbor publications office. The last meeting of the summer was a two-day intensive exploration of genetic manipulations in the cultivation and application of anaerobic bacteria. While noted as interesting pathogens, many of the anaerobes are also important potential sources of organic chemical intermediates and solvents. Organized by Ahmad Bukhari of Cold Spring Harbor Laboratory and Leonard E. Mortenson from Exxon Research and Engineering Company, this meeting proved to be an incisive and in-depth survey of this bacterial group and their possible industrial applications in production of acetone, butanol, methane, and volatile fatty acids.

The final three conferences of the year returned to more traditional Banbury topics, although in themselves encompassing a range almost as broad as the entire range of prior Banbury conferences. The first of these examined the application of recombinant DNA procedures to human disease. Organized by C. Thomas Caskey of the Baylor College of Medicine and Raymond L. White from the Howard Hughes Medical Institute, this meeting was, in a sense, an outgrowth of the earlier 1982 conference on gene therapy. The emphasis in this later meeting, however, was on the extremely rapid development of methods for the actual molecular identification and localization of disease-causing aberrant genes within the human genome. Given the extraordinary pace of development, this is probably the last time that a meeting of this small size and spontaneity of interaction could possibly even begin to encompass the essential features of this field. *Recombinant DNA Applications to Human Disease* will be appearing as Banbury Report 14 in the spring of 1983. This meeting was followed within three weeks by the second major conference of October, *Biological Aspects of Alzheimer's Disease*. The organizer for this meeting, Robert Katzman of Albert Einstein College of Medicine, brought together a heterogeneous mix of clinicians and basic researchers for a multidisciplinary approach to looking at the biology of this devastating affliction. The interaction of neuropathologists, psychiatrists, epidemiologists, virologists, and molecular geneticists in considering this major public health concern, responsible for at least half of all cases of dementia in the aged, led to both novel formulations and discussions arising from this stimulus of participatory "hybrid vigor." The proceedings of this conference will also be appearing in the spring of 1983 as Banbury Report 15.

The final 1982 meeting was a special workshop for congressional staff made possible through a grant from the Sloan Foundation. Judiciously held after the November elections, this conference recapped one of the most exciting and rapidly developing areas of cancer research—the nature of oncogenes and their implications for determining the mechanisms of human carcinogenesis. Congressional staff members seemed especially gratified at the opportunity to partake of the excitement of science so directly while both they and the scientists addressing them were appreciative of the opportunity of interacting outside of the almost automatically adversary setting of formal congressional hearings.

February 1, 1983

Michael Shodell
Director, Banbury Center

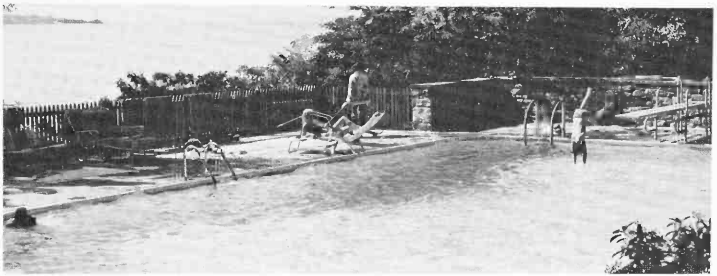


Banbury staff: Lynda Moran, Beatrice Toliver, Michael Shodell

Robertson House

1982 SUPPORT

Along with the donation of his estate, Charles S. Robertson also established a fund for the maintenance of the grounds and of Robertson House. Support for the activities of the Banbury Center, however, are dependent upon government and foundation grants and private and corporate contributions. In 1982, core support for operation of the Center in general came from the generous donations of the following twelve companies: The Bristol-Myers Fund, The Chevron Fund, Conoco, Inc., The Dow Chemical Company, E. I. du Pont de Nemours & Company, Exxon Corporation, Getty Oil Company, Grace Foundation Inc., Hoffman La Roche Inc., International Business Machines Corporation, Eli Lilly and Company, New York Life Foundation, and Rockwell International Corporation Trust. While support for specific meetings came partially from National Institutes of Health grants and from the National Science Foundation, these programs would have been impossible without major additional funding. The Kaiser Family Foundation was instrumental in enabling the holding of the Gene Therapy meeting, while an ongoing grant from the Sloan Foundation has supported science writers and congressional workshops. The Alzheimer's meeting and preparation of its publication was largely supported by the National Institute on Aging, the National Insitute of Neurological and Communicative Disorders and Stroke, and the Fogarty International Center, in conjunction with the Retirement Research Foundation (International Symposium on Aging and Cancer). The March of Dimes Birth Defects Foundation and The Burroughs Wellcome Fund joined with the National Cancer Institute, the Fogarty International Center, and the National Institute of General Medical Sciences in support of the conference on Recombinant DNA Applications to Human Disease, while the American Petroleum Institute largely supported the meeting on Genotoxic Indicators. Additional sources of funding for the holding of particular meetings were generously donated by the following: Bayer AG/Cutter/Miles, Biogen, S.A., Bristol-Myers Company, Cetus Corporation, E. I. du Pont de Nemours & Company, Merck Sharp & Dohme Research Laboratories, Schering-Plough, Searle Research and Development, SmithKline, Society for Microbiology, The Squibb Institute for Medical Research, Stauffer Chemical Company, and The Upjohn Company.



Prospects for Gene Therapy: Fact and Fiction

February 5–February 7

Opening Session

Chairperson: W.F. Anderson, National Heart, Lung and Blood Institute, Bethesda, Maryland

V.A. McKusick, Johns Hopkins University, Baltimore, Maryland: Review of genetic components of human disease.
Y.W. Kan, University of California, San Francisco: New approaches to diagnosis.

Session 1 Therapy

Chairperson: T. Friedmann, University of California, San Diego

W.L. Nyhan, University of California, San Diego: Current methods of therapy.
W.F. Anderson, National Heart, Lung and Blood Institute, Bethesda, Maryland: New approaches to therapy.

Session 2 Introduction of Genes into Cells and Complex Organisms

Chairperson: P. Berg, Stanford University, California

T. Maniatis, Harvard University, Cambridge, Massachusetts: Characterization and isolation of genes.
R. Axel, College of Physicians and Surgeons, Columbia University, New York, New York: Genes into cells.
F. Costantini, University of Oxford, England: Genes into complex organisms.

Session 3 Animal Models and Target Organs

Chairperson: U. Pettersson, University of Uppsala, Sweden

R.J. Desnick, Mount Sinai Medical Center, New York, New York: Animal models for human disease.
M.J. Cline, UCLA School of Medicine, Los Angeles, California: Potential human target organs.

Session 4 Approaches to Human Disease

Chairperson: L. Siminovitch, Hospital for Sick Children, Toronto, Canada

B.G. Forget, Yale University, New Haven, Connecticut: Hematological disease.
W.S. Sly, Stanford University, California: Lysosomal and other storage diseases.
M.S. Brown, University of Texas, Dallas: LDL receptor deficiency hypercholesterolemia.

P. Berg

B. Forget

J. Tooze

W. McKusick

The Possible Role of Nitrosamines in Human Cancer

April 4–April 7

Session 1 *Evidence Suggesting That Human Beings are Susceptible to Carcinogenesis by N-nitroso Compounds*

Chairperson: S. Goldfarb, University of Wisconsin, Madison

In vitro studies

G.E. Milo, Ohio State University, Columbus: In vitro transformation in cultured human diploid fibroblast cells.

I. Parsa, SUNY Downstate Medical Center, Brooklyn, New York: Transplantation of dimethylnitrosamine-transformed cultured tumor cells.

In vivo studies

R.D. Kimbrough, Centers for Disease Control, Atlanta, Georgia: Pathological changes in human beings acutely poisoned by dimethylnitrosamine.

W.E. Fleig, University of Ulm, Federal Republic of Germany: Pathological changes in a human subject chronically exposed to dimethylnitrosamine.

Session 2 *Comparative Metabolism and Alkylation Reactions*

Chairperson: A.E. Pegg, Pennsylvania State University, Hershey

P.F. Swann, Middlesex Hospital Medical School, London, England: Metabolism of *N*-nitroso compounds and alkylation of cellular macromolecules including DNA—An overview.

C.J. Michejda, Frederick Cancer Research Facility, Maryland: Metabolic formation of N_2 from *N*-nitroso compounds in vitro and in vivo.

M.C. Archer, University of Toronto, Canada: Metabolism of unsymmetrical nitrosamines.

S. Hecht, American Health Foundation, Valhalla, New York: Metabolism and activation of tobacco-associated nitrosamines in human and animal tissues.

C.C. Harris, National Cancer Institute, Bethesda, Maryland: Metabolism of *N*-nitrosamines by cultured human tissues and cells.

R. Montesano, International Agency for Research on Cancer, Lyon, France: Metabolism of nitrosamines by human liver slices in vitro.

R.C. Shank, California College of Medicine, Irvine: Liver nucleic acids after homicidal poisoning by dimethylnitrosamine.

R. Montesano[1] and A.E. Pegg[2], [1]International Agency for Research on Cancer, Lyon, France; [2]Pennsylvania State University, Hershey: Overview of repair by microbial and mammalian enzyme systems of DNA lesions induced by *N*-nitroso compounds, and recent studies with human enzymes.

Session 3 *Analytical Methods for Nitrosamines in Biological Media*

Chairperson: D.H. Fine, New England Institute for Life Sciences, Waltham, Massachusetts

D.H. Fine, New England Institute for Life Sciences, Waltham, Massachusetts: Analytical methods for nitrosamines—Overview.

G.W. Harrington, Temple University, Philadelphia, Pennsylvania: Electrochemical detection of *N*-nitroso compounds with high performance liquid chromatography (HPLC).

W. Garland and A.H. Conney, Hoffman-La Roche Inc., Nutley, New Jersey: Mass spectrometry in the analysis of nitrosamines in biological media.

Session 4 *Exposure of Human Beings to Nitrosamines*

Chairperson: J.H. Weisburger, American Health Foundation, Valhalla, New York

Exogenous sources of nitrosamines

D.H. Fine, New England Institute for Life Sciences, Waltham, Massachusetts: Nitrosamines in the general environment and food.

D. Hoffmann, American Health Foundation, Valhalla, New York: Nitrosamines in tobacco carcinogenesis.

Endogenous Formation

S. Mirvish, University of Nebraska, Omaha: Nitrosation reactions in vitro and in vivo.
B.C. Challis, Imperial College of Science and Technology, London, England: A kinetic model for the gastric synthesis of *N*-nitroso compounds.
W. Lijinsky, Frederick Cancer Research Facility, Maryland: Carcinogenesis by simultaneous exposure to nitrites and amines.
D.H. Fine, New England Institute for Life Sciences, Waltham, Massachusetts: Presence of nitrosamines in human beings.
M.L. Simenhoff, Jefferson Medical College, Philadelphia, Pennsylvania: Presence of nitrosamines in the blood and gastrointestinal fluids of normal and diseased human subjects.
H. Bartsch, International Agency for Research on Cancer, Lyon, France: Endogenous nitrosation in human beings—Proline I.
S.R. Tannenbaum, Massachusetts Institute of Technology, Cambridge: Endogenous nitrosation in human beings—Proline II.
P.I. Reed[1] and C. Walters[2], Wexham Park Hospital, [1]Berkshire, [2]Horsham, West Sussex, England: Possible effects of cimetidine on intragastric nitrosation in human beings.
J.B. Elder, University of Manchester, England: Possible role of cimetidine and its nitrosation products in human stomach cancer.
D.E. Jensen and C.T. Gombar, Temple University, Philadelphia, Pennsylvania: Chemistry, pharmacokinetics and biological actions of nitrosocimetidine—DNA methylation.
W. Lijinsky, Frederick Cancer Research Facility, Maryland: Carcinogenesis studies with nitrosocimetidine I.
R. Preussmann, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: Carcinogenesis studies with nitrosocimetidine II.

Session 5 Nitrites and Amines

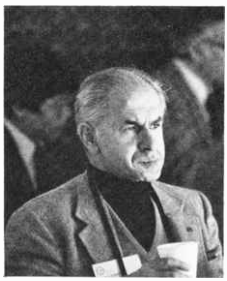
Chairperson: N.P. Sen, Health and Welfare Canada, Ottawa

P.E. Hartman, Johns Hopkins University, Baltimore, Maryland: Metabolism of nitrite—Overview.
S.R. Tannenbaum, Massachusetts Institute of Technology, Cambridge: Endogenous formation of nitrite.
C. Walters, Horsham, West Sussex, England: Endogenous amines—Amines in human gastric juice.

Session 6 Human Epidemiology

Chairperson: P. Correa, Louisiana State University, New Orleans

R.M. Hicks, Middlesex Hospital Medical School, London, England: Nitrosamines as possible etiological agents in bilharzial bladder cancer.
L.Y.-Y. Fong, University of Hong Kong: Possible relationship of nitrosamines in the diet to causation of cancer in Hong Kong.
C.S. Yang, New Jersey Medical School, Newark: Nitrosamines and other etiological factors in esophageal cancer in Northern China.
S.R. Tannenbaum, Massachusetts Institute of Technology, Cambridge: Stomach cancer.


J. Weisburger


P. Magee


R. Peto

Session 7 Dose-Response Relationships in Nitrosamine Carcinogenesis

Chairperson: R. Preussmann, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany

L.M. Anderson, Sloan-Kettering Institute for Cancer Research, Rye, New York: Dimethylnitrosamine metabolism and tumorigenesis during early life in the mouse.
W. Lijinsky, Frederick Cancer Research Facility, Maryland: Comparative carcinogenesis of some nitrosamines in rats and hamsters.
R. Peto, University of Oxford, England: Carcinogenesis study with dimethylnitrosamine and diethylnitrosamine in 4080 inbred rats—Experimental design and general nature of the dose-response relationship.
P. Grasso, BP Group Occupational Health Centre, Sunbury-on-Thames, Middlesex, England: Pathology of tumors observed in study with 4080 inbred rats.

Indicators of Genotoxic Exposure in Man and Animals

April 18–April 21

Session 1 Clinical Perspectives

Chairperson: B.A. Bridges, University of Sussex, England

I.B. Weinstein, College of Physicians and Surgeons, Columbia University, New York, New York: Molecular epidemiology—Combined laboratory and epidemiologic approaches to genetic toxicology.
E.B. Hook, Birth Defects Institute, New York State Department of Health, Albany: The value and limitations of clinical observations in assessing chemically induced genetic damage.

Session 2 Detection of Mutagens in Body Fluids

Chairperson: N.L. Petrakis, University of California, San Francisco

Urine

E. Eisenstadt, Harvard School of Public Health, Boston, Massachusetts: Urine as a monitor of mutagenic exposure of smokers.

Feces

R.D. Combes, Portsmouth Polytechnic, England: Rat, human and other animal samples.

Breast Fluid

N.L. Petrakis, University of California, San Francisco: Mutagens in nipple aspirates of breast fluid.

Session 3 DNA Damage and Repair

Chairperson: B.E. Butterworth, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina

Unscheduled DNA synthesis

J.C. Mirsalis, SRI International, Menlo Park, California: Rat hepatocytes.
B.E. Butterworth, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: Chemically induced DNA repair in rodent and animal cells.
M.J. Skinner, Mobil Oil Corporation, Princeton, New Jersey: Unscheduled DNA synthesis in rat lymphocytes.
C. Furihata, University of Tokyo, Japan: Unscheduled DNA synthesis in rat stomach—Short-term assay of potential stomach carcinogens.

Alkaline elution

S. Parodi, University of Genoa, Italy: Alkaline elution in vivo—Quantitative predictivity of carcinogenicity as compared with other, shorter-term tests.

Alkylated macromolecules

C.J. Calleman, University of Stockholm, Sweden: Alkylated hemoglobin.
P.B. Farmer, MRC Toxicology Unit, Carshalton, England: Significance of the occurrence of *S*-methyl cysteine in normal, untreated animals.
M.A. Pereira, United States Environmental Protection Agency, Cincinnati, Ohio: The use of alkylated hemoglobin as a dose monitor for chemical carcinogens and mutagens.

Session 4 DNA Adducts

Chairperson: I.B. Weinstein, College of Physicians and Surgeons, Columbia University, New York, New York.

W.K. Lutz, University of Zurich, Switzerland: DNA adducts as quantitative indicators of carcinogenic exposure.

Animal models

J.A. Swenberg, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: Cell specific effects—Application of new fluorometric techniques to detect adducts.
G.N. Wogan, Massachusetts Institute of Technology, Cambridge: Aflatoxin–DNA adducts and their detection in urine.
G. Theall, College of Physicians and Surgeons, Columbia University: Quantitative relationships between adduct formation and biological effects.

Human studies

D.C. Herron, Atlantic Richfield Company, Los Angeles, California: DNA adducts following a human DMN poisoning.
W.A. Haseltine, Sidney Farber Cancer Institute, Boston, Massachusetts: Studies using defined DNA sequences and postlabeling techniques.

Monoclonal antibodies to carcinogen-DNA adducts

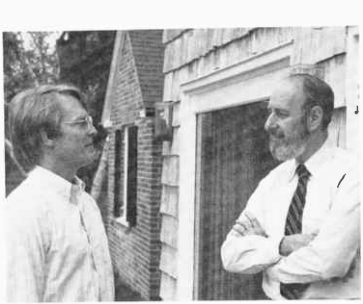
M.F. Rajewsky, University of Essen, Federal Republic of Germany: High-affinity monoclonal antibodies specific for DNA components structurally modified by alkylating agents.

Session 5 Cytogenetics and Sister-Chromatid Exchange (SCE)

Chairperson: H.J. Evans, MRC Clinical and Population Cytogenetics Unit, Edinburgh, Scotland

Sister chromatid exchange in animal models

A. Kligerman, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: In vivo and in vitro rat lymphocytes.
J.W. Allen, United States Environmental Protection Agency, Research Triangle Park, North Carolina: Species and organ specificity of sister-chromatid exchange in the mouse.



W. Thilly, J. Heddle

S. Parodi, A. Wyrobek

Human studies

A.V. Carrano, Lawrence Livermore National Laboratory, Livermore, California: Measurement of sister-chromatid exchange induction in human populations as an indicator of exposure.
W.H. McKenzie, North Carolina State University, Raleigh: Controlled human exposure studies.
H.J. Evans, MRC Clinical and Population Cytogenetics Unit, Edinburgh, Scotland: Industrially exposed populations.
M. Sorsa, Institute of Occupational Health, Helsinki, Finland: Sister chromatid exchange induction among nurses handling cytostatic drugs.
L.M. Henderson, University of Sussex, England: Measuring prenatal genotoxic effects in mice and men.

Micronucleus test

J.A. Heddle, Ludwig Institute for Cancer Research, Toronto, Canada: Application in human and animal systems.

Session 6 Mutagenesis

Chairperson: R.J. Albertini, University of Vermont, Burlington

Animal models

L.B. Russell, Oak Ridge National Laboratory, Tennessee: The Russell spot test.

Human studies

R.J. Albertini, University of Vermont, Burlington: Human lymphocytes.
G. Zetterberg, University of Uppsala, Sweden: Use of the cell sorter to concentrate mutants.
G.H. Strauss, University of Sussex, England: Development of a lymphocyte cloning assay.
H.D. Garcia, Philip Morris U.S.A., Richmond, Virginia: Development of a rat assay.
T.R. Skopek, Yale University, New Haven, Connecticut: Induced versus spontaneous mutations in human lymphocytes.
W.G. Thilly, Masssachusetts Institute of Technology, Cambridge: Use of mutational spectra to diagnose the causes of somatic mutation in man.

Altered gene products

H. Mohrenweiser, University of Michigan, Ann Arbor: Biochemical approaches to monitoring human populations for germinal mutation rates.

Session 7 Germ Cell Effects

Chairperson: A.J. Wyrobek, Lawrence Livermore National Laboratory, Livermore, California

Animal models

G. Sega, Oak Ridge National Laboratory, Tennessee: DNA repair in spermatocytes.
H.V. Malling, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Detecting sperm mutants.
R.J. Preston, Oak Ridge National Laboratory, Tennessee: Chromosome aberrations in decondensed sperm DNA.

Human studies

A.J. Wyrobek, Lawrence Livermore National Laboratory, Livermore, California: Sperm morphology (man and animals) and the fluorescent Y marker.
H.J. Evans, MRC Clinical and Population Cytogenetics Unit, Edinburgh, Scotland: Sperm morphology in smokers.
B.A. Bridges, University of Sussex, England: Summation—Future directions.

Prokaryotic Gene Expression—Symposium In Memory of Luigi Gorini

June 27–July 1

D. Fraenkel, Harvard Medical School, Boston, Massachusetts: Remembrance of Luigi Gorini.

Session 1 *Ribosome Function*

Chairperson: R.A. Zimmermann, University of Massachusetts, Amherst

Biosynthesis

M. Nomura, University of Wisconsin, Madison: Regulation of the synthesis of ribosomes and ribosomal components in *E. coli*.

J.D. Friesen, University of Toronto, Canada: Mutations affecting gene expression in the *rplJ* operon.

Structure and genes

K. Isono, Max-Planck-Institut, Berlin, Federal Republic of Germany: Genetics of the *E. coli* ribosomal protein—Some new aspects.

I.G. Wool, University of Chicago, Illinois: Alpha sarcin action and the structure and function of eukaryotic ribosomes.

J. Warner, Albert Einstein College of Medicine, Bronx, New York: Ribosomal protein genes of *S. cerevisiae* and their regulation.

Session 2 *Translational Fidelity I*

Chairperson: J. Davies, Biogen, S.A., Geneva, Switzerland

J.A. Gallant, University of Washington, Seattle: The ribosome's frame of mind.

C.G. Kurland, University of Uppsala, Sweden: Proofreading on ribosomes?

D. Elseviers, New York Medical College, Valhalla, New York: *E. coli* mutants with reduced misreading levels.

E.J. Murgola, University of Texas, Houston: tRNA, suppression, and the code.

Session 3 *Regulation of Gene Expression: Transcription and Translation*

Chairperson: W.K. Maas, New York University Medical Center, New York, New York

C. Yanofsky, Stanford University, California: Attenuation control of tryptophan operon expression.

B. Weisblum, University of Wisconsin, Madison: Translational control of resistance to MLS antibiotics.

M. Rosenberg, National Cancer Institute, Bethesda, Maryland: Regulation of gene expression by transcription termination and RNA processing.

K. Campbell, University of Colorado, Boulder: RegA—T4's amazing translational repressor.

H.E. Umbarger, Purdue University, West Lafayette, Indiana: The multivalent repression of isoleucine and valine biosynthesis in *E. coli*.

T. Eckhardt, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania: The *E. coli* argenine repressor gene (*argR*).

S. Adhya, National Cancer Institute, Bethesda, Maryland: Ribosomal proteins control transcription termination.

Prokaryotic Gene Meeting

Session 4 Translational Fidelity II

Chairperson: J.A. Gallant, University of Washington, Seattle

J. Parker, Southern Illinois University, Carbondale: Nonrandom codon misreading.
M. Yarus, University of Colorado, Boulder: Interaction of suppressor tRNA with the *strA* ribosome.
L. Bossi and J.R. Roth, University of Utah, Salt Lake City: Role of a tRNA modified base (anticodon loop ψ) in code translation and gene regulation.
R.C. Thompson, Temple University, Philadelphia, Pennsylvania: The reciprocal relationship between fidelity and speed in protein biosynthesis.

Session 5 Protein Secretion I

Chairperson: A. Torriani-Gorini, Massachusetts Institute of Technology, Cambridge

S.A. Benson, Frederick Cancer Research Center, Maryland: Genetic analysis of protein export.
L. Randall, Washington State University, Pullman: Processing of exported proteins in *E. coli*.
J. Beckwith, Harvard Medical School, Boston, Massachusetts: Genetics of the secretory apparatus of *E. coli*.
M. Schwartz, Institut Pasteur, Paris, France
J.O. Lampen, Rutgers University, Piscataway, New Jersey: Lipoprotein intermediates in secretion by Gram-positive bacteria.

Session 6 Protein Secretion II

Chairperson: P. Margolin, The Public Health Research Institute of the City of New York, Inc., New York.

B.D. Davis, Harvard Medical School, Boston, Massachusetts: Some aspects of protein secretion in bacteria.
W. Wickner, UCLA School of Medicine, Los Angeles, California: Studies of membrane assembly in *E. coli*.
G. Blobel, Rockefeller University, New York, New York: Mechanism of protein translocation across and integration into membranes.

Phycomyces

August 2–August 8

Session 1 Physiology I

Chairperson: E.D. Lipson, Syracuse University, New York

E.D. Lipson, Syracuse University, New York: Sensory transduction in *Phycomyces*.
D.S. Dennison, Dartmouth College, Hanover, New Hampshire: Phototropic perplexities.
P. Meyer, California Institute of Technology, Pasadena: *Phycomyces'* avoidance in a wind-free environment.
J.-F. Lafay and J. Matricon, University of Paris, France: Interferences between two simultaneous stimuli (barrier/wind; barrier/light).

Session 2 The Photoreceptor

Chairperson: D.E. Presti, University of Oregon, Eugene

D.E. Presti, University of Oregon, Eugene: The photoreceptor in *Phycomyces* photobiology considered in the light of flavin and carotene.
M. Jayaram, State University of New York, Stony Brook: Light-induced carotene synthesis in *Phycomyces*.
W. Shropshire, Jr., Smithsonian Radiation Biology Laboratory, Rockville, Maryland: Action spectra for light-induced carotenoid synthesis.
E. Schafer and G. Loser, University of Freiburg, Federal Republic of Germany: Photogeotropism of *Phycomyces*.
P.A. Galland, Syracuse University, New York: Action spectra of photogeotropic equilibrium in *Phycomyces* wild type and three behavioral mutants.

Session 3 Genetics

Chairperson: A.P. Eslava, University of Salamanca, Spain

M.I. Alvarez and A.P. Eslava, University of Salamanca, Spain: Mutants of *Phycomyces* with abnormal phototropism isolated with ICR-170.
M.I.G. Roncero and E. Cerda-Olmedo, University of Seville, Spain: Mutagenesis.
I. Lopez-Diaz and E.D. Lipson, Syracuse University, New York: Genetics of hypertropic mutants.
M. Orejas Suarez and A.P. Eslava, University of Salamanca, Spain: Mutagenesis with EMS in *Phycomyces*.
F. Rivero and E. Cerda-Olmedo, University of Seville, Spain: Spontaneously germinating mutants.

Session 4 Biochemistry I

Chairperson: R.J. Cohen, University of Florida, Gainesville

J. Ruiz-Herrera, University of Guanajuato, Mexico: Chitin biosynthesis in Mucorales with emphasis on *Phycomyces*.
J. Soler, D. De Arriaga, and F. Busto, University of Leon, Spain: Regulation of carbohydrate metabolism of *Phycomyces blakesleeanus* by lactic dehydrogenase.
G. Sandmann, University of Constance, Federal Republic of Germany: Inhibitory effect of continuous illumination on intermediary metabolism in *Phycomyces*.
A. Flores-Carreon, M.A. Avalos, and J. Ruiz-Herrera, University of Guanajuato, Mexico: Gluceronosyl transferase activity in cell free extracts of *Phycomyces blakesleeanus*.
J.A. Pollock, D.T. Sullivan, and E.D. Lipson, Syracuse University, New York: Characterization of plasma membrane flavoproteins from stage-1 sporangiophores of *Phycomyces* wild type and class-1 mutants by two-dimensional gel electrophoresis.

Session 5 Physiology II

Chairperson: D.S. Dennison, Dartmouth College, Hanover, New Hampshire

K.W. Foster, Mount Sinai School of Medicine, New York, New York: The signal processing of light in *Phycomyces*.
R.C. Poe and E.D. Lipson, Syracuse University, New York: White-noise analysis of *Phycomyces* photomutants as a probe of the internal dynamics of the light growth response.
P.A. Galland and E.D. Lipson, Syracuse University, New York: Wavelength dependence of phototropic dark adaptation in *Phycomyces*.
S.M. Block, J. Segall, and H. Berg, California Institute of Technology, Pasadena: Excitation and adaptation in bacterial chemotaxis.

Session 6 Special Lecture

E.-P. Fischer, University of Konstanz, Federal Republic of Germany: Max Delbrück—A physicist who looked at biology.

Session 7 Genetics and Sexual Development

Chairperson: R.P. Sutter, West Virginia University, Morgantown

M.I. Pelaez and A.P. Eslava, University of Salamanca, Spain: Recombination studies in *Phycomyces*.
R.P. Sutter, West Virginia University, Morgantown: Why do cultures of each mating type emit unique sex pheromones—Precursors of TAs (trisporic acids)?
W. Gauger, University of Nebraska, Lincoln: Mating-type heterokaryons and isogenicity in *Phycomyces*.
T. Suarez, A.P. Eslava, and A. Jimenez, University of Salamanca, Spain: Toward the isolation of a plasmid with a *Phycomyces* replication origin.

Session 8 Biochemistry/Genetics

Chairperson: E. Cerda-Olmedo, University of Seville, Spain

P.M. Bramley, Royal Holloway College, Egham, Surrey, England: Carotene biosynthesis in cell extracts of *Phycomyces*.

F. Parra, A. De La Concha, and F.J. Murillo, University of Murcia, Spain: Regulation of carotenogenesis in *Phycomyces*.

J.-L. Revuelta Doval[1] and A.P. Eslava[2], [1]University of Leon; [2]University of Salamanca, Spain: A new type of mutant disturbed in the photoindication of carotenoids in *Phycomyces*.

D. De Arriaga, J. Soler, F. Teixido, and E.G. Gallarraga, University of Leon, Spain: Pyridine-dependent dehydrogenases in *Phycomyces blakesleeanus*—Effects of light and vitamin A.

J.R. Medina, University of Seville, Spain: Genetics and regulation of alcohol-dehydrogenase in *Phycomyces*.

Session 9 Cytology and Development

Chairperson: T. Ootaki, Yamagata University, Japan

S.K. Malhotra and J.P. Tewari, University of Alberta, Canada: *Phycomyces blakesleeanus*—Plasma membrane as a model for studies on the structure and function.

A.J. Van Laere[1], B. Furch,[2] and J. Van Assche, [1]Catholic University of Louvain, Belgium; [2]University of Kiel, Federal Republic of Germany: Dormancy and germination of *Phycomyces* sporangiophores.

B. Furch, University of Kiel, Federal Republic of Germany: On the cell wall of sporangiophores of *Phycomyces blakesleeanus* in relation to heat-induced germination.

K. Koga and T. Ootaki, Yamagata University, Japan: Growth and behavior of piloboloid mutants in *Phycomyces*.

F. Gutierrez-Corona and E. Cerda-Olmedo, University of Seville, Spain: Microphorogenesis and carotene biosynthesis.

Session 10 Biochemistry II

Chairperson: P.V. Burke, University of California, Santa Cruz

R.J. Cohen, University of Florida, Gainesville: Cyclic nucleotide and polyamine metabolism.

L.S. Leutwiler and M. Brandt, California Institute of Technology, Pasadena: The absence of light-induced changes in cAMP concentration in the sporangiophore of *Phycomyces*.

M.V. Reddy, University of Florida, Gainesville: cAMP phosphodiesterase in *Phycomyces blakesleeanus*—A genetic and biochemical analysis.

W. Hilgenberg and F. Hoffman, J.W. Goethe-Universitat, Frankfurt, Federal Republic of Germany: Indoleacetic acid (IAA) in *Phycomyces blakesleeanus*.

Session 11 Physiology III

Chairperson: W. Shropshire, Jr., Smithsonian Radiation Biology Laboratory, Rockville, Maryland

K. Bergman, Northeastern University, Boston, Massachusetts: Controlled-release polymers—A new tool for the study of chemosensory systems.

R.I. Gamow, University of Colorado, Boulder: Fine structure analysis of the growing zone.

P.V. Burke, University of California, Santa Cruz: Geotropism in the hypertropic mutant C5.

Session 12 Directions of Future Research

Chairpersons: P.V. Burke, University of California, Santa Cruz, and E.D. Lipson, Syracuse University, New York.

E. Cerda-Olmedo[1] and E.D. Lipson[2], [1]University of Seville, Spain; [2]Syracuse University, New York: Status report on the *Phycomyces* monograph.

Panel led by session chairpersons: *Directions of future research.*

Anaerobic Genetics

August 16–August 17

Session 1

Chairperson: L.E. Mortenson, Exxon Research and Engineering Company, Linden, New Jersey

Clostridium

J.L. Johnson, Virginia Polytechnic Institute and State University, Blacksburg: General taxonomy.

C. acetobutylicum-pasteurianum

G. Gottschalk, University of Gottingen, Federal Republic of Germany: A continuous process for the production of butanol and acetone.

J.-S. Chen, Virginia Polytechnic Institute and State University, Blacksburg: (1) Acetone-butanol fermentation by *C. beijerinckii* (*C. butylicum*) in cultures maintained at neutral pH. (2) Study of nitrogenase genes and nitrogen-fixation in *C. pasteurianum*.

J.G. Morris, University College of Wales, Aberystwyth: The physiology of solvent production in continuous flow cultures of *C. acetobutylicum*.

D.R. Woods, University of Cape Town, South Africa: Molecular genetic studies on *C. acetobutylicum*.

M. Hermann, Institut Francais du Petrole, Rueil-Malmaison, France: Isolation of butanol resistant mutants of *C. acetobutylicum*.

Session 2

Chairperson: J.G. Morris, University College Wales, Aberystwyth, United Kingdom

C. thermocellum

E.A. Johnson and A.L. Demain, Massachusetts Institute of Technology, Cambridge: Anaerobic nature of *C. thermocellum* cellulase.

R.F. Gomez, Genentech, Inc., South San Francisco, California: An insertion seqence from *C. thermocellum*.

Other clostridia

L.G. Ljungdahl, University of Georgia, Athens: Aspects of electron transfer processes in acetogenic bacteria.

M.L. Britz, Masssachusetts Institute of Technology, Cambridge: Production of larger varied (C_4 to C_6) volatile fatty acids by anaerobes.

J.N. Grindley, Biogen Inc., Cambridge, Masssachusetts: Attempts to create a transformation system and cloning vector for *C. thermosaccharolyticum*.

M. Sebald, Institut Pasteur, Paris, France: Present status of genetics in clostridia—Emphasis on pathogens.

Other systems

A. Matin, Stanford University, California: Physiology of bacterial acidophilism.

B.L. Marrs, St. Louis University, Missouri: Development of genetic systems for photosynthetic bacteria.

Participants at Anaerobic Genetics Meeting

Session 3

Chairperson: G. Gottschalk, University of Gottingen, Federal Republic of Germany

Methanogens

M.J. Wolin, New York State Department of Health, Albany: Overview of methanogens—Methane-producing bacteria of the human gut.
S. Yamazaki, National Institutes of Health, Bethesda, Maryland: Properties of selenium-containing hydrogenase from *Methanococcus vannielii*.
G. Bertani and L. Baresi, Jet Propulsion Laboratory, Pasadena, California: Some mutants of *Methanococcus voltae*.
W.B. Whitman, University of Georgia, Athens: Physiology of *Methanococcus voltae*.
J. Konisky, University of Illinois at Urbana-Champaign: Expression of *Methanococcus voltae* genes in *E. coli*.
P. Hamilton, Ohio State University, Columbus: Cloning and expression of methanogen DNA.

Other systems

C.E. Nord, National Bacteriology Laboratory, Stockholm, Sweden: β-lactamases in anaerobic bacteria.

Session 4

Chairperson: M. Sebald, Institut Pasteur, Paris, France

Bacteroides

F. Tally, Tufts University, Boston, Massachusetts: Introduction.
F. Tally and M. Malamy, Tufts University, Boston, Massachusetts: Drug-resistance transfer in *Bacteroides fragilis*.
J.L. Johnson, Virginia Polytechnic Institute and State University, Blacksburg: Plasmid distribution in *Bacteroides*.
F.L. Macrina, Virginia Commonwealth University, Richmond: Plasmid- and nonplasmid-mediated gene transmission in *Bacteroides*.
C.J. Smith, Bethesda Research Laboratories, Gaithersburg, Maryland: Transferable Clindamycin resistance in *B. ovatus*.
D.G. Guiney, University of California, San Diego: Cloning and expression in *E. coli* of a tetracycline-resistance gene from *B. fragilis*.
D.R. Woods, University of Cape Town, South Africa: UV repair systems in *B. fragilis*.

Session 5 Summary and Discussion

Chairperson: A.I. Bukhari, Cold Spring Harbor Laboratory, New York

J.E. Brenchley, Genex Corporation, Gaithersburg, Maryland
J.G. Morris, University College of Wales, Aberystwyth
L.E. Mortenson, Exxon Research and Engineering Company, Linden, New Jersey
J. Konisky, University of Illinois at Urbana-Champaign
B.L. Marrs, St. Louis University, Missouri

Comments

E.B. Priestley, Exxon Research and Engineering Company, Linden, New Jersey
O.H. Smith, United States Department of Energy, Washington, D.C.

Recombinant DNA Applications to Human Disease

October 3–October 6

Session 1 Globin Genes, HLA, and Immunoglobins

Chairperson: S.M. Weissman, Yale University, New Haven, Connecticut

B.G. Forget, Yale University, New Haven, Connecticut: Normal human globin gene structure and mutations causing different thalassemia syndromes.

S. Orkin, The Children's Hospital Medical Center, Boston, Massachusetts: A review of β-thalassemias—The spectrum of gene mutations.
H.H. Kazazian, Jr., Johns Hopkins Hospital, Baltimore, Maryland: DNA polymorphisms in the β-globin gene cluster: Use in discovery and in prenatal diagnosis.
S.M. Weissman, Yale University, New Haven, Connecticut: Molecular studies of the genes of the human major histocompatibility complex.
T.A. Waldmann, National Cancer Institute, Bethesda, Maryland: The arrangement of immunoglobulin genes in human lymphoid leukemias.
D. Pious, University of Washington, Seattle: Analysis of the HLA region using somatic cell mutants.
D.E. Housman, Massachusetts Institute of Technology, Cambridge: Discussion.

Session 2 Specific Disease Entities

Chairperson: C.T. Caskey, Baylor College of Medicine, Houston, Texas

C.T. Caskey, Baylor College of Medicine, Houston, Texas: Mutation at the *hprt* locus in rodent and man.
T. Friedmann, University of California, San Diego: Characterization of an expressible human HPRT cDNA.
A.L. Beaudet, Baylor College of Medicine, Houston, Texas: Altered mRNA for argininosuccinate synthetase in citrullinemia.
S.L.C. Woo, Baylor College of Medicine, Houston, Texas: α_1-antitrypsin deficience and pulmonary emphysema—Identification of recessive homozygotes by direct analysis of the mutation site in the chromosomal gene.
D. Pious, University of Washington, Seattle: Discussion.

Session 3 Oncogenes and Heritable Cancers

Chairperson: D. Botstein, Massachusetts Institute of Technology, Cambridge

F. Dautry, Massachusetts Institute of Technology, Cambridge: Mechanism of activation of an oncogene from a human bladder carcinoma.
M. Wigler, Cold Spring Harbor Laboratory, New York: Human transforming genes.
W.F. Benedict, Children's Hospital of Los Angeles: Human retinoblastoma—A prototype of a suppressor class of human cancer genes.
L.C. Strong, University of Texas Cancer Center, Houston: Genetic heterogeneity and retinoblastoma.
S.M. Weissman, Yale University, New Haven, Connecticut: Discussion.

Session 4 Chromosome Alteration and Mapping

Chairperson: P.L. Pearson, Sylvius Laboratories, Leiden, The Netherlands

M.E. Harper, The Agouron Institute, La Jolla, California: Chromosome mapping of single copy sequences by in situ hybridization.
T.B. Shows, Roswell Park Memorial Institute, Buffalo, New York: Chromosome mapping of cloned genes and DNA polymorphisms associated with disease.

S. Brenner, K.E. Davies, M. Shodell

M. Siniscalco, U. Francke, D. Botstein

U. Francke, Yale University, New Haven, Connecticut: Mapping of DNA sequences to chromosome regions in somatic cell hybrids.
S.A. Latt, Children's Hospital Medical Center, Boston, Massachusetts: Construction, analysis, and utilization of recombinant phage libraries enriched for the human X chromosome by florescence activated flow sorting.
T.T. Puck, Eleanor Roosevelt Institute for Cancer Research, Inc., Denver, Colorado: Human gene mapping, exposure, and expression.
D. Patterson, Eleanor Roosevelt Institute for Cancer Research, Inc., Denver, Colorado: Somatic cell genetics and molecular analysis of Down's syndrome.
P.L. Pearson, Sylvius Laboratories, Leiden, The Netherlands: Discussion.

Session 5 Specific Linkage Associations

Chairperson: R.L. White, University of Utah, Salt Lake City

R.L. White, University of Utah, Salt Lake City: Approaches to linkage analysis in the human.
M. Skolnick, LDS Hospital, Salt Lake City, Utah: The variation in expected LOD score by degree of polymorphism and recombination.
J.F. Gusella, Massachusetts General Hospital, Boston: The use of restriction fragment length polymorphisms to map the Huntington's disease gene.
D.C. Page, Massachusetts Institute of Technology, Cambridge: Homologous single-copy sequences on the human X and Y chromosomes.
H. Erlich, Cetus Corporation, Berkeley, California: Discussion.
R. Williamson, St. Mary's Hospital Medical School, London, England: The study of the basic defects in cystic fibrosis.
K.E. Davies, St. Mary's Hospital Medical School, London, England: An X chromosome probe linked to Duchennes muscular dystrophy.
M. Siniscalco, Memorial Sloan-Kettering Cancer Center, New York, New York: Molecular mapping of the human genome—A crossroad between basic and applied research.
R.L. Nussbaum, Baylor College of Medicine, Houston, Texas: Molecular analysis of the *hprt* locus.
B.G. Forget, Yale University, New Haven, Connecticut: Discussion.

Session 6 Growth Hormone and Therapies

Chairperson: S. Brenner, Cambridge University, England

J.D. Baxter, University of California, San Francisco: Expression of growth hormone-related genes.
J.A. Phillips, Johns Hopkins Hospital, Baltimore, Maryland: The growth hormone gene in human disease.
G.I. Bell, University of California, San Francisco: The polymorphic locus adjacent to the human insulin gene and its association with diabetes mellitus—A population study.
H. Erlich, Cetus Corporation, Berkeley, California: Restriction fragment length polymorphism analysis of HLA-typed families using cloned HLA probes.
H.I. Miller, Food and Drug Administration, Rockville, Maryland: The role of the Food and Drug Administration in the regulation of the products of recombinant DNA technology—Update 1983.
D. Botstein, Massachusetts Institute of Technology, Cambridge: Growth hormone therapies.
S. Brenner, Cambridge University, England: Discussion.

Biological Aspects of Alzheimer's Disease

October 24–October 27

Session 1 The Alzheimer's Patient—The Human Dimension

R. Katzman, Albert Einstein College of Medicine, Bronx, New York.
Panel Discussion—G.D. Cohen, National Institute of Mental Health, Rockville, Maryland; J.P. Blass, The Burke Rehabilitation Center, White Plains, New York: D.A. Drachman, University of Massachusetts, Worcester; M.F. Folstein, Johns Hopkins Hospital, Baltimore, Maryland.

Session 2 Cell and Tissue Changes in Alzheimer's Disease

Chairperson: R. Katzman, Albert Einstein College of Medicine, Bronx, New York

T.L. Kemper, Boston City Hospital, Massachusetts: Organization of the neuropathology of the amygdala in Alzheimer's disease.
P.C. Burger, Duke University, Raleigh, North Carolina: Limbic system in Alzheimer's disease.
M.J. Ball, University of Western Ontario, Canada: Hippocampal morphometry in Alzheimer's dementia—Implications for neurochemical hypotheses.
G.D. Cohen, National Institute of Mental Health, Rockville, Maryland: Discussion.
D.L. Price, Johns Hopkins University, Baltimore, Maryland: Basal forebrain cholinergic neurons and neuritic plaques in primate brain.
M. Mesulam, Beth Israel Hospital, Boston, Massachusetts: Cortical projections from the nucleus basalis in primates.
R.D. Terry, Albert Einstein College of Medicine, Bronx, New York: Cortical morphometry in Alzheimer's disease.
R. Katzman, Albert Einstein College of Medicine, Bronx, New York: Discussion.

Session 3 Fibrous Proteins

Chairperson: R.J. Lasek, Case Western Reserve University, Cleveland, Ohio

W.W. Schlaepfer, University of Pennsylvania, Philadelphia: Some observations on the structural and chemical nature of neurofilaments.
L. Autilio-Gambetti, Case Western Reserve University, Cleveland, Ohio: Paired helical filaments—Relatedness to neurofilaments shown by silver staining and reactivity with monoclonal antibodies.
D.J. Selkoe, McLean Hospital, Belmont, Massachusetts: Protein chemistry of paired helical filaments.
G.G. Glenner, University of California, San Diego: Alzheimer's disease—Multiple cerebral amyloidosis.
H.M. Wisniewski, New York State Institute for Basic Research in Mental Retardation, Staten Island: Pathogenesis of neuritic and amyloid plaques.
S. Yen, Albert Einstein College of Medicine, Bronx, New York: Discussion.

Session 4 Genetics and Biochemistry of Alzheimer's and Related Disorders

Chairperson: X.O. Breakefield, Yale University, New Haven, Connecticut

C.J. Epstein, University of California, San Francisco: Down's syndrome and Alzheimer's disease—Implications and approaches.
L.L. Heston, University of Minnesota, Minneapolis: Dementia of the Alzheimer's type—Perspectives from studies of families.
C.A. Marotta, McLean Hospital, Belmont, Massachusetts: In vitro protein synthesis by messenger RNA from the Alzheimer's disease brain.
D.E. Housman, Massachusetts Institute of Technology, Cambridge: Molecular genetic approaches.
X.O. Breakefield, Yale University, New Haven, Connecticut: Discussion.

Session 5 Neurotransmitter Changes in Alzheimer's Disease

Chairperson: P. Davies, Albert Einstein College of Medicine, Bronx, New York

R.T. Bartus, Lederle Laboratories, Pearl River, New York: Evidence for a cholinergic deficiency using animal models of aging—Implications for studying and treating Alzheimer's disease.
D.M. Bowen, Institute of Neurology, London, England: Neurotransmitters and metabolism in Alzheimer's disease.
J.T. Coyle, Johns Hopkins University, Baltimore, Maryland: Synaptic neurochemistry of the basal forebrain cholinergic projections.
I. Hanin, University of Pittsburgh, Pennsylvania: Chemically induced cholinotoxicity in vivo—Studies utilizing ethylcholine aziridinium ion (AF64A).
P. Davies, Albert Einstein College of Medicine, Bronx, New York: Neurotransmitters and neuropeptides in Alzheimer's disease.
R.H. Perry, Newcastle General Hospital, England: Discussion.

Session 6

Chairperson: C.W. Cotman, University of California, Irvine

Trophic factors

R. Katzman, Albert Einstein College of Medicine, Bronx, New York: In vivo cholino-trophic activity.
C.W. Cotman, University of California, Irvine: Increase in neurotrophic factor following brain injury.
S.H. Appel, Baylor College of Medicine, Houston, Texas: Neurotrophic effects of hippocampal extracts on medial septal nucleus.

Metabolism

J.P. Blass, The Burke Rehabilitation Center, White Plains, New York: Fibroblast phosphofructokinase in Alzheimer's disease and Down's syndrome.
D.F. Benson, University of California, Los Angeles: Alterations in glucose metabolism in Alzheimer's disease.
F.S.J. Frackowiak, Hammersmith Hospital, London, England: O^{15} metabolism.
C.W. Cotman, University of California, Irvine: Discussion.

Session 7 Behavioral Correlates

Chairperson: C.W. Cotman, University of California, Irvine

M.F. Folstein, The Johns Hopkins Hospital, Baltimore, Maryland: Differential cognitive changes in the hereditary form of Alzheimer's disease.
R.H. Perry, Newcastle General Hospital, England: Relation of cholinergic loss to dementia.
D.A. Drachman, University of Massachusetts, Worcester: Aging and dementia—Insights from the study of anticholinergic drugs.
R. Katzman, Albert Einstein College of Medicine, Bronx, New York: Discussion.

Session 8 Viral and Environmental Agents

Chairperson: R.G. Rohwer, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland

S.B. Prusiner, University of California, San Francisco: On the molecular biology of prions.
A.G. Dickinson, ARC and MRC Neuropathogenesis Unit, Edinburgh, Scotland: The relevance of scrapie as an experimental model for Alzheimer's disease.
R.G. Rohwer, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland: Discussion.
E.E. Manuelidis, Yale University, New Haven, Connecticut: Novel biological properties of Creutzfeldt-Jakob brains in vitro.
L. Manuelidis, Yale University, New Haven, Connecticut: Fractionation and infectivity studies in Creutzfeldt-Jakob disease.
D.C. Gajdusek, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland: Environmental factors responsible for ALS, P-D, and other neurological syndromes in high incidence foci.
D.P. Perl, University of Vermont, Burlington: Aluminum in Alzheimer's disease.

R. Katzman, H. Wisniewski

L. Manuelidis, M. Folstein, G. Glenner

R.G. Rohwer, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland: Discussion.

Session 9 Therapeutic Interventions

Chairperson: R.J. Wurtman, Massachusetts Institute of Technology, Cambridge

K.L. Davis, Veterans Administration Medical Center, Bronx, New York: Pharmacological and biological studies of Alzheimer's disease.
J.H. Growdon, Massachusetts General Hospital, Boston, Precursor therapies.
L.J. Thal, Albert Einstein College of Medicine, Bronx, New York: Oral physostigmine and lecithin improve memory in Alzheimer's disease.
R.J. Wurtman, Massachusetts Institute of Technology, Cambridge: Discussion.

Congressional Workshop: Carcinogenesis—From the Environment to the Gene

November 19–November 21

Session 1

J. Cairns, Harvard School of Public Health, Boston, Massachusetts: Epidemiology and molecular biology—More than just good friends?

Session 2

A.J. Levine, State University of New York at Stony Brook: What we now know about the organization of the human genome.
J. Sambrook, Cold Spring Harbor Laboratory, New York: How we know what we now know about the organization of the human genome.

Session 3

R.A. Weinberg, Massachusetts Institute of Technology, Cambridge: Finding oncogenes by "transfection."
R.L. Erikson, University of Colorado, Denver: Retroviruses and oncogenes.
D.E. Housman, Massachusetts Institute of Technology, Cambridge: Probing the human genome.

Session 4

R.C. Gallo, National Cancer Institute, Bethesda, Maryland: Viruses and human cancer.
R. Wilson, Harvard University, Cambridge, Massachusetts: New parameters for the health-risk equation?
J.M. Bishop, University of California, San Francisco: Summary.


R. Gallo


A. Levine, J. Tuchman-Mathews


M. Bishop

Postgraduate Training Program

SUMMER 1982

The Postgraduate Training Program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects which are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Principles of Developmental Neurobiology, June 12–June 25

INSTRUCTORS

Purves, D., M.D., Washington University School of Medicine, St. Louis, Missouri
Patterson, P.H., Ph.D., Harvard Medical School, Cambridge, Massachusetts

The aim of this intensive two-week course was to expose a group of 18 students to the classical literature of neural development, with special emphasis on recent advances and controversies in this rapidly growing field. The selection of students was based primarily on recommendations and potential rather than background; thus this year's group included 11 postdoctoral fellows, 5 graduate students, and 2 fulltime faculty members from the United States and Europe involved in widely different research programs. The common denominator was an interest in pursuing research related to neural development.

The usual format was a detailed review of particular subjects given by invited lecturers in a three-hour morning session. The speaker then chose several important papers, generally ones not discussed in the morning, for the students to read during the afternoon. In the late afternoon the speaker (and instructors) met with groups of 6 students to discuss the reading and prepare one of the students in each group to present the papers at an evening meeting of the whole class. The evening then consisted of several student presentations of aspects of the morning's subject not covered by the invited lecturer, as well as a general discussion of the issues raised during the day. Most evenings ended with one or two student research seminars in which an individual's work in progress was described for discussion and criticism by fellow students, instructors and the guest lecturers. In general, we attempted to have such presentations on an evening when the day's guest lecturer had a special interest in the student's field. An occasional variant of this format was to end the evening with a research seminar by an additional invited speaker whose material was deemed of special interest, but not broad enough to devote an entire day to.

PARTICIPANTS

Bernhardt, Robert, M.S., Friedrich Meischer Institut, Basel, Switzerland
Blanks, Janet, Ph.D., University of Southern California, Los Angeles
Caudy, Michael, B.S., University of California, Berkeley
Diaz-Arrastia, Ramon, B.A., Baylor College of Medicine, Houston, Texas
Forehand, Cynthia, Ph.D., Washington University School of Medicine, St. Louis, Missouri
Johnson, Jim, B.S., Bowman Gray School of Medicine, Winston-Salem, North Carolina
Kalcheim, Chaya, M.Sc., Weizmann Institute of Science, Rehovot, Israel
Kinnamon, Sue, Ph.D., University of Colorado Medical Center, Denver
Korsching, Sigrun, M.S., Max-Planck Institute for Psychiatry, Munich, Federal Republic of Germany

Maeder, Jacques, M.Sc., Friedrich Miescher Institut, Basel, Switzerland
Pearson, John, M.D., New York University Medical Center, New York
Price, Jack, B.A., University College of London, England
Rosenheimer, Julie, B.A., University of Wisconsin, Madison
Stürmer, Claudia, Ph.D., University of Michigan, Ann Arbor
Scherer, Steve, B.S., University of Michigan, Ann Arbor
Tomaselli, Kevin, B.S., University of California, San Francisco
Weber, Art, B.A., University of Wisconsin, Madison
Wolinsky, Eve, B.S., Harvard Medical School, Boston, Massachusetts

SEMINARS

Cowan, W.M., Salk Institute. *An overview of neural development.*
Rakic, P., Yale University Medical School. *Cell proliferation.*
——. *Neuronal migration.*
Lawrence, P.A., MCR Laboratory of Molecular Biology. *Pattern formation: Insect cuticle.*
——. *Compartments, segments, and postitional information.*
Spitzer, N.C., University of California, San Diego. *Development of the grasshopper nervous system: Morphology, chemosensitivity, and electrical properties.*
Horvitz, R., Massachusetts Institute of Technology. *Development of* C. elegans: 1) *Cell diversity,* 2) *Cell death,* 3) *Egg-laying.*
Spitzer, N.C., University of California, San Diego. *Development of Rohan-Beard cells.*
Patterson, P.H., Harvard Medical School. *Influence of environment on phenotype in vivo and in vitro.*
Landmesser, L.T., Yale University. *Axon guidance in vertebrates.*
Landis, S., Harvard University Medical School. *The growth cone.*
Bentley, D., University of California, Berkeley. *Insect pioneer axons.*
Aguayo, A.J., McGill University. *Axon regeneration; interactions of nerve and glia.*
Purves, D., Washington University School of Medicine, St. Louis. *Neuronal death.*
Patterson, P.H., Harvard Medical School. *Nerve growth factor.*
Hall, Z.W., University of California School of Medicine, San Francisco. *Synapse formation.*
Patterson, P.H., Harvard Medical School. *Trophic effects in sensory systems.*
Purves, D., Washington University School of Medicine. *Specificity in the peripheral nervous system.*
Easter, S., University of Michigan. *Specificity in the retinotectal system.*
Gottlieb, D.I., Washington University School of Medicine. *Molecular basis of cell recognition I.*
Sanes, J., Washington University School of Medicine. *Molecular basis of cell recognition II.*
Raff, M., Yale University School of Medicine. *Molecular basis of cell recognition III.*
Purves, D., Washington University School of Medicine. *Synaptic rearrangement; synaptic maintenance.*
Stent, G.S., University of California, Berkeley. *Development of the leech.*
Stryker, M.P., University of California Medical School, San Francisco. *Development of the visual system I.*
——. *Development of the visual system II.*
Drager, U., Harvard Medical School. *Mutations in visual development.*
Nottebohm, F., Rockefeller University. *Development of birdsong.*
Hildebrand, J.G., Columbia University. *Development of insect behavior.*

Nervous System of the Leech, June 22–July 1

INSTRUCTORS

Nicholls, John, M.D., Ph.D., Stanford University, California
Parnas, Itzhak, Ph.D., Hebrew University, Jerusalem, Israel
Muller, Ken, Ph.D., Carnegie Institution of Washington, Baltimore, Maryland
Zipser, Birgit, Ph.D., Cold Spring Harbor Laboratory, New York

The aim of this workshop was to provide students with an intensive lab and seminar course that would enable them to pursue independent work on the leech. To this end, we hoped to provide the students with techniques for recording from leech cells, now considered straightforward and relatively easy, that took much time and effort to be refined. With this knowledge, they might avoid many of the trivial technical difficulties that bedevil anyone starting on the nervous system of the leech or other animals.

The initial work was devoted mainly to recognizing the individual cells, learning how to record from them with intracellular and extracellular electrodes, getting familiar with the equipment, and performing dissections. The students then progressed to more difficult experiments, such as recording synaptic potentials while changing the fluid, bathing the preparation, or injecting individual cells with marker substances to study their geometry.

PARTICIPANTS

Drapeau, Pierre, Ph.D., University of Maryland, College Park
Flaster, Murray S., B.S., Cold Spring Harbor Laboratory, New York
Hoppe, Pamela E., B.A., Princeton University, New Jersey
Johansen, Jorgen, M.S., Cold Spring Harbor Laboratory, New York
McGlade, Ellen, M.S., University of Maryland, College Park
Mehaffey, Leathem, Ph.D., Vassar College, Poughkeepsie, New York
Metzmer, Henri, M.S., Hebrew University, Jerusalem, Israel
Soileau, Carmen L., M.S., University of Texas, Austin
Ways, Nancy R., B.A., Stanford University, California
Wordeman, Linda G., B.A., University of California, Berkeley

SEMINARS

Kristen, W., University of California, San Diego. *The swimming leech.*
——. *Circuitry that generates leech swimming.*

Stent, G., University of California, Berkeley. *Embryonic development.*
Friesen, W.O., University of Virginia, Charlottesville. *Wave detectors.*
Hockfield, S. and R. McKay, Cold Spring Harbor Laboratory. *Monoclonal antibodies to identified neurons.*
Payton, B., Memorial University. *Giant leech in Newfoundland.*
Salzberg, B., University of Pennsylvania Dental School. *Optical recording.*

Molecular Biology of Plants, June 12–July 2

INSTRUCTORS
Bedbrook, John R., Ph.D., CSIRO, Canberra, Australia
Ausubel, Frederick, Ph.D., Harvard University, Cambridge, Massachusetts

ASSISTANTS
Waldren, John, CSIRO, Canberra, Australia
Schoene, Vinni, Harvard University, Cambridge, Massachusetts
Brown, Susan E., Harvard University, Cambridge, Massachusetts
Dunn, Barbara, B.S., Harvard University, Cambridge, Massachusetts
Buikema, Bill, Harvard University, Cambridge, Massachusetts

The three week Plant Molecular Biology Course consisted of a series of lectures by the staff and by distinguished invited speakers and a series of laboratory exercises designed to acquaint the students with the fundamentals of current plant molecular biology research. The course assumed that most students come with a good background in molecular genetic techniques but with a poor understanding of general botany, plant physiology, plant sexual reproduction, and plant development. Therefore, the course began with lectures to acquaint the students with some of the fundamental differences between plants and animals. The introductory topics were tied together by considering plants from a developmental point of view. Laboratory experiments involving whole plant anatomy, embryo and meristem culture, and somatic cell tissue culture complemented the introductory lectures.

The introductory section was followed by a series of detailed lectures covering topics which were relatively unique to plants including photosynthesis, seed formation and germination, and regeneration of intact plants from somatic cultures. Among the topics covered were classical transmission genetics including transposable genetic elements, cytogenetics, plant viruses, plant-bacterial interactions, and the molecular biology of the plant organelle and nuclear genomes. The laboratory projects paralleled the lectures and included experiments on (1) the mechanism of α-amylase induction in germinating seeds; (2) the manipulation of plant tissue cultures; (3) the generation of plant mutants; (4) the isolation, characterization and manipulation of plant DNAs and RNAs; and (5) cytogenetics.

PARTICIPANTS

Barta, Andrea, Ph.D., University of Vienna, Austria
Bonitz, Susan G., Ph.D., Genentech, San Francisco, California
Daniell, Ellen, Ph.D., University of California, Berkeley
Digan, Mary Ellen, Ph.D., National Institutes of Health, Bethesda, Maryland
Grabau, Elizabeth A., Ph.D., University of Utah, Salt Lake City
Hernalsteens, Jean-Pierre, Ph.D., Vrije Universiteit Brussel, Belgium
Higuchi, Russell G., Ph.D., University of California, Berkeley
Huang, Wai Mun, Ph.D., University of Utah, Salt Lake City
Ladin, Beth, Ph.D., Washington University, St. Louis, Missouri
Leutwiler, Leslie S., Ph.D., California Institute of Technology, Pasadena
Lu, Deru, National Institutes of Health, Bethesda, Maryland
Machida, Yasunori, Ph.D., State University of New York, Stony Brook
Muskavitch, Karen, M.T., Ph.D., Harvard University, Cambridge, Massachusetts
Nixon, B. Tracy, M.S., Harvard Medical School, Boston, Massachusetts
Riazuddin, Sheikh, Johns Hopkins University, Baltimore, Maryland
Robinson, Steven J., Ph.D., University of Chicago, Illinois
Tabor, Paul S., Ph.D., Naval Research Laboratory, Bethesda, Maryland

SEMINARS

Schmidt, G., University of Georgia. *Chloroplast assembly.*
Steinback, K., Michigan State University. *Structure of the chloroplast membrane.*
Bogorad, L., Harvard University. *Molecular biology of the chloroplast.*
Goldberg, B., University of California, Los Angeles. *Seed storage protein genes.*
Varner, J., Washington University. *Structure of the plant cell wall.*
Miles, D., University of Missouri. *Genetics of photosynthesis.*
Ho, D., University of Illinois, Urbana. *Molecular biology of alpha amylase.*
Burr, B. and F., Brookhaven National Laboratory. *Controlling elements in maize.*
Shepard, J., Kansas State University. *Plant tissue culture and agriculture.*

Animal Cell Culture, June 19–July 2

INSTRUCTORS

Barnes, David, Ph.D., University of California, San Diego
Mather, Jennie, Ph.D., The Population Council, Rockefeller University, New York, New York

ASSISTANTS

Byers, Alicia, B.S., The Population Council, Rockefeller University, New York, New York
Kaczorowski, Flo, B.S., The Population Council, Rockefeller University, New York, New York

The course was designed to introduce investigators familiar with conventional culture methodology to the special techniques and approaches of hormone-supplemented serum-free cell culture. Techniques for the handling of cells for serum-free culture and details of the preparation of media etc. were discussed. The handling, storage and methods of purification of various growth factors, hormones, and attachment factors were discussed. A large number of the currently available hormones, attachment factors, growth factors media, and growth surfaces were made available for participants to use in devising the supplements required for the growth of their own cell lines or those provided for use in the course. Exercises and demonstrations taught primary culture techniques for the isolation and culture of neuroblasts, thyroid, kidney, liver, and testicular cells. Clonal cell lines available were derived from a variety of tissues and species and were of both normal and transformed phenotype.

Seminars were designed to acquaint the participants with various approaches to the study of hormone action in cells and to stimulate thinking on approaches to studies of differentiation, cell function, and hormone action which can only be performed in a serum-free culture system.

The course was designed with maximum flexibility so as to allow participants to concentrate on areas of maximum interest to them with advice and discussion with the instructors, speakers and other course participants.

PARTICIPANTS

Angadi, Channabasappa, Ph.D., Nassau County Medical Center, East Meadow, New York
Anzano, Mario A., Ph.D., National Cancer Institute, Bethesda, Maryland
Brodie, Ann E., Ph.D., Oregon State University, Corvallis
Glaser, Janet, Ph.D., University of Illinois, Chicago
Kudo, Yoshihisa, Ph.D., Mitsubishi-Kasei Institute, Tokyo, Japan
Pannier-Poulain, Claude, M.D., Paris-South University, France
Pinset, Christian, M.D., Pasteur Institute, Paris, France
Van deWalle, Philippe, Universite Libre de Bruxelles, Belgium

SEMINARS

McKeehan, W., W. Alton Jones Cell Science Center. *Nutrition in cell culture.*
Strickland, S., Rockefeller University. *Differentiation of embryonal carcinoma cells in vitro.*
Pawelek, J., Yale University. *Effects of insulin on melanoma cell cultures.*
Phillips, D., The Population Council. *Use of morphological techniques for the study of hormone action in vitro.*
Reid, L., Albert Einstein College of Medicine. *Biomatrix in cell culture.*
Taub, M., State University of New York Buffalo. *Kidney cell culture in serum-free medium.*
Coon, H., National Cancer Institute. *Growth and function of thyroid and neuroblast cells in vitro.*
Sato, G., University of California, San Diego. *The endocrine system/hormone effect in vitro.*
Solomon, Y., Weizmann Institute. *Adenylate cyclase.*
Breitman, T.R., National Cancer Institute. *Terminal differentiation of HL-60 and leukemic cells by retinoic acid.*
Glass, J., Beth Israel Hospital. *Transferrin.*
Kaplan, J., University of Utah Medical School. *Control of $\alpha=m$ and transferrin receptors in culture.*
Werb, Z. University of California, San Francisco. *Collagenase and elastace secretion by cultured cells.*

Single-Channel Recording, July 5–July 23

INSTRUCTORS

Corey, David P., Ph.D., Yale University, New Haven, Connecticut
Dionne, Vincent E., Ph.D., University of California, San Diego
Ohmori, Harunori, M.D., Ph.D., University of California, Los Angeles
Pallotta, Barry, Ph.D., University of Miami, Florida
Patlak, Joseph, Ph.D., University of Vermont, Burlington
Stevens, Charles F., M.D., Ph.D., Yale University, New Haven, Connecticut
Yellen, Gary, B.A., Yale University, New Haven, Connecticut

ASSISTANT

Stevens, Meg, Harvard University, Boston, Massachusetts

The Single-Channel Recording Course brought together ten students whose backgrounds ranged from graduate student to department chairman, with seven instructors currently using the patch-clamp technique in their own research. The goals were two: Students, through intensive lab work and lectures, became proficient at the new patch-clamp technique, which is presently revolutionizing electrophysiology. Instructors, by comparing methods used in different laboratories, reached consensus on some aspects of the technique, and laid the groundwork for further development.

Lectures, by instructors and by invited lecturers, covered both the detailed methods for recording currents through single membrane channels and from whole cells, and the theoretical basis for analyzing and understanding the records. Laboratory work, at five electro-physiological setups, enabled students to learn and practice the various permutations of the patch-clamp technique, to become proficient at recognizing and specifically activating different membrane channel species, and to carry out short research projects on the tissues used in their own laboratories. Research projects included such diverse studies as development of Na^+ and Ca^{++} currents in embryonic frog neurons, Cl^- transport in red blood cells, an inwardly rectifying K^+ channel in adult frog muscle, Na^+ channels in dissociated mammalian cardiac muscle, and the observation of a large (500 pS), hitherto unreported channel in a skeletal muscle cell line.

PARTICIPANTS

Cook, Daniel L., Ph.D., Seattle VA Medical Center, Washington
Coronado, Roberto B., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Datyner, Nicholas, Ph.D., State University of New York, Stony Brook
Freschi, Joseph E., M.D., National Naval Medical Center, Bethesda, Maryland
Gray, Richard A., B.S., Baylor College of Medicine, Houston, Texas
Gruol, Donna L., Ph.D., Salk Institute, San Diego, California
Gunn, Robert B., M.D., Emory University, Atlanta, Georgia
O'Dowd, Diane, B.S., University of California, San Diego
Stanfield, Peter R., Ph.D., University of Leicester, England
Willard, Alan, L., Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Aldrich, R.W., Yale University. *Stochastic Processes.*
Breakfield, X.O., Yale University. *Tissue preparation: Primary cultures and cell lines.*

Advanced Neuroanatomical Methods, July 5–July 25

INSTRUCTORS

Jones, Edward G., Ph.D., Washington University Medical School, St. Louis, Missouri
Hand, Peter J., Ph.D., University of Pennsylvania, Philadelphia
Pickel, Virginia M., Ph.D., Cornell Medical College, New York, New York
Wise, Steven P., Ph.D., National Institutes of Mental Health, Bethesda, Maryland
Swanson, Larry W., Ph.D., Salk Institute, San Diego, California

ASSISTANTS

McClure, Bertha, Washington University, St. Louis, Missouri
Hendry, Stewart, B.A., Washington University, St. Louis, Missouri

The course for 1982 was conducted along new lines with students individually or in pairs carrying out a morphological investigation of selected areas of the central nervous system. The fundamental neuroanatomical techniques were not neglected but incorporated within the framework of advanced technology. Students were intensively supported by a relatively large group of teachers and assistants who, with the visiting faculty, also provided lectures on selected topics in current research.

Methods covered included autoradiography of axoplasmic transport, transmitter uptake and receptor localization, anterograde and retrograde labeling with horseradish peroxidase and fluorescent dyes, light and electron microscopic immunocytochemistry using a number of different polyclonal and monoclonal antibodies, the 2-deoxy-D-glucose method and localization of oxidative enzymes. Where feasible, methods for physiological localization were employed and the material carried to both light and electron microscopic levels.

PARTICIPANTS

Bielajew, Catherine H., M.A., Concordia University, Montreal, Canada
Blakely, Randy D., B.A., Emory University, Atlanta, Georgia
Fairbanks, Mary E., Dartmouth College, Hanover, New Hampshire
Gibson, Candace J., Ph.D., Massachusetts Institute of Technology, Cambridge
Gizzi, Martin S., M.A., New York University, New York
Kilduff, Thomas S., Ph.D., Stanford University, California
Lang, William J., B.S., University of Oxford, England
Priestley, John V., B.A., University of Oxford, England
Schuz, Almut, Ph.D., Max-Planck Institut, Berlin, Federal Republic of Germany
Wysocki, Charles J., Ph.D., Monell Chemical Senses Center, Philadelphia, Pennsylvania

SEMINARS

Hendrickson, A.E., University of Washington. *Study of the visual system by autoradiographic techniques.*
Priestley, J.V., Oxford University. *Immunocytochemical techniques as applied to the study of spinal cord organization.*
Raviola, E., Harvard University. *Freeze fracture and related techniques in the study of the retina.*
Peters, A., Boston University. *The golgi-EM technique in the study of the cerebral cortex.*
MacKay, R.W., Cold Spring Harbor Laboratory. *Neuroimmunology and hybridoma technology.*
Kitai, S.P., Michigan State University. *Intracellular injection studies of the basal ganglia.*

Advanced Bacterial Genetics, July 5–July 25

INSTRUCTORS

Silhavy, Thomas J., Ph.D., NCI, Frederick Cancer Research Facility, Frederick, Maryland
Berman, Michael L., Ph.D., NCI, Frederick Cancer Research Facility, Frederick, Maryland
Enquist, Lynn W., Ph.D., Molecular Genetics, Inc., Minnetonka, Minnesota

ASSISTANTS

Bear, Susan, Ph.D., National Institutes of Health, Bethesda, Maryland
Benson, Spencer, Ph.D., NCI, Frederick Cancer Research Facility, Frederick, Maryland
Taylor, Ronald, B.S., NCI, Frederick Cancer Research Facility, Frederick, Maryland

The Advanced Bacterial Genetics course was designed to teach students how to isolate and characterize defined mutations in any gene in *Escherichia coli*, even if the mutation alters a gene whose product is essential for cellular viability. Such defined mutations include Tn10 insertions, nonsense mutations and deletions. Experimental protocols for performing the various types of mutagenesis on chromosomal genes, genes on transducing phages, and genes on multi-copy plasmids were presented. The course emphasized the use of gene fusions to effect genetic analysis. Accordingly, students were taught how to construct gene fusions *in vivo* and *in vitro*, isolate specialized λ transducing phage and clone the gene fusion onto a multi-copy plasmid vector. In addition, bacteriophage λ vectors were also employed to construct transducing phage that carry any gene. Clones of interest were identified using several different genetic and biochemical techniques.

The model system employed for demonstrating these techniques is the *omp* regulon of *E. coli*. The regulon contains two regulatory genes and two structural genes. The latter specify the major outer membrane porin proteins of the bacterium. As in many cases, the phenotypes conferred by mutations in this regulon are difficult to score. To permit genetic analysis, gene fusions of the porin structural genes to the lactose operon are employed. Such fusions confer readily identifiable phenotypes which facilitate mutant isolation and characterization. Using bacteriophage λ vectors, *envZ*$^+$ transducing phage were constructed and identified by lytic and lysogenic complementation. Students isolated mutations using a number of

mutagenic protocols that abolished expression of one of the porin regulatory genes, *envZ*. By using appropriate diploid strains, students were able to characterize these mutations despite the fact that in wild-type strains they appear to affect viability. In addition, gene libraries of several other Gram-negative bacteria were constructed using the same vectors. Both the libraries and genomic DNAs were screened for the presence of DNA homologous to *envZ* by either plaque hydridization or Southern blot analysis.

PARTICIPANTS

Arps, Peggy J., Ph.D., Johns Hopkins University, Baltimore, Maryland
Clarke, Robert C., B.S., Ontario Veterinarian College, Canada
DeVeaux, Linda C., B.S., University of Virginia, Charlottesville
Franco, Robert J., M.S., University of Rochester, New York
Freundlieb, Sabine, B.S., University of Konstanz, Federal Republic of Germany
Garcia, Alphonse, M.A., Pasteur Institute, Paris, France
Kaplan, Heidi Beth, B.A., Cornell University, Ithaca, New York
Kellman, Susan I., B.S., Public Health Research Institute, New York, New York
Meier, Joseph T., B.A., University of California, San Diego
Nazos, Penelope M., M.S., University of Michigan, Ann Arbor
Ohman, Dennis, E., Ph.D., University of California, Berkeley
Progulske, Ann, Ph.D., University of Massachusetts, Amherst
Ryan, James Patrick, B.S., University of North Carolina, Chapel Hill
Sen, Jyoti, M.S., Columbia University, New York, New York
Trun, Nancy J., B.S., Ohio State University, Columbus
Whoriskey, Susan K., B.S., Harvard Medical School, Boston, Massachusetts

SEMINARS

Kleckner, N., Harvard University. *Mechanism and regulation Tn10 transposition.*
Beckwith, J., Harvard Medical School. *The* lac *operon is terrific.*
Gottesman, S., National Institutes of Health. *Coping with emergencies.*
Shapiro, J., University of Chicago. *Two types of translocatable elements.*
Court, D., National Institutes of Health. *The tail of lambda* int *regulation.*
Roth, J., University of Utah. *Behavior of three transposable elements in* Salmonella.
Botstein, D., Massachusetts Institute of Technology. *New approaches for the study of old genetic problems.*

Molecular Cloning of Eukaryotic Genes, July 5–July 25

INSTRUCTORS

Fritsch, Edward F., Ph.D., Genetics Institute, Boston, Massachusetts
Engel, Doug, Ph.D., Northwestern University, Chicago, Illinois
Dodgson, Jerry, Ph.D., Michigan State University, East Lansing

ASSISTANTS

Fischer, David, Ph.D., Northwestern University, Chicago, Illinois
Grandy, David, B.A., Michigan State University, East Lansing
Friderici, Karen, B.S., Michigan State University, East Lansing

The course was designed to give "hands on" experience in the isolation and analysis of eukaryotic genes. Experiments performed during the course included double-strand cDNA synthesis: poly(dG):poly(dC) tailing; DNA fragment isolation from gels; P labeling of nucleic acids in vitro; preparation and isolation of λ arms and preparation of eukaryotic DNA inserts for λ cloning; preparation of in vitro packaging extracts; formation of eukaryotic λ libraries; screening of the eukaryotic DNA libraries in λ phage by plaque hybridization screening and by recombinational screening; formation of libraries in cosmid vectors; specific methods of subcloning fragments of eukaryotic DNA in plasmid vectors; screening of plasmids for insert fragments by size and by filter colony hybridization screening; and Northern and chromosomal Southern blotting analysis. Laboratory demonstrations, prepared by outside experts, detailed the identification of recombinants by RNA filter selection and subsequent in vitro translation. Topics covered include the transient replication and expression of genes in SV40 subclones of chromosomal genes transfected into simian cells and the expression of isolated genes introduced into cultured cells by microinjection. Through the seminar series, students were also introduced to a wide variety of topics in which recombinant DNA studies have proven to be indispensable in the analysis of the organization and expression of genes in a wide variety of organisms and eukaryotic viruses.

PARTICIPANTS

Bewley, Glen C., Ph.D., North Carolina State University, Greensboro
Chang, Long-Sheng, B.S., University of Medicine and Dentistry, Newark, New Jersey
Dean, Jurrien, M.D., National Institutes of Health, Bethesda, Maryland
Greene, Warner C., M.D., National Institutes of Health, Bethesda, Maryland
Kalthoff, Klaus O., Ph.D., University of Texas, Austin
Lindqvist, Bjorn, Ph.D., University of Tromso, Norway
Miller, Louis H., M.D., National Institutes of Health, Bethesda, Maryland
O'Farrell, Minnie K., Ph.D., University of Essex, Colchester, England
Rao, Anjana, Ph.D., Sidney Farber Cancer Institute, Boston, Massachusetts
Redmond, Shelagh, M.S., B.A., Imperial Cancer Research Fund, London, England
Searls, David, B., Ph.D., Wistar Institute, Philadelphia, Pennsylvania
Sher, Franklin A., Ph.D., National Institutes of Health, Bethesda, Maryland
Strauss, William L., Ph.D., National Institutes of Health, Bethesda, Maryland
Valerio, D., B.A., Sylvius Laboratories, The Netherlands
White, Judith M., Ph.D., Yale Medical School, New Haven, Connecticut
Worton, Ronald G., Ph.D., Hospital for Sick Children, Toronto, Canada

SEMINARS

Seed, B., Harvard University. *Screening bacteriophage libraries by recombination in* E. coli.
Dodgson, J., Michigan State University. *Chicken histone genes.*
Sambrook, J., Cold Spring Harbor Laboratory. *Expression of a cloned influenza.*
Gething, M.J., Cold Spring Harbor Laboratory. *Hemmagglutinin gene in prokaryotic and eukaryotic cells.*
Korman, A., Harvard University. *Application of immunopurification of polysomes to gene cloning.*
Wigler, M., Cold Spring Harbor Laboratory. *Human transforming genes.*
Irwin, N., Harvard University. *Expression of eukaryotic genes in* E. coli.
Shortle, D., State University of New York, Stony Brook. *Site-directed mutagenesis.*
Levings, C., North Carolina State University. *Plasmid-like DNA in the corn mitochondrial genome.*
Baltimore, D., Massachusetts Institute of Technology. *Immunoglobulin genes and gene cloning.*
Shenk, T., State University of New York, Stony Brook. *Genetic manipulation of the adenovirus genome.*
McKnight, S., Fred Hutchinson Cancer Research Center. *Transcriptional control signals of a constitutively expressed eukaryotic protein-coding gene.*
Capecchi, M., University of Utah. *Patterns of integration of DNA microinjected into cultured mammalian cells.*
Goodman, H., Harvard Medical School. *Structure, evolution, and expression of mammalian polypeptide genes.*
Spradling, A., Carnegie Institution. *P. elements as transformation vectors in* Drosophila.
Bender, W., Harvard Medical School. *Transposable elements in the Bithorax locus of* Drosophila.
Engel, D., Northwestern University. *Avian red cell gene expression.*

The Synapse: Cellular and Molecular Neurobiology, July 12–August 1

INSTRUCTORS

Rahamimoff, Rami, M.D., Hebrew University, Hadassah Medical School, Jerusalem, Israel
McMahan, U. Jack, Ph.D., Stanford University School of Medicine, California

PART-TIME INSTRUCTORS

Stevens, Charles F., M.D., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Yoshikami, Doju, Ph.D., University of Utah, Salt Lake City

This intensive lecture course was intended for graduate students and research workers interested in an interdisciplinary understanding of cellular and molecular biology of the synapse. The topics covered included synaptic excitation and inhibition; structure of presynaptic and postsynaptic elements; release, synthesis, storage, and inactivation of neurotransmitters; noise analysis of transmitter action; synthesis properties and distribution of receptor molecules; synaptic immunology; electrical synapses and gap junctions; slow synaptic potentials; principles of neuronal development, denervation, and reinnervation; synaptic pharmacology; role of peptides in neuronal function; principles of sensory transduction; synaptic transmission at the retina; and pathophysiology of synapses. The course consisted of lectures, seminars, reading of papers, small group discussions, presentations by the course participants, and workshops on morphological techniques, analysis of synaptic activity, noise analysis, principles of the usage of digital computers in data collection and analysis, and quantal analysis.

PARTICIPANTS

Ashe, John H., Ph.D., University of California, Riverside
Blaxter, Timothy J., B.S., St. Andrews University, Winnipeg, Canada
Carman, George John, B.A., California Institute of Technology, Pasadena
Carroll, Paul T., Ph.D., Texas Tech University, Lubbock
Constantini, Schlomo, B.S., Hadassah Medical School, Jerusalem, Israel
Coopersmith, Robert, M., University of California, Irvine
Covault, Jon, M., Ph.D., University of Iowa, Iowa City
Craft, Cheryl M., B.S., University of Texas, Corpus Christi
Hoshi, Toshinori, B.A., Yale University, New Haven, Connecticut
Kudo, Yoshihisa, Ph.D., Mitsubishi-Kasei Institute, Tokyo, Japan
Leonard, Reid J., B.S., Purdue University, West Lafayette, Indiana
Lisak, Joan W., B.S., New York University Medical College, New York
Murakami, Fujio, Ph.D., National Institute for Sciences, Tokyo, Japan
Nitkin, Ralph M., Ph.D., Stanford Medical School, California
Owen, David G., Ph.D., National Institutes of Health, Bethesda, Maryland
Radvanyi, Francois, Institut Pasteur, Paris, France
Smith, Kirk E., B.S., University of Florida, Gainesville
Ueno, Ryuji, M.D., Kyoto University, Japan
Ways, Nancy R., B.A., Stanford University, California

SEMINARS

McMahan, U.J., Stanford University School of Medicine. *Structure of the nervous system: The neuromuscular junction; structure of central synapses.*

Rahamimoff, R., Hebrew University, Hadassah Medical School. *Principles of signaling in the nervous system; Transport across membranes: Forces and Fluxes; Determinants of the membrane potential; Resting potential: Passive and active mechanisms; Generation of the action potential; Cable properties of nerve and the conduction of the action potential; Ionic basis of the action potential: 1) the currents, 2) the conductances and the Hodgkin-Huxley model.*

Yoshikami, Doju, University of Utah. *The excitatory post-synaptic response.*

——. *Ionic basis of the excitatory post-synaptic response.*

——. *Visualization of synaptic activity of neurons by autoradiography.*

McMahan, U.J., Stanford University. *Localization of acetylcholine receptors at the neuromuscular junction.*

——. *Localization of acetylcholinesterase at the neuromuscular junction.*

Yoshikami, D., University of Utah. *Ionic basis of synaptic inhibition.*

Goodenough, D., Harvard Medical School. *Cell biology of gap junctions.*

——. *Structure of gap junctions.*

Bennett, M., Albert Einstein College of Medicine. *Coupling between cells.*

———. *The role of H^+ ions and calcium in coupling and uncoupling of cells.*
McKay, R., Cold Spring Harbor Laboratory. *Principles of neuro-immunology.*
Hockfield, S., Cold Spring Harbor Laboratory. *Immunology of the cat spinal cord.*
McMahan, U.J., Stanford University School of Medicine. *Vesicle hypothesis.*
Rahamimoff, R., Hebrew University, Hadassah Medical School. *Quantal transmitter release.*
———. *Frequency modulation of transmitter release.*
———. *The role of calcium ions in transmitter release.*
———. *Slow regulation of transmitter release.*
———. *The role of the sodium calcium exchanger in transmitter release at the neuromuscular junction.*
Yoshikami, D., University of Utah. *Fate of acetylcholine released from the nerve.*
———. *Number of acetylcholine molecules in a quantum.*
Stevens, C., Yale University School of Medicine. *Principles of single-channel recording.*
Yoshikami, D., University of Utah. *Slow synaptic potentials.*
Stevens, C., Yale University School of Medicine. *Fluctuation analysis of the acetylcholine post synaptic response.*
Dionne, V., University of California, San Diego. *Kinetics of the acetylcholine channel.*
Kado, R., CNRS. *Voltage induced membrane conductance changes in crayfish and tunicate oocyte.*
Stevens, C., Yale University School of Medicine. *Molecular basis for endplate current time course and its voltage sensitivity.*
———. *Reverse voltage dependence of response amplitudes for nerve released and ontophorectically applied ACh.*
Ohmori, H., Tokyo University. *Properties of Ca^{++} channels in CH cells—evidence from whole cell voltage clamp and single channel recording.*
Palatta, B., University of Miami. *Studies of Ca^{++} activated K channels using the single channel recording method.*
Lindstrom, J., Salk Institute. *Structure and biosynthesis of acetycholine receptors.*
———. *Myasthenia gravis, EAMG and the use of monoclonal antibodies to study acetylcholine receptors.*
Rotundo, R., Carnegie Institution of Washington. *Structure and function of the acetylcholine estarase.*
———. *Synthesis and assembly of acetylcholine estarase molecules.*
Black, I., Cornell University. *The catecholamine synapse.*
———. *Postnatal development of the catecholamine synapse.*
———. *Target regulation of the catecholamine system.*
———. *Catecholamine embryogenesis-phenotypic expression.*
———. *Catecholamine-neuropeptide relations.*
Adams, P., State University of New York, Stony Brook. *Slow synaptic potentials in the frog autonomic ganglia: The properties of the M–current.*
Black, I. Cornell University. *Pathophysiology of the catecholaminergic synapse: Parkinsonism, hyperkinetic disorder, idiopathis orthostatic hypotension.*
Jan, L., University of California, San Francisco. *Methodology for the study of the role of peptides in the nervous system: Eukephalin and substance P.*
———. *Peptidergic transmission in autonomic ganglia.*
Yellen, G., Yale University. *The calcium channels in heart dissociated muscle cells.*
Herbert, E., University of Oregon. *Neuroactive peptides: Protein processing and secretion.*
———. *Neuroactive peptides: Use of recombinant DNA and molecular biology techniques to study peptide gene expression.*
Landmesser, L., Yale University. *Development of the motor pattern to muscle: Possible errors in connection and mechanism of removal.*
———. *Pathway selection in contributing to appropriate connectivity.*
Purves, D., Washington University. *Specificity of neuronal connections: Qualitative aspects.*
———. *Specificity of Neuronal connections: Quantitative aspects.*
Patterson, P., Harvard Medical School. *Migration of neural crest cells: Effects of European lineage and of American influence of environment.*
———. *External factors in determination of self in autonomic nervous system.*
Rubin, L., Rockefeller University. *Early events in nerve muscle synapse formation.*
———. *Regulation of acetylcholine receptor and acetylcholine esterase appearance at newly formed synapses.*
McMahan, U.J., Stanford University School of Medicine. *Sequence of steps in muscle denervation and reinnervation.*
———. *Factors that influence regeneration of the neuromuscular junction.*
Frank, E., Harvard Medical School. *Structure and functional specificity of spinal sensory-motoneuronal synapses.*
———. *Quantal transmission at spinal sensory motoneuronal synapses.*

Greenberg, A., State University of New York, Stony Brook. *Development of AChR single channel properties in non-innervated embryonic* Xenopus *muscle.*
Hudspeth, A.J., California Institute of Technology. *Structure and connection of the retina.*
——. *Cell biology of photoreceptor cells.*
Kehoe, J.S., Ecole Normale Superieure. *Diminution of membrane conductance by synaptic actions.*
Hudspeth, A.J., California Institute of Technology. *Photochemistry and extracellular signals of photoreceptors.*
Corye, D., Yale Medical School. *Intracellular signals of photoreceptors.*
——. *Intermediate messengers in photoreceptors.*
Hudspeth, A.J., California Institute of Technology. *Structure and operation of the inner ear.*
Stefani, E., Instituto Politesnico Nacional, Mexico City. *Calcium channels in muscle membrane.*
Corey, D., Yale Medical School. *Responses of hair cells to hair-bundel defection.*
——. *Ionic basis and kinetics of the hair-cell response.*
Hudspeth, A.J., California Institute of Technology. *Membrane conductances and frequency tuning in hair cells.*

Electrophysiological Methods, July 29–August 17

INSTRUCTORS
Kehoe, JacSue, Ph.D., Ecole Normale Superieure, Paris, France
Chiarandini, Dante, M.D., New York University, New York
Stefani, Enrique, M.D., Instituto Politecnico Nacional, Mexico City, Mexico

GUEST LECTURER
Kado, Ray, Ph.D., CNRS, France

This laboratory course focused on methods of introducing biologically active macromolecules into living cells. Emphasis was placed on transferring DNA into cultured cells by transfection and by nuclear injection using glass micropipettes. Laboratory exercises included (1) transfection of DNA containing viral and cellular transforming genes; (2) microinjection and transfection of DNAs containing selectable biochemical markers; (3) introduction of proteins into cells by fusion with loaded red cell ghosts and liposomes.

These exercises were supplemented with group discussion and lectures by invited speakers who considered the application of these methods to the isolation and functional characterization of structural genes and their regulatory sequences, evaluation of in vitro mutagenesis, and analysis of the biological activity of proteins and messenger RNAs.

PARTICIPANTS

Duerr, Janet S., M.S., Princeton University, New Jersey
Fung, Sek-chung, M.S., University Laboratory of Physiology, Oxford, England
Holt, Christine E., Ph.D., University of California, San Diego
Luini, Alberto, M.D., Weizmann Institute, Rehovot, Israel
Malenka, Robert C., B.A., Stanford University, California
Moody-Corbett, Frances, Ph.D., Harvard Medical School, Boston, Massachusetts
Muller, Dominique, M.D., University of Geneva, Switzerland
Sonderegger, Peter, M.D., National Institutes of Health, Bethesda, Maryland
Storm, Johan, M.D., University of Oslo, Norway
Weiner, Joseph S., B.A., New York University, New York

Yeast Genetics, July 28–August 17

INSTRUCTORS

Sherman, Fred, Ph.D., University of Rochester, New York
Fink, Gerald, Ph.D., Cornell University, Ithaca, New York
Hicks, James, Ph.D., Cold Spring Harbor Laboratory, New York

ASSISTANT

Laiken, Robert, B.A., University of Rochester, New York

The major laboratory techniques used in the genetic analysis of yeast were studied, including the isolation and characterization of chromosomal and mitochondrial mutants, tetrad analysis, chromosomal mapping, mitotic recombination, and test of allelism and complementation. Micromanipulation used in tetrad analysis were carried out by all students. Recombinant DNA techniques, including yeast transformation, filter hydridization, and gel electrophoresis were applied to cloning and genetic analysis of yeast DNA. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Berman, Judith G., B.S., Weizmann Institute, Rehovot, Israel
Chakraburtty, Kalpana, Ph.D., Medical College of Wisconsin, Madison
Couto, Fernando, B.S., Massachusetts Institute of Technology, Cambridge
Hall, Barry G., Ph.D., University of Connecticut, Storrs
Margolin, Paul, Ph.D., Public Health Research Institute, Bethesda, Maryland

Peebles, Craig L., Ph.D., University of Pittsburgh, Pennsylvania
Poso, Hannu J., Ph.D., State Alcohol Monopoly, Helsinki, Finland
Rosbash, Michael M., Ph.D., Brandeis University, Waltham, Massachusetts
Sancar, Gwendolyn B., Ph.D., Yale University, New Haven, Connecticut
Segall, Jacqueline M., Ph.D., University of Toronto, Canada
Silver, Pamela A., Ph.D., University of California, Los Angeles
Sugino, Akio, Ph.D., National Institutes of Health, Bethesda, Maryland
Tittawella, Ivor, Ph.D., University of Goteborg, Sweden
Witkin, Evelyn M., Ph.D., Rutgers University, New Brunswick, New Jersey
Yang, Elizabeth W., M.S., Stanford University, California
Zoller, Mark J., Ph.D., University of British Columbia, Vancouver, Canada

SEMINARS

Dutcher, S.K., Rockefeller University. *Genetic analysis of the cell division cycle.*
Botstein, D., Massachusetts Institute of Technology. *Cloning of yeast genes.*
Hicks, J., Cold Spring Harbor Laboratory. *Regulation of mating type.*
Broach, J., State University of New York, Stony Brook. *Yeast plasmid 2 μm circle.*
Sherman, F., University of Rochester. *Evolution and regulation of iso-cytochromes c.*
Warner, J., Albert Einstein College of Medicine. *Yeast ribosomal protein genes and their regulation.*
Guthrie, C., University of California, San Francisco. *Genetics and biochemistry RNA biosynthesis in yeast.*
Szostak, J., Sidney Farber Cancer Institute. *Structure and function of telomeres.*
Hereford, L., Brandeis University. *Regulation of histone gene expression.*
Fink, G., Cornell University. *Transposable elements and reiterated sequences.*
Petes, T., University of Chicago. *Genetic interactions among repeated yeast genes.*
Klar, A., Cold Spring Harbor Laboratory. *Mechanism of cell type switching in yeast.*
Fox, T., Cornell University. *Mitochondrial genetic codes.*
Strathern, J., Cold Spring Harbor Laboratory. *Mechanism of cell type switching in yeast (II).*
Grunstein, M., University of California, Los Angeles. *Genetic approach to histone function.*
Esposito, R., University of Chicago. *Defining genes controlling meiotic exchange and segregation.*
Bloom, K., University of California, Santa Barbara. *The structure of yeast centromere.*

Introduction of Macromolecules into Mammalian Cells

July 28–August 17

INSTRUCTORS

Capecchi, Mario, Ph.D., University of Utah, Salt Lake City
Mulligan, Richard, Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANTS

Fraser, Laurie, B.S., University of Utah, Salt Lake City
Mann, Richard, Massachusetts Institute of Technology, Cambridge

This laboratory course focused on methods of introducing biologically active macromolecules into living cells. Emphasis was placed on transferring DNA into cultured mammalian cells by transfection, viral infection and microinjection. Laboratory exercises included:

Use of retrovirus vectors for the study of expression and transformation, transfection, and microinjection of DNA containing selectable biochemical markers into cultured mammalian cells, introduction of proteins into cells by fusion with loaded red cell ghosts, and introduction of DNA into plant cells using liposomes.

These exercises were supplemented with group discussions and lectures by invited speakers who considered the application of these methods to problems in cellular and developmental biology.

PARTICIPANTS

Avivi, Abraham, Ph.D., Weizmann Institute, Rehovot, Israel
Baldacci, Patricia A., M.S., Institut Pasteur, Paris, France
Beckingham, Kathleen M., Ph.D., Rice University, Houston, Texas
Brennand, John, Ph.D., Baylor College of Medicine, Houston, Texas
Clegg, Christopher H., B.A., University of Washington, Seattle
Daubas, Philippe, Ph.D., Pasteur Institute, Paris, France
Flores-Menendez, Carlos, Ph.D., University of El Salvador, San Salvador
Gottlieb, Ellen, B.A., Yale University, New Haven, Connecticut
Kondor-Koch, Claudia, Ph.D., EMBO, Heidelberg, Federal Republic of Germany
Lupski, James R., B.A., New York University, New York
Korman, Allen, Harvard University, Cambridge, Massachusetts
Nagamine, Yoshikuni, Rockefeller University, New York, New York
Post, Leonard E., Ph.D., Upjohn Company, Kalamazoo, Michigan
Seiler, Anne F., Ph.D., National Cancer Institute, Bethesda, Maryland
Steinmetz, Michael, Ph.D., California Institute of Technology, Pasadena
Tramontano, Donatella, M.D., University of Naples, Italy

SEMINARS

McKnight, S., Fred Hutchinson Cancer Research Center. *Transcription control signals of a constituitively expressed eukaryotic protein-coding gene.*
Botchan, M., University of California, Berkeley. *Enhancer sequences of SV40 and BPV.*
Axel, R., Columbia University. *Expression and regulation of cloned genes introduced into mammalian cells via DNA transformation.*
Maniatis, T., Harvard University. *The expression of normal and abnormal globingenes in cells in culture.*
Gluzman, Y., Cold Spring Harbor Laboratory. *Helper independent adeno vectors.*
Gething, M.J., Cold Spring Harbor Laboratory. *Expression of the influenza hemagglutinate protein in mammalian cells.*
Hager, G., National Cancer Institute. *Characterization of the hormone-inducible promoter of MMTV.*
Howley, P., National Cancer Institute. *BPV as a cell replicating eukaryotic vector.*
Szostak, J., Sidney Farber Cancer Institute. *On the mechanism of recombination in yeast.*
Bishop, M., University of California, San Francisco. *Retroviruses and cancer.*
Rechsteiner, M., University of Utah. *On the mechanism of protein degradation in cultured mammalian cells.*
DiMaio, D., Harvard University. *BPV vectors.*
Khoury, G., National Cancer Institute. *On the mechanism of SV40 enhancer sequences.*
Sorge, J., Cold Spring Harbor Laboratory. *Retroviruses as a eukaryotic vector.*
Wigler, M., Cold Spring Harbor Laboratory. *Human transforming genes.*
Ruddle, F., Yale University. *Introduction of new genetic information into the germ line of mice.*
Rubin, G., Carnegie Institution of Washington. *P elements as transformation vectors in* Drosophila.

Molecular Approaches to the Nervous System, August 9–August 15

INSTRUCTORS

Brockes, Jeremy, California Institute of Technology, Pasadena
McKay, Ronald, Cold Spring Harbor Laboratory, New York

Lecturers in the course considered a variety of topics in cellular and molecular neurobiology. These included fundamental studies on receptors and in channels, the diversity and lineages of cell types in the nervous system, and the molecular organization of synapses and possible mechanisms involved in synaptic

specificity. A particular focus was the application of recombinant DNA methodology and monoclonal antibodies to these and other issues. This included studies on gene expression in the vertebrate CNS, the genetic organization of peptides, and recent progress in cloning genes for the subunits of the acetylcholine receptor. Monoclonal antibodies were considered as probes for extracellular and intracellular components at the neuromuscular junction and other synapses, as labels for discrete classes of neurons or neuronal processes in vertebrate and invertebrate nervous systems, and as markers for morphogenetic patterns such as gradients. The continued application of these techniques clearly promises to have considerable impact in molecular neurobiology.

PARTICIPANTS

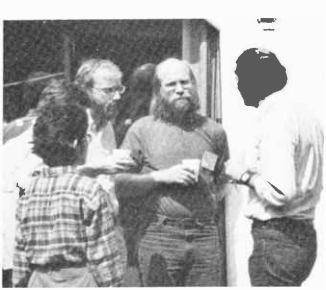
Bernhardt, Robert, M.S., Friedrich Miescher Institute, Tubingen, Federal Republic of Germany
Botticelli, Lawrence J., Ph.D., Stanford Medical School, California
Buonanno, Andres L., M.S., IVIC, Caracas, Venezuela
Early, Philip, Ph.D., University of California, Berkeley
Flaster, Murray S., Ph.D., Cold Spring Harbor Laboratory, New York
Fukada, Keiko, Ph.D., Harvard Medical School, Cambridge, Massachusetts
Johnson, Carl D., Ph.D., University of Wisconsin, Madison
Kanazir, Selma D., M.S., Yale University, New Haven, Connecticut
Lo, Mathew, MS., Ph.D., Johns Hopkins University, Baltimore, Maryland
Macagno, Eduardo R., Ph.D., Columbia University, New York, New York
O'Donohue, Thomas L., Ph.D., National Institutes of Health, Bethesda, Maryland
Sommer, Ernst W., Ph.D., Columbia University, New York, New York
Zipursky, Stephen, Ph.D., California Institute of Technology, Pasadena

SEMINARS

Fundamental Studies on Receptors and Channels
Stevens, C.F., Yale University
Karlin, A., Columbia University
Davidson, N., California Institute of Technology
Balivet, M., Salk Institute
Heinemann, S., Salk Institute
Catteral, W., University of Washington Medical School
Schwartz, J., Columbia University of Physicians and Surgeons
Spitzer, N.C., University of California
Goldin, S., Harvard Medical School

Genetic Organization of Neuropeptides
Roberts, J., Columbia University College of Physicians and Surgeons
Scheller, R., Columbia University College of Physicians and Surgeons

Specificity and Heterogeneity in the Nervous System
Zipser, B., Cold Spring Harbor Laboratory
Nirenberg, M., National Institutes of Health
Karten, H.J., State University of New York, Stony Brook
Hockfield, S., Cold Spring Harbor Laboratory
Jessel, T., Harvard Medical School



General Features of Gene Expression in the Vertebrate CNS
Hahn, W.E., University of Colorado Medical Center
Sutcliffe, G., Scripps Clinic

The Neuromuscular Junction
Burden, S., Harvard Medical School
Fambrough, D.M., Carnegie institution of Washington
Sanes, J., Washington University
Rubin, L., Rockefeller University
Anderson, D., Rockefeller University

Mechanisms of Steroid Function
Kurtz, D., Cold Spring Harbor Laboratory
De Vellis, J., University of California
Gurney, M., University of Chicago Medical School

Neural Lineages
Horvitz, R., Massachusetts Institute of Technology
Goodman, C., Stanford University
Patterson, P., Harvard Medical School
Raff, Martin, University College London

Growth Factors
Gurney, M., University of Chicago Medical School
Patterson, P., Harvard Medical School
Villa-Komarov, L., University of Massachusetts

Axonal Extension and Guidance
Lasek, R.J., Case Western Reserve University
Reichardt, L., University of California
Rutishauser, U., Rockefeller University
Goodman, C., Stanford University

UNDERGRADUATE SUMMER RESEARCH PROGRAM

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, 239 students have completed the course, and many have gone on to creative careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology, (2) an increased awareness of major problem areas under investigation, (3) better understanding of the physical and intellectual tools for modern research and the pertinence of this information to future training, and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from a large number of applicants, took part in the program, which was supported by The Camille and Henry Dreyfus Foundation, Inc. They are listed below with their laboratory sponsors and topics of research.

Tania Ann Baker, University of Wisconsin *Research Advisor:* A.I. Bukhari	Cloning of the Mu *A* gene
David Campanelli, Wesleyan University *Research Advisor:* L. Chow	Gene expression of human papilloma virus type 1
Brad Cookson, University of Utah *Research Advisor:* M. Wigler	Construction of a transforming gene under control of a metallothionein promoter
Andrew Gray, Princeton University *Research Advisor:* P. Thomas	Genomic clones of human heat shock genes
Jill Heemskerk, University of California, Berkeley *Research Advisor:* M. So	DNA rearrangement and pathogenicity in *N. gonorrhoeae*
Eva Nozik, University of Colorado *Research Advisor:* D. Kurtz	Hormones and methylation patterns in gene expression
Philip Starr, Princeton University *Research Advisor:* T. Gingeras	Cloning bacterial restriction/modification genes
Nick Theodorakis, Washington University *Research Advisor:* J. Feramisco	Analysis of structural proteins in non-muscle cells
Peter Weinstein, University of Michigan *Research Advisor:* L. Silver	Mapping of an MMTV provirus on mouse chromosome 7
Olney Fellow Kenneth Howard, University of Cambridge *Research Advisor:* J. Fiddes	Aspects of expression of the multigene family for the beta subunit of human chorionic gonadotropin

IN-HOUSE SEMINARS

Lee Silver

Cold Spring Harbor In-House 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 post-graduate 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.

1981-1982

October

Lindley C. Blair, University of California, Berkeley: Processing and secretion of yeast sex factors.

Roberto Weinmann, Wistar Institute, Philadelphia, Pennsylvania: Mechanism of initiation of transcription by RNA polymerase II.

November

Seymour Fogel, University of California, Berkeley: Repetitive DNA and its relationship to metal resistance in yeast.

Martin Marinus, University of Massachusetts Medical Center, Amherst: The deoxyadenosine methylase (dam) function of *E. coli*.

Nancy Hoag, Imperial Cancer Research Fund, London, England: Human monocytes have a role in coagulation.

Mary Jane Gething, Imperial Cancer Research Fund, London, England: Expression of a cloned copy of the influenza hemagglutinin gene in prokaryotes and eukaryotes.

January

Janice Chow, National Institutes of Health, Bethesda, Maryland: Study of liver differentiation in vitro with SV40 *tsA* mutant transformed cells.

Andrei Laszlo, Lawrence Berkeley Laboratory, Berkeley, California: Tumor promoter-induced alterations in gene expression and protein phosphorylation in avian cells.

Jack Szostak, Sidney Farber Cancer Center, Boston, Massachusetts: The structure of the ends of chromosomes—Analysis of cloned yeast telomeres.

Frank Stahl, University of Oregon, Eugene: Recombination in fungi.

Ihor Lemischky, Massachusetts Institute of Technology, Cambridge: The structure and evolution of rat alpha-tubulin genes.

Mark Cochran, Queens University, Kingston, Canada: The molecular biology of baculoviruses.

February

Miercio Pereira, Tufts New England Medical Center, Boston, Massachusetts: Lectin receptors as tools for studying the developmental stages of *T. cruzi*.

Judy White, Yale University, New Haven, Connecticut: Virus-mediated cell fusion.

David Anderson, Rockefeller University, New York, New York: Biosynthesis of acetylcholine receptors in cell-free systems.

March

Edward Wagner, University of California, Irvine: Overlapping mRNA families in herpes simplex virus type I.

April

Mike Fried, Imperial Cancer Research Fund, London, England: The annual lecture on integration and expression of polyoma virus.

Lincoln T. Potter, University of Miami, Florida: Two populations of muscarine receptors.

Paolo Dotto, Rockefeller University, New York, New York: Functional dissection of the replication origins of bacteriophage F1.

Nick Proudfoot, University of Oxford, England: Structure and expression of normal and mutant hemoglobin genes.

John Gordon, Yale University, New Haven, Connecticut: Gene transfer into mouse embryos.

David Ward, Yale University, New Haven, Connecticut: Immunological methods for in situ localization of nucleic acids.

June

Mike Leffak, Wright State University, Dayton, Ohio: Chromatin assembly.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and high school students the opportunity to acquire a greater 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 program programs such as Marine Biology, Nature Photography, and the Geology of Long Island.

During the summer of 1982 a new high total of 450 students participated in the Nature Study Program. Most classes were held outdoors, when weather permitted, or at the Uplands Farm Nature Preserve of the Long Island Chapter of the Nature Conservancy. The Laboratory has equipped and maintains a darkroom and classroom-laboratories at Uplands Farm for the study of field specimens collected by the students. Field classes are also held on Laboratory grounds, St. John's Preserve, Shu Swamp Preserve, Montauk Point State Park, and in the Long Island pine barrens.

In addition to the four-week courses, a series of one-day marine biology workshops was offered to students. Studies on the marine ocology of Long Island Sound were conducted aboard the 66-foot schooner J.N. Carter chartered from Schooner, Inc. of New Haven, Connecticut. Students were able to study the sound chemically, physically, and biologically using the ship's instrumentation. The three-day Adventure Education class took children on an 18-mile bike hike to Caumsett State Park, a six-mile canoe trip on the Nissequogue River, and a day of sailing on the J.W. Carter.

PROGRAM DIRECTOR

Edward Tronolone, M.S., P.D., Science Curriculum Associates, East Williston Public Schools

INSTRUCTORS

Kathryn Bott, M.S., science teacher, Friends Academy
Ruth Burgess, B.A., naturalist, Nassau County BOCES
Gail Claydon, B.S., teacher, United Methodist Church of Huntington, Cold Spring Harbor
Robert Jaeger, M.S., science teacher, Mineola High School
Fred Maasch, M.Ed., science teacher, Islip High School
Linda Payoski, B.A., naturalist, Nassau County BOCES
Debra Peters, B.S., naturalist, Nassau County BOCES
James Romansky, M.S., science teacher, Bay Shore High School

COURSES

Nature Bugs
Nature Detectives
Advanced Nature Study
Pebble Pups
Geology of Long Island
They Swim, Crawl, and Walk
Seashore Life
Marine Biology
Nature Photography I & II
Fresh Water Life
Frogs, Flippers, and Fins
Bird Study
Adventure Education
Marine Biology Workshop

LABORATORY STAFF

DIRECTOR
J.D. Watson

ADMINISTRATIVE DIRECTOR
William R. Udry

ASSISTANT DIRECTOR FOR RESEARCH
Joseph Sambrook

DIRECTOR—BANBURY CENTER
Michael Shodell

RESEARCH SCIENTISTS

Stephen Blose
Susan Bonitz
Thomas Broker
Ahmad Bukhari
Louise Chow
Fevzi Daldal
James Feramisco
John Fiddes
James Garrels
Mary-Jane Gething
Thomas Gingeras
Yakov Gluzman
Mitchell Goldfarb
Terri Grodzicker
Edward Harlow
Rasika Harshey
Fred Heffron
James Hicks
Susan Hockfield
Stephen Hughes
Amar Klar
David Kurtz
Jim Lin
Russell Malmberg
Michael Mathews
Fumio Matsumura
Ronald McKay
Richard Roberts
Mara Rossini
Lee Silver
Magdalene So
Bruce Stillman
Jeffrey Strathern
Fuyuhiko Tamanoi
William Topp
Michael Wigler
Birgit Zipser

VISITING SCIENTISTS

Jorgen Johansen
Yoshiki Katoh
Jin-Zhao Li
Pei-Mao Lin
Kenji Shimizu

POSTDOCTORAL FELLOWS

Judith Abraham
William Addison
David Beach
Ashok Bhagwat
Daniel Birnbaum
Janet Brandsma
Joan Brooks
Carolyn Deal
Stephen Dellaporta
Ottavio Fasano
Thomas Flanagan
Murray Flaster
Michele Francoeur
Robert Franza
Greg Freyer
Richard Guilfoyle
Mary Harper
David Helfman
William Huse
John Ivy
Tohru Kamata
Prasad Koka
Thomas Kost
Michael Krangel
Chao-Hung Lee
John Lewis
Janet MacInnes
Catherine Monaghan
Michael Roth
Earl Ruley
Joseph Sorge
Yolande Suard
Olof Sundin
Karen Talmadge
Elizabeth Taparowsky
G. Paul Thomas
William Welch
Stuart Weisbrod
Masao Yamada

GRADUATE STUDENTS

Carolyn Doyle
Douglas Hanahan
Margaret Kelly
Richard Kostricken
Michele Manos
Zarina Manzoor
Kent Matlack
Robert O'Malley
Nora Sarvetnick
Ellyn Segal
Carmella Stephens
Kevin Van Doren

RESEARCH ASSISTANTS

Barbara Ahrens
Elizabeth Billyard
Georgia Binns
Gladys Blose
Angela Calasso
Lynn Cascio
Terrance Chisum
Lois Cisek
Barbara Daniels
Deborah Danna
Mark Deluca
Marlene Dermody-Weisbrod
Anne Devine
Louise Edlund
Jane Emanuele
Deborah Foster
Cecilia Fraser
Rachel Galli
Laurel Garbarini
Nadia Gural
Nancy Haffner
Marie Hallaran
Christine Herrmann
Deborah Keller
Lynn Kleina
Elaine Kosik
Deborah Kwak
Patricia Lalik
Geraldine LoFranco
Diana Matkovich
Carolyn McGill
Robert McGuirk
Jean McIndoo
Mary Merle
Doris Moll
Janet Monnier
Carolyn Moomaw
Phyllis Myers
Maureen O'Halloran
Diana O'Loane
Kathy O'Neill
Wendy Osheroff
Karen Pantina
Diana Pascucci

Staff as of December 1982

Anthony Pelletier
Gina Penzi
Doris Prudente
Stephen Raps
William Ricci
Michael Riggs
Linda Rodgers
Mary Ann Ruley
Jan Scal
Carol Schley
Patricia Smith
Deborah Taylor
Jane Uman
Linda Van der Wal
Bruce Vogel
Martin Walsh
Elizabeth Waldvogel
Margaret Wallace
Jeanne Wiggins
Jonathan Wood

ADMINISTRATIVE AND GENERAL STAFF

Charlaine Apsel
Deon Baker
Guy Cozza
Sandra D'Arcangelo
Joan Doherty
Ann Felten
Douglas Gensel
Patrick Hickey
Eric Kaufman
William Keen
Stephen Kron
Patricia Kurfess
Dorrine Lonardo
John Maroney
Carlos Mendez
David Micklos
Neil Penn
June Polistina
James Sabin
Roberta Salant
Susan Schultz
Martin Spiegel
Jacqueline Terrence
Barbara Terry

GENETICS RESEARCH UNIT CARNEGIE INSTITUTION OF WASHINGTON

Alfred D. Hershey
Barbara McClintock

SUPPORTING STAFF

Michael Balamuth
Deborah Balke
Patricia Barkley
Maureen Berejka
Robert Borruso
Arthur Brings
Louisa Dalessandro
Joanne Dellaporta
August Dulis
Marilyn Goodwin
Catherine Jackson
Christopher Keller
Gladys Kist
Eric Lockhart
Deborah Lukralle
Raymond Mickus
Patrick Nevins
Michael Ockler
Constance Parra
Patricia Pugliese
Herb Parsons
Philip Renna
Marlene Rubino
Regina Schwarz
Lorine Seyffarth
Andrea Stephenson
Clifford Sutkevich
Madeline Szadkowski
Barbara Ward

PUBLICATIONS DEPARTMENT

Dorothy Brown
Elizabeth Cattaneo
Michaela Cooney
Judith Cuddihy
Nadine Dumser
Nancy Ford
Annette Kirk
Douglas Owen
Elizabeth Ritcey
Karen Sundin

LIBRARY STAFF/ MARKETING

Carol Aznakian
Jennifer Carri
Ellen DeWeerdt
Cynthia Elwell
Genemary Falvey
Elaine Gaveglia
Susan Gensel
Amy Gibson
David Gibson
Karen Herrmann
Laura Hyman
Colleen Lanahan
Ronald Letta
Audrey Powers

BUILDINGS AND GROUNDS DEPARTMENT

John Bach
Joseph Brodawchuk
Carol Caldarelli
Joseph Canner
Vincent Carey
Ronald Chambers
Barbara Cuff
Warren Eddy
Joseph Ellis
Bruce Fahlbusch
Willie Gardner
Louis Gruebel
Douglas Haskett
Mary Hill
Thomas Lyden
Charles Marshall
Christopher McEvoy
John Meyer
Alfred Nickel
Dirk Peterson
Alfred Pfeiffer
Joseph Pirnak
Gerald Reichert
Peter Rice
Jack Richards
Donald Rose
Diamond Scarduzio
Charles Schneider
Lane Smith
Peter Stahl
Owen Stewart
Mark Strazay
Patrick Sullivan
Peter Tierney
Hans Trede
Archibald Walker
Dorothy Youngs

BANBURY CENTER

Christine Dacier
Katya Davey
Margaret McEvoy
Lynda Moran
Beatrice Toliver

First row: T. Lyden, J. Meyer; M. Balamuth, J. Dellaporta; W. Keen; C. Mendez, P. Kurfess, S. Kron
Second row: D. Lonardo; D. Gensel, S. Gensel; J.D. Watson, E. Watson; L. Moran, M. Shodell, B. Toliver
Third row: D. Micklos, R. Wilson; C. Keller, L. Dalessandro; C. Schneider, B. Cuff; N. Ford, D. Brown, E. Cattaneo, E. Ritcey
Fourth row: S. D'Arcangelo, A. Felten; M. Goodwin, P. Barkley; M. Berejka, M. Simkins, B. Ward, G. Kist; D. Baker, B. Terry

First row: A. Brings; E. Kaufman, J. Maroney; M. Szadowski; L. Gruebel
Second row: J. Pirnak; J. Terrence; D. Owen; O. Stewart; G. Cozza
Third row: A. Kirk, N. Dumser, K. Sundin, M. Cooney, J. Cuddihy; H. Parsons; L. Hyman, K. Herrmann, G. Falvey, E. Deweerdt, A. Gibson, E. Gaveglia, A. Powers
Fourth row: M. Speigel; J. Doherty; J. Sabin; R. Salant; A. Stephenson

FINANCIAL SUPPORT OF THE LABORATORY

The Cold Spring Harbor Laboratory is a publicly supported educational institution chartered by the University of the State of New York and may receive contributions which are tax exempt under the provisions of the Internal Revenue Code, particularly Section 501C. In addition, the Laboratory has been formally designated a "public charity" by the Internal Revenue Service. Accordingly, it is an acceptable recipient of funds which would result from the termination of "private" foundations.

The Laboratory depends upon the generous contributions of its sponsors, participating institutions, and friends for central institutional needs and capital improvements. In addition, the development of any new programs, such as year-round research in neurobiology or the marine sciences, can be undertaken only with 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, if the appreciation would have been a long-term gain, to the extent of 50% of your adjusted gross income and you need pay no capital gains tax on the stock's appreciation.

We recommend either of the following methods:

(1) 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.
(2) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, New York 11724. In a separate envelope send an exe*cuted* 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 could be 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 Administrative Director, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8300.

CONTRIBUTORS

to the Cold Spring Harbor Laboratory

Dr. Bruce Alberts
Ande, Inc.
Mr. & Mrs. C.V. Angadi
Mr. & Mrs. E. Bench
Biogen, S.A.
Bristol-Myers Fund
Mr. & Mrs. S.R. Callaway
Patricia Casentini
Cetus Corp.
Chevron Fund
Dr. Bayard Clarkson
Columbia University
Conoco, Inc.
Cunningham Family
Dr. Bernard Davis
Mr. & Mrs. Charles F. Dolan
Dow Chemical Co.
Dr. Lester Dubnick
Duke University
E.I. du Pont de Nemours & Co.
Eli Lilly and Co.
Exxon Corp.
Exxon Research & Engineering Co.
Walter N. Frank, Jr.
Getty Oil Co.
Dr. & Mrs. H. Bentley Glass
Glenmede Trust Co.
Dr. & Mrs. Richard L. Golden
Grace Foundation, Inc.
Oliver R. Grace Charitable Trust
Dr. Alfred D. Hershey
Dr. Ira Herskowitz
Hoffman-La Roche, Inc.
Dr. Alexander Hollaender
Laura J. Ingraham
International Business Machine Corp.
Antonio Jimenez
Arthur Landy
Mr. & Mrs. Richard Leckerling
Mr. & Mrs. George Lindsay
Mr. & Mrs. Robert V. Lindsay
Mrs. V.S. Littauer
Mr. & Mrs. Michael Luckey
Massachusetts Institute of Technology
Leila McKnight
Merck Sharp & Dohme Research Laboratories
Arthur Merrill
Mrs. Anges Meyer & Family
Miles Laboratory
Morgan Guaranty Trust
R.V. Myers
New York Life Foundation
New York University Medical Center
Linda Peyton
Mr. & Mrs. Michael G. Pfeifer
Pamela P. Post
Rockwell International Corp. Trust
Marjorie Robertson Rose
Schering-Plough Corp.
Dr. T. Schopf
Searle Research and Development
Dr. & Mrs. W. Shropshire, Jr.
Dr. & Mrs. Robert Smirnow
Squibb Institute for Medical Research
Dr. A.R. Srinivasan
Stauffer Chemical Co.
Mr. & Mrs. Stuart Symington
Mrs. Robert W. Tilney, Jr.
University of Wisconsin
Upjohn Co.
Mr. & Mrs. Colton P. Wagner
Mr. & Mrs. Kenneth Wagner
Robert A. Weisberg
Yale University
Yeshiva University

FINANCIAL STATEMENT

BALANCE SHEET
year ended December 31, 1982
with comparative figures for year ended December 31, 1981

ASSETS

	1982	1981
CURRENT FUNDS		
Unrestricted		
Cash and Short-term investments	$ 2,658,863	$ 1,062,039
Accounts Receivable	464,261	267,888
Prepaid expenses and other assets	258,587	349,506
Inventory of books	203,899	131,461
Due from restricted fund	286,290	—
Due from Banbury Center	284,630	146,373
Total unrestricted	4,156,530	1,957,267
Restricted		
Grants and contracts receivable	4,615,780	2,845,237
Due from unrestricted fund	—	110,702
Total restricted	4,615,780	2,955,939
Total current funds	$ 8,772,310	4,913,206
ENDOWMENT FUNDS		
Robertson Research Fund		
Cash	508,041	395,840
Marketable securities (quoted market 1982—$13,987,572; 1981—$11,111,405)	11,585,922	10,507,785
Total Robertson Research Fund	12,093,963	10,903,625

LIABILITIES AND FUND BALANCES

	1982	1981
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 171,326	$ 438,074
Mortgage payable	33,300	66,600
Due to plant fund	961,628	30,850
Due to restricted fund	—	110,702
Fund balance	2,990,276	1,311,041
Total unrestricted	4,156,530	1,957,267
Restricted		
Due to unrestricted fund	286,290	—
Fund balance	4,329,490	2,955,939
Total restricted	4,615,780	2,955,939
Total current funds	$ 8,772,310	$ 4,913,206

Olney Memorial Fund					
Cash	1,420	90			
Marketable Securities (quoted market 1982—$23,284; 1981—$20,355)	27,538	27,538			
Total Olney Memorial Fund	28,958	27628			
Total endowment funds	$ 12,122,921	$ 10,931,253	Fund balance	$ 12,122,921	$ 10,931,253
PLANT FUNDS			**PLANT FUNDS**		
Investments	$ 461,239	$ 517,021			
Due from unrestricted fund	961,628	30,850			
Land and improvements	1,111,213	966,110			
Buildings	7,307,583	6,513,210			
Furniture, fixtures and equipment	1,647,248	1,408,503			
Books and periodicals	365,630	365,630			
Construction in progress	727,245	585,779			
	12,581,786	10,387,103			
Less allowance for depreciation and amortization	3,371,273	2,865,421			
Total plant funds	$ 9,210,513	$ 7,521,682	Fund balance	$ 9,210,513	$ 7,521,682
BANBURY CENTER			**BANBURY CENTER**		
Current funds			**Current funds**		
Unrestricted			*Unrestricted*		
Cash	$ 700	$ 700	Accounts payable	$ 11,376	$ 85,406
Prepaid and deferred expenses	70,001	8,101	Due to CSHL unrestricted fund	284,630	146,373
Inventory of books	25,777	47,419	Fund balance	(163,529)	(133,245)
Due from Banbury restricted fund	35,999	42,314	Total unrestricted	132,477	98,534
Total unrestricted	132,477	98,534	*Restricted*		
Restricted			Due to Banbury unrestricted	35,999	42,314
Grants and contracts receivable	91,046	113,440	Fund balance	55,047	71,126
Total restricted	91,046	113,440	Total restricted	91,046	113,440
Total current funds	223,523	211,974	**Total current funds**	223,523	211,974
Endowment Funds			**Endowment funds**		
Robertson Maintenance Fund					
Cash	89,768	21,660			
Marketable securities (quoted market 1982—$2,385,039; 1981—$1,937,629)	1,971,628	1,882,356			

Plant funds		
Land	$ 772,500	$ 772,500
Buildings	846,028	792,757
Furniture, fixtures and equipment	176,284	176,285
Construction in progress	1,386	1,386
	1,796,198	1,742,928
Less allowance for depreciation	275,311	221,356
Total plant funds	1,520,887	1,521,572
Total Banbury Center	$ 3,805,806	$ 3,637,562
Total—All funds	$33,911,550	$27,003,703

Plant funds		
Plant funds balance	1,520,887	1,521,572
Total Banbury Center	$ 3,805,806	3,637,562
Total—All funds	$33,911,550	$27,003,703

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1982
with comparative figures for year ended December 31, 1981

COLD SPRING HARBOR LABORATORY

	1982	1981
REVENUES		
Grants and contracts	$ 7,689,982	$ 5,691,966
Indirect cost allowances on grants and contracts	3,381,795	2,402,569
Contributions		
Unrestricted	30,682	51,273
Restricted and capital	225,000	33,367
Long Island Biological Association	46,500	—
Robertson Research Fund Distribution	453,000	412,000
Summer programs	433,188	363,967
Laboratory rental	20,732	20,732
Marina rental	53,192	48,401
Investment income	376,587	266,752
Publications sales	1,650,263	838,687
Dining Hall	469,196	387,436
Rooms and apartments	269,061	235,798
Other sources	12,347	26,211
Total revenues	$15,111,525	$10,779,148
EXPENSES		
Research*	5,716,197	4,616,825
Summer programs*	759,832	747,519
Library	191,344	182,990
Operation and maintenance of plant	1,719,729	1,378,817
General and Administrative	1,409,112	1,100,037
Depreciation	505,252	475,136
Publications sales*	1,132,856	655,911
Dining hall*	508,560	425,887
Total expenses	$11,942,882	$ 9,583,122

BANBURY CENTER

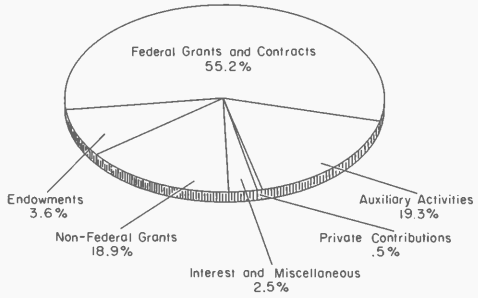
	1982	1981
REVENUES		
Endowment income	$ 99,000	$ 90,000
Grants & contributions	288,226	277,003
Indirect cost allowances on grants and contracts	19,880	—
Rooms and apartments	46,353	40,196
Publications	136,391	139,455
Conference fees	20,375	—
Dining hall	6,484	—
Transfer from Cold Spring Harbor Laboratory	—	60,264
Total revenues	616,709	606,918
EXPENSES		
Conferences	151,631	118,715
Publications	168,011	182,079
Operation and maintenance of plant	109,474	109,199
Program administration	163,312	183,610
Depreciation	53,954	48,537
Capital plant	53,271	13,315
Total expenditures	699,653	655,455
Excess (deficit) of revenues over expenses	$ (82,944)	$ (48,537)

TRANSFERS		
Capital building projects	2,003,518	1,115,471
Banbury Center	—	60,264
Total transfers	2,003,518	1,175,735
Total expenses and transfers	13,946,400	10,758,857
Excess (deficit) of revenues over expenses and transfers	$ 1,165,125	20,291

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

Note: Copies of our complete, audited financial statements, certified by our independent auditors, Peat, Marwick, Mitchell & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
Year End December, 31, 1982



GRANTS

January 1–December 31, 1982

NEW GRANTS

COLD SPRING HARBOR LABORATORY

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
National Institutes of Health	Dr. Watson—General Research Support	$ 104,822	4/1/82–3/31/83
	Dr. Watson—Summer Workshops	657,174	6/1/82–12/31/86
	Dr. Brandsma—Fellowship	17,040	
	Dr. Flaster—Fellowship	34,776	9/15/82–8/14/84
	Dr. Hockfield—Research	282,400	2/1/82–1/31/82
	Dr. Krangel—Fellowship	55,156	10/1/82–9/30/85
	Dr. Lin—Research	290,636	9/24/82–8/31/85
	Dr. Silver—Research	625,803	2/1/82–1/31/85
	Dr. So—Research	379,182	9/1/82–8/31/85
	Dr. Taparowsky—Fellowship	55,156	10/16/82–10/15/85
	Dr. Topp—Research	279,134	3/1/82–2/28/84
	Dr. Welch—Fellowship	57,156	9/1/82–8/31/85
	Dr. B. Zipser—Neurobiology Course Support	331,891	5/1/82–4/30/85
	Dr. B. Zipser—Neurobiology Course Support	473,925	4/1/82–3/31/87
	1982 Papilloma Virus Meeting Dr. Broker	9,500	7/23/82–6/30/83
	1982 RNA Processing Meeting Dr. Matthews	10,000	4/15/82–3/31/83
	1982 Heat Shock Meeting Dr. Watson	8,000	4/15/82–3/31/83
National Science Foundation	Dr. Bukhari—Research	60,000	7/1/82–6/30/83
	Dr. Harshey—Research	115,000	1/1/82–12/31/83
	Drs. Hicks/Malmberg/Strathern/ Klar—Research	165,682	7/1/82–6/30/83
	Dr. So—Research	217,000	7/1/82–6/30/85
	Drs. Stillman/Tamanoi	88,000	7/1/82–6/30/84
	1982 Herpes Virus Workshop —Dr. Watson	4,000	7/15/82–6/30/83
	1982 Symposium—Dr. Watson	5,000	6/1/82–5/31/83
	1982 Heat Shock Induction Conference—Dr. Watson	7,000	5/1/82–4/30/83
	1982 In Vitro Mutagenesis Meeting—Dr. Watson	5,000	5/1/82–4/30/83
	1982 Phycomyces Meeting —Dr. Watson	2,000	6/1/82–5/31/83
	1982 Papilloma Virus Conference —Dr. Broker	3,000	7/15/82–12/31/82
	1982 RNA Processing Meeting —Dr. Mathews	5,000	5/15/82–4/30/83
A.B.C. Foundation	Dr. Wigler—Research	600,000	5/1/82–4/30/87
Abbott Laboratories	1982 Papilloma Virus Meeting Support	500	
	1982 In Vitro Mutagenesis Meeting Support	500	

COLD SPRING HARBOR LABORATORY

American Cancer Society	Dr. Chow—Research	150,000	1/1/82–12/31/83
	Dr. Rossini—Research	110,000	1/1/82–12/31/83
	Dr. Topp—Institutional	50,000	7/1/82–6/30/84
Applied Molecular Genetics	1982 Papilloma Virus Meeting Support	500	
	1982 In Vitro Mutagenesis Meeting Support	1,000	
Biogen, Inc.	1982 Papilloma Virus Meeting Support	500	
Cell Biology Corporation	Dr. Sambrook—Research	394,100	1/1/82–12/31/82
	Dr. Topp—Research	53,646	6/1/82–12/31/82
Cetus Corporation	1982 Papilloma Virus Meeting Support	1,000	
	1982 In Vitro Mutagenesis Support	1,000	
Jane Coffin Childs	Dr. Sundin—Fellowship	32,000	7/1/82–6/30/84
Ciba-Geigy	1982 Papilloma Virus Meeting Support	500	
Columbia University	Dr. Fiddes—Research Subcontract	20,000	1/1/82–12/31/82
Council for Tobacco Research-USA	1982 Papilloma Virus Meeting Support	3,500	
Department of Energy	1982 Symposium Support	9,000	9/15/82–8/31/83
Exxon	Research	7,500,000	1/1/82–12/31/86
Foundation for Microbiology	1982 Phycomyces Meeting Support	5,000	
Genentech	1982 Papilloma Virus Meeting Support	500	
Esther A. and Joseph Klingenstein Fund	Year round neurobiology support	150,000	1982–1984
Leukemia Society of America, Inc.	Dr. Kost—Fellowship	37,000	7/1/82–6/30/84
Eli Lilly & Co.	1982 In Vitro Mutagenesis Meeting Support	500	
	1982 Papilloma Virus Meeting Support	500	
Merck & Co.	1982 In Vitro Mutagenesis Meeting Support	1,000	
Monsanto-External Research & Development Division	1982 Heat Shock Meeting Support	1,000	
Agricultural Products Co-Research Division	1982 Heat Shock Meeting Support	2,000	

NEW GRANTS

COLD SPRING HARBOR LABORATORY

New England Biolabs	1982 In Vitro Mutagenesis Meeting Support	2,000	
New England Nuclear	1982 In Vitro Mutagenesis Meeting Support	100	
Pfizer Inc.	1982 In Vitro Mutagenesis Meeting Support	5,000	
Schleicher & Schuell, Inc.	1982 In Vitro Mutagenesis Meeting Support	100	
Alfred P. Sloan Foundation	Neurobiology Workshop Support	20,000	1/6/82–12/31/82
Smith Kline & French	1982 Phycomyces Meeting Support	500	
State University of New York, Stony Brook	Dr. Topp—Research Sub–contract	7,267	8/1/82–7/31/83
Stauffer Chemical	Dr. Hicks—Research	15,000	1982
Damon Runyon–Walter Winchell Cancer Fund	Dr. Bhagwat—Fellowship Dr. Roth—Fellowship	35,000 35,000	7/1/82–6/30/84 9/1/82–8/31/84

BANBURY CENTER

Grantor	*Program*	*Total Award*	*Duration of Grant*
National Institutes of Health	1982 Recombinant DNA Applications Meeting Support	$ 18,200	9/22/82–8/31/83
	1982 Conference on Biological Aspects of Alzheimer's Disease	30,672	9/30/82–8/31/83
March of Dimes	1982 Recombinant DNA Applications Meeting Support	15,000	7/1/82–12/31/82
Henry J. Kaiser Family Foundation	1982 Gene Therapy Meeting and Publication Support	60,000	1/1/82–12/31/82
Burroughs Wellcome Fund	1982 Recombinant DNA Applications Meeting Support	15,000	1/1/82–12/31/82
American Petroleum Institute	1982 Genotoxic Indicators Meeting Support	12,500	1/1/82–12/31/82

COLD SPRING HARBOR LABORATORY

Grantor	Principal Investigator and Program	Total Award	Duration of Grant
National Institutes of Health	Dr. Watson—Gene Organization	$ 5,626,997	4/1/81–3/31/86
	Dr. Watson—General Research Support	104,513	4/1/81–3/31/82
	Dr. Watson—Summer Workshops	470,340	4/1/77–3/31/82
	Dr. Watson—Symposium Support	187,433	4/1/79–3/31/84
	Dr. Watson—Neurobiology Course Support	258,500	6/1/79–3/31/82
	Mr. Udry—Cancer Research Facility	1,411,011	9/15/77–indefinite
	Dr. Albrecht-Buehler—Research	67,014	5/1/81–4/30/82
	Dr. Blose—Research	326,982	12/1/81–11/30/84
	Dr. Bukhari—Genetics Program Project	1,661,439	6/1/80–5/31/83
	Dr. Feramisco—Research	214,758	7/1/80–6/30/83
	Dr. Garrels—Research	323,073	4/1/79–3/31/82
	Drs. Gingeras/Roberts—Research	408,085	3/1/80–2/28/83
	Dr. Grodzicker—Fellowship Training	852,578	7/1/78–6/30/83
	Dr. Hicks—Advanced Bacterial Genetics Course	97,081	4/1/80–3/31/83
	Dr. Hicks—Research	1,196,529	7/1/81–6/30/86
	Dr. Ivy—Fellowship	38,776	1/27/82–1/26/84
	Dr. Klar—Research	876,786	7/1/81–6/30/86
	Dr. Kurtz—Research	179,542	4/1/80–3/31/83
	Dr. Lemaster—Fellowship	34,681	6/1/81–2/15/83
	Dr. Mathews—Research	266,570	4/1/80–3/31/83
	Dr. McKay-Research	288,546	7/1/81–6/30/84
	Dr. Sambrook—Cancer Research Center	18,604,426	1/1/82–12/31/86
	Dr. So—Research	124,370	9/1/80–8/31/82
	Dr. Sorge—Fellowship	58,332	6/1/81–1/31/83
	Dr. Topp—Research	303,452	7/1/80–6/30/83
	Dr. Welch—Fellowship	57,156	9/14/82–9/13/85
	Dr. B. Zipser—Research	299,219	12/1/81–11/30/84
National Science Foundation	Dr. Bukhari—Research	330,000	6/15/79–5/30/82
	Dr. Garrels—Research	140,000	9/1/80–8/31/82
	Dr. Heffron—Research	147,000	2/15/81–1/31/83
	Dr. Hicks—Research	99,600	5/15/81–4/30/84
	Dr. Roberts—Research	345,000	8/15/80–7/31/83
	Dr. Roberts—Research	210,000	12/15/79–11/30/82
	Dr. Strathern—Equipment	64,845	5/15/81–4/30/82
	Dr. B. Zipser—Neurobiology Course Support	60,000	7/1/79–12/31/82
	Dr. D. Zipser—Research	157,000	5/15/80–4/30/83
	Dr. D. Zipser—Research	100,000	1/1/81–12/31/82
American Cancer Society	Dr. Topp—Research	15,000	2/1/81–1/31/82
Rita Allen Foundation	Dr. Hockfield—Research	30,000	7/1/81–6/30/82
Cystic Fibrosis	Dr. Garrels—Research	87,230	7/1/80–6/30/82
Grass Foundation	Dr. B. Zipser—Neurobiology Scholarship	25,790	1980–1982

COLD SPRING HARBOR LABORATORY

The Camille and Henry Dreyfus Foundation	Dr. D. Zipser—1982 Undergraduate Research Participation	20,000	1/1/82–12/31/82
Department of Energy	1981 Symposium Support	9,000	5/1/81–4/30/82
Esther A. and Joseph Klingenstein Fund	Dr. Watson—Neurobiology Course Support	60,000	5/1/79–4/30/82
	1982 Advanced Neuro–Anatomical Course and Single–Channel Recording Workshop	45,000	
Leukemia Society of America, Inc.	Dr. Engler—Fellowship	18,000	7/1/81–6/30/82
Merck & Co.	Dr. So—Research	5,000	
Muscular Dystrophy Association	Dr. Garrels—Research	40,000	1/1/81–12/31/82
	Dr. Lin—Research	58,743	1/1/81–12/31/83
	Dr. Matsumura—Fellowship	36,000	1/1/81–12/31/83
	Dr. Welch—Fellowship	32,000	1/1/81–12/31/82
New England Biolabs, Inc.	Dr. Roberts—Research	14,000	
Alfred P. Sloan Foundation	Dr. D. Zipser—Computer Workshop	31,000	6/1/79–12/31/82
Volkswagen Foundation	Dr. Watson—Neurobiology Scholarships	78,696	1980–1982
Whitehall Foundation	Dr. B. Zipser—Research	135,000	10/1/80–9/30/83
Damon Runyon–Walter Winchell Cancer Fund	Dr. Abraham—Fellowship	35,000	8/1/81–7/31/83
	Dr. B. Anderson—Fellowship	25,250	10/1/80–3/31/82
	Dr. Brandsma—Fellowship	35,000	9/1/81–8/31/83
	Dr. Goldfarb—Fellowship	35,000	10/1/80–9/30/82
Yamasa Shoyo Co., Ltd.	Dr. Katoh—Research	94,480	10/1/81–9/30/83

BANBURY CENTER

Grantor	*Program*	*Total Award*	*Duration of Grant*
National Institutes of Health	1981 Gene Amplification Conference	31,120	9/1/81–8/31/82
	1981 Nitrosamines Conference	32,320	9/30/81–8/31/82
Environmental Protection Agency	1981 Meeting on Response of the Developing Organism to Environmental Risks	50,000	9/1/81–8/31/82
Alfred P. Sloan Foundation	Public Information Workshops	100,000	1/1/80–12/31/82

THE 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 was looking for a place to set up a summer laboratory as the headquarters of its Department of Zoology. Representatives of the Institute were invited by Eugene S. Blackford, at that time the First Commissioner of the State of New York, to inspect a site on Bungtown Road across Northern Boulevard (25A) from the Fish Hatchery. The site was found to be ideal, and so the original Laboratory was organized with Mr. Blackford as president of the Board of Trustees. The land was leased from Mr. John D. Jones, whose family since 1819 had operated various industries including shipbuilding, coopering, and textile manufacture (later whaling, also) at the head of Cold Spring Harbor. Bungtown Road, which runs through the Lab property, got its name from the factory that specialized in making bungs—or stoppers—for barrels.

In 1892 the Laboratory's land was leased for a dollar a year from the Wawepex Society, which Mr. Jones had organized as a corporation for holding real estate and for investing funds for the propagation of fishes and for scientific research. In 1904 the Wawepex Society leased additional land to the Carnegie Institution of Washington, which wanted to locate a Department of Experimental Evolution in the Cold Spring Harbor area. Charles B. Davenport, who had been directing the Laboratory since 1896, assumed the additional duties of director of the Carnegie Institution's experimental station. It is interesting to note that Mr. Davenport lived in what came to be known later as the Carnegie Dormitory, the Victorian house on 25A built by John D. Jones before the turn of the century, and recently repainted in its original colors.

The Long Island Biological Association was established in 1924 when the Brooklyn Institute decided to discontinue its research at Cold Spring Harbor and offered its laboratory to two universities. Fortunately, a local group of interested neighbors decided to assume responsibility for the Lab, and thus LIBA came into being. For 38 years LIBA actually operated the Laboratory in conjunction with the Carnegie Institution, but in 1962 it seemed advisable for the Laboratory to be reorganized as an independent unit. Therefore, the property on which it now stands was conveyed to it by LIBA, which, however, still retains reversionary rights. Today LIBA is one of twelve institutions participating in the support of the Laboratory, each institution being represented on the Laboratory's Board of Trustees.

What has happened, in effect, is that LIBA has become an expanding group of local "Friends of the Laboratory" who help support it through annual contributions. Also, from time to time, the Association undertakes campaigns to finance special important projects for which the Lab cannot obtain funds from the Federal Government or from other sources. For instance, in 1974, LIBA made possible building the James Laboratory Annex and the renovation of Blackford Hall; and in 1976 the rebuilding of Williams House.

The affairs of LIBA are handled by a board of 28 directors who are elected to office by the membership at an annual meeting. At least twice a year LIBA members are invited to bring their friends to a lecture or an open house at the Lab.

Membership in LIBA requires a minimum annual contribution (tax deductible) of $25 for a husband and wife, $15 for a single adult, $5 for a junior member (under 21). Further information can be obtained from the Long Island Biological Association, Box 100, Cold Spring Harbor, N.Y. 11724, or by telephoning the Laboratory's administrative director, Mr. William R. Udry, at (516) 367-8300.

OFFICERS

Chairman	Mr. Edward Pulling
Vice-Chairman	Mr. George J. Hossfeld, Jr.
Treasurer	Mr. James A. Eisenman
Secretary	Mrs. James J. Pirtle, Jr.
Asst. Secretary-Treasurer	Mr. William R. Udry

EXECUTIVE COMMITTEE

The Officers, also
Mrs. Charles O. Ames
Mrs. Henry U. Harris, Jr.
Mrs. Richardson Pratt

DIRECTORS

Mrs. Charles O. Ames
Dr. Alfred A. Azzoni
Mrs. Gilbert A. Ball
Mr. Edmund Bartlett
Mr. Samuel R. Callaway
Mrs. John P. Campbell
Mr. David C. Clark
Mrs. Miner D. Crary, Jr.
Mr. Roderick H. Cushman
Mr. Norris W. Darrell, Jr.
Mr. Joseph C. Dey, Jr.
Mr. Charles Dolan
Mr. James A. Eisenman
Mr. Walter N. Frank, Jr.
Mr. Clarence E. Galston
Mr. Charles S. Gay
Mrs. Henry U. Harris, Jr.
Mr. George J. Hossfeld, Jr.
Mr. Grinnell Morris
Mr. William Parsons, Jr.
Mrs. James J. Pirtle, Jr.
Mrs. Richardson Pratt
Mr. Edward Pulling
Mr. John R. Reese
Mr. Stanley S. Trotman, Jr.
Mrs. Stephen Ulman
Dr. James D. Watson
Mrs. Alex M. White

REPORT OF THE CHAIRMAN FOR 1982

The big event for LIBA in 1982 was the launching of another major project, this time to raise $500,000 from our members over a two-year period to help the Lab finance the construction of an urgently needed new auditorium.

As stated in the appeal brochure, the Cold Spring Harbor Laboratory has become the world center for the exchange and distribution of information about the latest discoveries in molecular biology. As the number of molecular biologists from all over the world who apply for participation in the Symposium and other seminar meetings continues to increase, the need for an auditorium large enough to accommodate them has become increasingly urgent. In 1982 eleven meetings were held attended by 2,400 scientists from many countries.

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

I am happy to report that the response to our appeal has been very generous. As of February 15, 1983 we have already passed the halfway mark toward our goal.

As usual, members and their guests were invited to two lectures during the year. At the first, the annual Dorcas Cummings Memorial Lecture, on May 9th, our speaker was Robert D. Ballard, of the Woods Hole Oceanographic Institution. He fascinated his audience by an account of explorations on the ocean floor.

Then at the Annual Meeting, held on December 12th, we were addressed by Professor Bob Brier, chairman of the Philosophy Department at C.W. Post College. He pointed out ways in which ethical dilemmas created by modern science can be delineated and clarified for making moral decisions. A lively question and answer period took place after Professor Brier had concluded his lecture.

At the directors' meeting on April 4th an informal talk was given by Jeff Strathern, a member of the Laboratory's scientific staff, who explained the

Architect's sketch of new auditorium

technical and complicated process and the implications of his work on molecular genetics.

The custom of giving dinner parties during the June Symposium for visiting scientists was successfully continued in 1982. It is a custom enjoyed equally by the scientists and by their hosts and hostesses. This year's parties were given by

Mrs. Frank R. Berman
Mrs. J. Sedgwick Browne
Mr. and Mrs. Sidney M.G. Butler
Mr. and Mrs. Roderick H. Cushman
Mr. and Mrs. George W. Cutting, Jr.
Mr. and Mrs. Norris W. Darrell, Jr.
Mr. and Mrs. James A. Eisenman
Mrs. Mario A. Fog
Mr. and Mrs. Henry U. Harris, Jr.
Mr. and Mrs. George J. Hossfeld, Jr.
Mr. and Mrs. George N. Lindsay, Jr.
Mr. and Mrs. Angus P. McIntyre
Mr. and Mrs. Walter H. Page
Mr. and Mrs. James J. Pirtle, Jr.
Mrs. E.W. Taylor
Mr. and Mrs. Stanley S. Trotman, Jr.
Mr. and Mrs. Stephen Ulman
Mr. and Mrs. Robert P. Walton
Mrs. Ethelbert Warfield
Mr. and Mrs. R. Ray Weeks
Mr. and Mrs. Richard J. Weghorn

At the annual meeting regret was expressed that the terms of office as directors for Messrs. J.A. McCurdy II, Walter H. Page, Edward Everett Post, and Richard J. Weghorn had terminated after eight years of faithful service. Mr. Clarence E. Galston, chairman of the Nominating Committee, placed in nomination to fill the vacant places: Mrs. Gilbert A. Ball, Mrs. John P. Campbell, Mr. Grinnell Morris, and Mr. John R. Reese, who were then duly elected to the Board.

Edward Pulling, *Chairman*
Long Island Biological Association
February 1, 1983

LIBA members tour Laboratory facilities at Annual Open House

Dr. Jeffrey Strathern at Director's Meeting

MEMBERS
of the Long Island Biological Association

Mr. & Mrs. Thomas Ackerman
Mr. & Mrs. Amyas Ames
Mrs. Charles E. Ames
Mrs. Charles O. Ames
Mr. & Mrs. Hoyt Ammidon
Drs. Harold & Shirley Andersen
Mr. Robert W. Anthony
Mr. & Mrs. J.S. Armentrout
Mrs. Donald Arthur
Dr. & Mrs. Joseph T. August
Mrs. Robert W. Ayer
Dr. & Mrs. Alfred Azzoni
Mr. Henry D. Babcock, Jr.
Mr. & Mrs. Benjamin H. Balkind
Mrs. Gilbert Ball
Miss Marion S. Bannard
Mrs. George C. Barclay
Dr. & Mrs. Henry H. Bard
Mrs. Lila Barnes
Mr. & Mrs. D.R. Barr
Mr. & Mrs. Edmund Bartlett
Mrs. Robert E. Belknap, Jr.
Dr. & Mrs. Stephen Belman
Miss Linda Bennetta
Dr. & Mrs. Alan Bernheimer
Mr. & Mrs. Loren C. Berry
Mr. & Mrs. R. Beuerlein
Mr. & Mrs. Nicholas Biddle, Jr.
Mr. & Mrs. William Binnian
Mr. F. Roberts Blair
Miss Mary Lenore Blair
Mr. & Mrs. Bache Bleecker
Mrs. Margery Blumenthal
Mr. & Mrs. Elito Bongarzone
Mr. & Mrs. A.L. Boorstein
Dr. & Mrs. George Bowen
Mr. & Mrs. William Braden
Dr. & Mrs. Arik Brissenden
Mrs. Horace Brock
Mr. F. Sedgwick Browne
Mr. & Mrs. G. Morgan Browne, Jr.
Mr. & Mrs. Frank Bruder
Mr. & Mrs. James Bryan, Jr.
Mr. & Mrs. Julian Buckley
Mr. & Mrs. Vincent Burns
Mrs. R.P. Burr
Mr. & Mrs. John Busby
Mr. & Mrs. Albert Bush-Brown
Mr. & Mrs. Sidney G. Butler
Mr. & Mrs. T.J. Calabrese, Jr.
Mr. & Mrs. S.R. Callaway
Mr. & Mrs. John P. Campbell
Mr. & Mrs. Ward C. Campbell
Martha Worth Carder
Mr. & Mrs. John Carr
Mr. & Mrs. T.C. Cattrall, Jr.
Mr. & Mrs. Lionel Chaikin
Mr. & Mrs. Beverly C. Chew
Mr. & Mrs. Charles M. Clark
Mr. & Mrs. David C. Clark
Dr. & Mrs. Bayard Clarkson
Mr. & Mrs. Thomas N. Cochran
Dr. & Mrs. Peter Cohn
Mrs. C. Payson Coleman
Mr. & Mrs. Francis X. Coleman, Jr.
Mr. & Mrs. John H. Coleman
Mrs. John K. Colgate
Mr. & Mrs. Emilio G. Collado
Mr. Bradley Collins
Mr. & Mrs. Patrick Collins
Mrs. Ralph C. Colyer
Mr. & Mrs. Kingsley Colton
Dr. & Mrs. Crispin Cooke
Mr. & Mrs. Howard Corning, Jr.
Mr. Duncan B. Cox
Mr. & Mrs. Charles L. Craig
Mr. & Mrs. Miner D. Crary, Jr.
Mr. & Mrs. Arthur M. Crocker
Mr. & Mrs. Robert Cuddeback
Mr. Robert L. Cummings
Mr. Robert L. Cummings III
Mr. & Mrs. Richard T. Cunniff
Dr. & Mrs. Paul Cushman, Jr.
Mr. & Mrs. Roderick H. Cushman
Mr. & Mrs. George W. Cutting, Jr.
Mrs. Howard Dana
Mr. Theodore H. Danforth
Mr. & Mrs. Van Ness Darling
Mr. & Mrs. Norris Darrell, Jr.
Mr. & Mrs. A. George Dartt
Miss Katya Davey
Mr. & Mrs. Lawrence Davis
Mrs. F. Trubee Davison
Mr. & Mrs. Jay DeBow
Mr. & Mrs. Raymond DeClairville
Mr. & Mrs. Donald L. Deming
Mr. & Mrs. Douglas Despard, Jr.
Mr. & Mrs. Joseph C. Dey, Jr.
Mr. & Mrs. Charles Dolan
Russell & Janet Doubleday Fund
Mrs. Halsey S. Downer
Mr. & Mrs. Clark M. Drasher, Jr.
Dr. & Mrs. Arthur Drazan
Dr. & Mrs. Lester Dubnick
Mrs. Eugene DuBois
Mr. & Mrs. James C. Dudley
Dr. & Mrs. John L. Duffy
Mr. & Mrs. Edgar P. Dunlaevy
Mr. & Mrs. William T. Dunn, Jr.
Dr. & Mrs. Gerard L. Eastman
Dr. & Mrs. James D. Ebert
Mr. & Mrs. Paul S. Eckhoff
Mr. & Mrs. James A. Eisenman
Mrs. Fred J. Eissler
Mr. & Mrs. Duncan Elder
Mr. & Mrs. Ashton G. Eldredge
Mr. & Mrs. Martin Elias
Mr. & Mrs. John Evans
Mr. Henri Eyl
Mr. & Mrs. Raymond Z. Fahs
Mrs. B. Tappen Fairchild
Mr. & Mrs. Joel M. Fairman
Mr. H.L. Fates
Mrs. Allston Flagg
Mrs. June L. Fog
Mr. & Mrs. Henry L. Fox
Mr. Walter N. Frank, Jr.
Mr. & Mrs. George S. Franklin
Mr. & Mrs. Jack B. Friedman
Mr. Louis M. Fuccio
Mr. D. Kent Gale
Mr. & Mrs. Clarence E. Galston
Mr. & Mrs. John W. Galston
Mrs. James E. Gardner, Jr.
Mr. & Mrs. Charles S. Gay
Mr. & Mrs. Robert A. Gay
Mrs. Louis F. Geissler, Jr.
Mrs. Ruth Reed Gillespie
Dr. & Mrs. H. Bentley Glass
Mrs. J. Wooderson Glenn
Mr. & Mrs. Bernard Gloisten
Mr. & Mrs. E. Rawson Godfrey
Mr. C.F. Gordon
Mr. & Mrs. Kilbourn Gordon, Jr.
Mr. & Mrs. Oliver R. Grace
Mr. & Mrs. William R. Grant
Mr. & Mrs. Alfred T. Gregory
Mr. & Mrs. G.P. Griffin
Mr. & Mrs. Sidney Hack
Mrs. Joanna Hadden
Mr. & Mrs. Lawrence Hahn
Mrs. Beverly Hanson
Mr. & Mrs. Gordon Hargraves
Mr. & Mrs. Henry U. Harris
Mr. & Mrs. Henry U. Harris, Jr.
Miss Maxine Harrison
Dr. & Mrs. C. Hartenstein
Mr. & Mrs. R.D. Hartmann
Mr. & Mrs. Horace Havemeyer, Jr.

Mr. Huyler C. Held
Mr. & Mrs. James E. Hellier
Heritage Federal Savings & Loan
Dr. & Mrs. William W. Heroy
Mr. & Mrs. Charles L. Hewitt
Mrs. Herman A. Heydt
Mr. & Mrs. Charles R. Holcomb
Mr. & Mrs. Karl Holtzschue
Mr. & Mrs. Frederick G. Hoppin
Mr. & Mrs. George J. Hossfeld
Mr. & Mrs. George J. Hossfeld, Jr.
Mrs. Chandler Hovey, Jr.
Mr. & Mrs. Ralph E. Howe
Mrs. J. Taylor Howell
Mr. & Mrs. Charles J. Hubbard
Mr. & Mrs. Philip G. Hull
Mr. John P. Humes
Mr. & Mrs. J.A.V. Hyatt
Mrs. Elmira S. Ingersoll
Mrs. David Ingraham
Mr. & Mrs. Frederic B. Ingraham
Mr. & Mrs. Richard M. Jackson
Mr. & Mrs. Valdemar F. Jacobsen
Mr. Irving D. Jakobson
Walter B. James Fund
Mr. & Mrs. Robert D. Jay
Mrs. B. Brewster Jennings
Mr. & Mrs. L.S. Johnson
Mr. & Mrs. John J. Juda
Mr. & Mrs. Morris I. Karpen
Mr. & Mrs. Francis E. Kelly, Jr.
Mrs. Walter A. Kernan
Mr. & Mrs. Francis S. Kinney
Mr. & Mrs. John P. Kipp
Mr. & Mrs. Richard L. Kline
Dr. & Mrs. Rudolf W. Knoepfel
Mr. & Mrs. Robert P. Koenig
Mr. & Mrs. Edward W. Kozlik
Mr. & Mrs. Paul Kramer
Mrs. Carol Lamb
Mr. & Mrs. David Lamb
Mr. & Mrs. Gordon Lamb
Mr. & Mrs. Edward M. Lamont
Ms. Laurie J. Landeau
Mrs. Orin T. Leach
Mr. & Mrs. Leslie S. Learned
Mrs. Randall J. LeBoeuf, Jr.
Mr. & Mrs. Henry C. Lee
Rev. & Mrs. T. Carleton Lee
Mr. & Mrs. Thomas Leon
Dr. & Mrs. R.V. Lewis
Mrs. Elizabeth A. Lindsay
Mr. & Mrs. George N. Lindsay, Jr.
Mr. & Mrs. Robert V. Lindsay
Mr. & Mrs. Bernard Lippman
Mr. & Mrs. V.S. Littauer
Mr. & Mrs. Angus Littlejohn
Mr. & Mrs. John H. Livingston
Mr. Alan J. Long
Dr. S.E. Luria
Mr. & Mrs. John F. MacKay
Mrs. Malcolm MacKay
Mr. Marshall MacLeod
Mr. & Mrs. Sayre MacLeod
Mrs. Henry R. Macy
Mrs. J.R. Mahoney
Mr. & Mrs. John Mariani
Mrs. John B. Marsh
Mr. & Mrs. John M. Martin
Mr. & Mrs. William H. Mathers
Mr. & Mrs. John D. Maxwell
Dr. Ernst Mayr
Mr. & Mrs. J.A. McCurdy II
Mr. & Mrs. Richard McDaniel
Mr. & Mrs. Victor McElheny
Miss Diana McIlvaine
Mr. & Mrs. Angus P. McIntyre
Mr. & Mrs. Randall P. McIntyre
Dr. & Mrs. Walter Meier
Mr. Arthur C. Merrill
Mrs. Frances Meurer
Dr. & Mrs. Leo M. Meyer
Mr. & Mrs. Kennedy B. Middendorf
Mr. & Mrs. William H. Miller
Mr. Dudley H. Mills
Mrs. A.M. Milton
Mr. & Mrs. George G. Montgomery, Jr.
Mr. & Mrs. F. Franklin Moon
Mr. & Mrs. Richard K. Moore
Mrs. Henry S. Morgan
Mr. & Mrs. George Morgese
Mr. & Mrs. Grinnell Morris
Mrs. Robert C. Muhlhausen
Mr. & Mrs. Allan F. Munro
Mr. & Mrs. Francis W. Murray III
Dr. Larry Nathanson
Mr. & Mrs. Adams Nickerson
Mrs. John W. Nields
Mr. & Mrs. William S. Niven
Mr. Lawrence W. Northam
Mr. & Mrs. Charles P. Noyes
Mr. & Mrs. David C. Noyes, Jr.
Mr. & Mrs. George D. O'Neill
Mr. & Mrs. Grover O'Neill, Jr.
Mr. & Mrs. Richard B. Opsahl
Mr. & Mrs. Robert Osterhus
Mr. & Mrs. Arthur W. Page, Jr.
Dr. & Mrs. David A. Page
Mr. & Mrs. John H. Page
Mr. & Mrs. Walter H. Page
Mr. & Mrs. Fred W. Pain
Mr. & Mrs. Samuel D. Parkinson
Mr. & Mrs. Joseph M. Parriott
Mr. & Mrs. William Parsons
Mr. & Mrs. William Parsons, Jr.
Mr. & Mrs. Charles F. Paul
Mr. & Mrs. William H. Peck, Jr.
Mr. & Mrs. Ernest Penchuk
Mrs. Paul G. Pennoyer
Mr. & Mrs. Paul Pennoyer, Jr.
Mr. & Mrs. John M. Perkins
Mr. & Mrs. Bernard Peyton III
Miss Linda Peyton
Mr. & Mrs. Howard Phipps, Jr.
Mr. John Pickett
Mrs. Richard N. Pierson
Mr. & Mrs. James J. Pirtle
Mr. & Mrs. Collier Platt
Mr. Graham L. Platt
Mr. & Mrs. Francis T.P. Plimpton
Mr. & Mrs. Martin Pollak
Mr. & Mrs. Edward Everett Post
Mr. Samuel H. Post
Mrs. William S. Post
Mrs. H. Irving Pratt
Mr. & Mrs. Richardson Pratt
Dr. William Preston
Mr. Edward Pulling
Mr. Thomas L. Pulling
Mr. & Mrs. Preston V. Pumphrey
Mr. & Mrs. Tarrant Putnam
Mr. & Mrs. James T. Pyle
Mr. & Mrs. William Rand
Dr. John H. Ray III
Mrs. Sara Redmond
Mr. & Mrs. E. Corprew Reed
Mr. & Mrs. John R. Reese
Mr. & Mrs. Willis L.M. Reese
Mr. A.G. Reid
Mrs. Cornelius J. Reid
Dr. Robert M. Reiss
Mr. Jose L. Reissig
Mr. Bernard J. Reveredin
Mr. & Mrs. John T. Ricks
Mr. & Mrs. J.G. Riley
Mrs. Penny Ringquist
Mr. & Mrs. Samuel B. Rogers
Miss Elizabeth E. Roosevelt
Mrs. Phillip J. Roosevelt
Mrs. Quentin Roosevelt
Mr. & Mrs. Charles W. Root
Mrs. Donald Rose
Mr. & Mrs. Arthur W. Rossiter, Jr.
Mr. & Mrs. Walter N. Rothschild, Jr.
Mr. & Mrs. Francis J. Rue, Jr.
Mrs. F.E. Ruland
Mr. & Mrs. Harold P. Salembier
Mr. & Mrs. Bernard Salzberg
Mr. & Mrs. Harvey E. Sampson
Mr. & Mrs. John K. Sands
Mr. John M. Schiff
Dr. & Mrs. Carl J. Schmidlapp II
Mr. & Mrs. Frederick P. Schmitt
Mr. & Mrs. Franz Schneider
Dr. Irving M. Schneider
Mr. & Mrs. Hermann C. Schwab
Mr. & Mrs. David R. Schwarz
Mr. & Mrs. Frederick E. Seymour
Mr. & Mrs. Marvin Sharfin
Mr. & Mrs. Dale Sharp
Mr. & Mrs. Paul C. Sheeline
Dr. & Mrs. Edward M. Shepard
Mr. & Mrs. Douglas A. Shepardson, Jr.
Mr. & Mrs. Robert E. Sheridan
Dr. & Mrs. Paul Siminoff
Mr. & Mrs. Richard Simmons
Mr. & Mrs. W.O. Simpson
Mrs. James C. Skiff
Mr. & Mrs. Rowland G. Skinner
Mr. & Mrs. H. Turner Slocum
Mr. & Mrs. Robert Smirnow
Mr. & Mrs. William S. Smoot
Mr. & Mrs. James Sommermeyer
Mr. W.O. Stanton
Mr. & Mrs. James F. Stebbins
Mrs. Theodore E. Stebbins
Mr. John C. Stevenson
Mr. & Mrs. Norman W. Stewart
Mr. Dudley W. Stoddard
Mr. & Mrs. F. Page Storment
Mr. & Mrs. Richard S. Storrs
Mr. & Mrs. Joseph S. Stout
Mr. & Mrs. Joseph S. Stout, Jr.
Mr. Theodore C. Streibert
Mrs. Terry Sullivan
Mr. Scott Sutcliffe
Mr. & Mrs. Robert Swiggert

Dr. Basil Tangredi
Mr. & Mrs. Benjamin Taylor
Mrs. E.W. Taylor
Mr. & Mrs. Frederick Taylor
Mrs. Henry C. Taylor
Mr. & Mrs. John W. Taylor
Miss Jeanie Tengelson
Mr. & Mrs. Daniel G. Tenney, Jr.
Mr. & Mrs. D.B. Tenney
Mr. & Mrs. Fred Thielking
Miss Denyse Duval Thors
Dr. & Mrs. Robert Tilney
Mr. & Mrs. Warren I. Titus, Jr.
Mr. Alexander C. Tomlinson
Mr. & Mrs. Perry D. Trafford, Jr.
Mr. M.B. Travis
Mr. Bronson Trevor, Jr.
Mr. & Mrs. Stanley Trotman
Mr. & Mrs. Godfrey G. Truslow
Mr. & Mrs. E.T. Turner
Mr. & Mrs. William R. Udry
Mr. & Mrs. Stephen Van R. Ulman
Dr. & Mrs. Thornton Vandersall
Mr. & Mrs. Martin Victor
Mr. Eric Von Raits
Mr. & Mrs. Colton P. Wagner
Ms. Christine M. Walker
Mr. & Mrs. George Walker
Mr. & Mrs. R.W. Walker
Mrs. Robert P. Walton
Dr. & Mrs. David E. Warden
Mrs. Ethelbert Warfield
Mr. & Mrs. Bradford A. Warner
Mr. & Mrs. Harold L. Warner, Jr.
Dr. & Mrs. James D. Watson
Edwin S. Webster Foundation
Mrs. Arthur D. Weekes, Jr.
Mr. & Mrs. B.G. Weekes
Mr. & Mrs. R. Ray Weeks
Mrs. F. Carrington Weems
Mr. John C. Weghorn
Mr. & Mrs. Richard J. Weghorn
Mr. & Mrs. Taggart Whipple
Mrs. Alex M. White
Mr. & Mrs. Warren D. White
Mr. & Mrs. William F. Whitman, Jr.
Mr. Theodore S. Wickersham
Mr. Malcolm D. Widenor
Mr. & Mrs. Douglas Williams
Mr. & Mrs. Duane N. Williams
Mr. Henry S. Williams
Mr. & Mrs. Ichabod Williams
Mr. & Mrs. Thorndike Williams
Mrs. John C. Wilmerding
Mr. & Mrs. Henry S. Wingate
Mrs. Scudder Winslow
Mr. & Mrs. William A. Woodcock
Mr. & Mrs. James A. Woods
Mrs. Ford Wright
Miss Flavia Helen Wyeth
Mr. & Mrs. Woodhull Young