COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1975

Cover: Ruffling lamellipodium and several filopodia extending from a 3T3 mouse fibroblast 15 minutes after plating on a glass substratum. Scanning electron microscopy; horizontal magnification, 31,500×. *(Photo by G. Albrecht-Buehler)*

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COLD SPRING HARBOR, NEW YORK

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Cold Spring Harbor, Long Island, New York

DIRECTOR'S REPORT

No one can do first-rate science without possessing a deep sense of curiosity. We can be told that a given problem is important and should be solved, but unless it tickles our fancy we had best leave it alone. For some forty years we have been diligently investigating the molecular nature of genes, never seriously worrying whether it warranted such prime attention. As the carriers of heredity, genes must be at the center of cellular existence, and a deep understanding of them is bound to provide fundamental insights into the nature of life itself and possibly even into the origin of life. Happily we have discovered basic facts about cell biochemistry at a much faster rate than anyone would have predicted, and the rules of genetics are now seen to be unambiguous expressions of the underlying laws of physics and chemistry.

The question thus can and has been raised whether as molecular geneticists we have become intellectually somewhat passé. Do we in fact already understand so many of the important generalizations about the molecular basis of the living state that the future is bound to be a letdown? Should we continue to tell our brightest students that molecular genetics still has a future, or should we tell them that only by great leaps into the unknown, say toward the brain and the dilemma of human consciousness, will they ever achieve the real respect of their elders? Are *E. coli* and its genetics best left to the textbooks, and will true answers come only if we take on the brain, hoping to so master it that at long last we will no longer have to put up with the philosophers and their endless prattles about free will?

Alas, there is no simple way to settle this argument, and if a former protagonist has lost his curiosity about how genes work and wishes now to conquer the brain, we should wish him well. Most clearly he is not moving from a difficult to an easy career. Moreover, unless his immediate objectives are modest, he may suffer from acute withdrawal symptoms brought on by the fact that he is no longer deluged by the information explosion within molecular biology. Admittedly, most new facts about DNA and RNA in journal articles are not shattering. Nonetheless, there still appear each month, if not each week, one to several papers on molecular genetics that smell of real excitement. Neurobiology, however, still does not possess simple Mendelian or base-pairing type rules that can be used to understand how we perceive complex stimuli, sometimes to memorize and later to use in rational thinking. Of course, this does not mean that our friends who have moved into neurobiology have done so unwisely. Quite deliberately they have chosen a seemingly slow-moving field, arguing that the inherent profundity of their long-range goals more than compensates for the fact that their path through the woods is not clear.

As for those of us who still wish to worry about how genes function, I feel there has been much too much pessimism about our future. No one can but be enormously impressed by the intellectual vigor of the past decade. The solving of the genetic code has not initiated a downward path in which each new discovery seems more and more of the same old stale way of proceeding. Both DNA replication per se and the molecular structure and functioning of chromosomes are much more exciting fields than they were a decade ago. And there is no reason to believe that they have already peaked. Instead, one could easily imagine the next 5–10 years to be as exciting as any in the past several decades. Such optimism appears particularly justified once we understand that we need to relate these subjects to the processes of cell growth and division. Our only real mistake may come in feeling that we should be able to work out all the true answers over the next decade. This is not because our science will suddenly slow down—in fact, it will probably go faster than we predict. But undoubtedly new complexities not yet dreamed of will be discovered, further delaying the day when we can finally comprehend the structure, replication and function of chromosomes with the same all-embracing certainty that accompanied our first views of the sixty-four codon assignments.

Thus it still makes great sense for many of our best students to opt for the gene and its mode of functioning. Here we must be careful not to talk so much about higher cells that we forget that *E. coli* and its phages remain powerful systems for establishing fundamental facts about how cells grow and divide. I'm afraid that we now pay far too much attention to the assertion that most research must be at least semirelated to the human condition, and that we can most certainly sell research on higher cells more easily than we can peddle a bacterium that all proclaim to be harmless. We shall sell both ourselves and the general public short if we let politicians believe that their own superficial reactions should influence the way we do science. Not knowing any science, they are completely unqualified to judge what areas of pure science are in the long term likely to benefit humans most. At best they will opt for what on the surface appears to be the most direct approach, but we have to remember that we seldom solve problems by direct assault.

It is not obvious, for example, that the best approach to understanding the control of DNA replication in human cells is to mount a crash program that allocates most of our nucleic acid money for research with higher organisms. Even now we do not have any stable of vertebrate cell lines that contains genetically well-characterized mutations which block specific steps in DNA replication. So to the extent that mutants play a key role in the understanding of complex biochemical pathways, we should still invest a significant fraction of our money in the working out of the fundamental mechanism(s) of DNA replication in both *E. coli* and yeast. This is not to say that research with higher cells is still too difficult—I believe just the opposite—but I fear that unless we are vigilant, the higher cell bandwagon, coupled with our chronic lack of money for "pure research," may effectively choke off the many crucial experiments that still need to be done with microorganisms.

We must also take care that the great satisfaction we continue to get from the genetic approach does not blind us to the great advances that may soon occur in understanding at the molecular level how single cells move about in response to specific external stimuli. Such directed movements are of immense selective advantage to cells, and their evolution must have been a key step in the creation of life as we now comprehend it. Although it may appear that many cell movements are highly random, they are, in effect, highly coordinated efforts that depend upon the successful interaction of large numbers of different proteins, many closely related to those that can now be isolated from muscle tissue. Particularly important must have been the development of amoeboidlike motion. Until recently, the generality of this form of motion has been underappreciated. This can no longer be the case as we face up to the recently discovered fact that some 15–20% of the proteins of the average animal cell are typical musclelike proteins (e.g., actin and myosin).

A major biological goal of the next several decades will thus be to establish the structural features of a cell that permit its various muscle proteins to bring about

directed cell movements. A forward extension in one direction, for example, must soon be followed up by a pulling back of the posterior tail-like segment. When so phrased, we might imagine that we are studying the neurobiology of the single cell. So the establishment of its molecular basis is bound to bring great intellectual satisfaction to all who make major contributions. Increasingly large numbers of our most talented young scientists are already so involved, and we happily note the recent exploitation here by Lazarides and Weber of fluorescent immunological techniques that provide a powerful tool to scan large numbers of cells for the location of their organized musclelike fibers.

We would like to accelerate our program of research in this area and have taken the first step of setting up a Cell Biology section, whose primary purpose will be the study of those features of cell structure that bear upon cell movements. For the time being it must work out of both Demerec and McClintock laboratories. Ultimately, however, it will require especially equipped facilities, in particular to house the most modern light and electron microscopes. An ideal location for this laboratory is the site of our long disused and decayed greenhouses. We are therefore preparing tentative plans for a modestly sized lab with direct access to Demerec. The existence of such a facility would allow us to attack from still another important direction the question of how cells, both normal and cancerous, grow, divide and differentiate, so we have high hopes that some enlightened philanthropic source will come to our aid to help make these plans a reality.

The possession of a modern cell biology lab would also add impetus to our plans for the establishment of a major, year-round neurobiology program at Cold Spring Harbor. Many of the most exciting aspects of neurobiology now lie in understanding the nerve cell per se; in particular, how they can establish synapses with neighboring nerve (muscle) cells. So the techniques used to study nerve cells often overlap those involved in studying other cell types, and it has become impossible at times to distinguish much of cellular neurobiology from cell biology. Thus the stronger we become in cell biology, the greater our opportunity for inspired neurobiology, and vice versa. Of course, we also hope that in studying cellular neurobiology per se, we shall find unexpected new facts about nerve cell behavior that will provide important information as to how the organized sets of nerve cells function.

A prime factor in our move toward year-round research on nerve cells will be the newly created Marie Robertson Fund for Neurobiology, a marvelous new gift from the Robertson family of Lloyd Harbor. The income from this fund will be used initially for the purchase of much new equipment for Jones lab, so as to provide a capability for research on the vertebrate central nervous system itself. In particular we wish to explore the visual cortex, that portion of the brain that can now be studied most directly. So if all goes well in the next few years, we expect to have a capability for neurobiology research both at the cellular level and at the complex level of the brain itself. As my opening remarks indicated, we have no illusions that the problems we have taken on will be quickly solved. But that is what we are for.

Highlights of the Year

The DNA Tumor Virus Problem Becomes More Well Defined

The umbrella support provided by our large NCI Cancer Research Center Grant has allowed us to continue our broad attack on the way in which DNA-containing animal viruses can transform normal cells into their cancerous equivalents. With both SV40 and the adenoviruses, it appears most likely that the cancerous transformation is caused by the insertion into a host chromosome of a viral gene necessary for the replication of viral DNA. Exactly what these genes do at the enzymatic level is still unclear. Over the last year our protein synthesis group, Ray Gesteland, Jim Lewis, John Atkins and Carl Anderson, has narrowed down the list of potentially oncogenic adenovirus proteins to two candidates, one of MW~50,000 and the other of MW~15,000. Now we want to find out where they function in the infected (transformed) cell; in particular, whether they bind

to DNA. Simultaneously, Rich Roberts and Sharyn Endow have prepared a very detailed restriction enzyme map of the extreme left end of the adeno genome, the region now known to carry the oncogenic trait. They, together with Rich Gelinas, intend now to proceed to map exactly the starting points for the early adenovirus mRNAs that code for the potential oncogenic proteins. With luck, much useful DNA and RNA sequence data will be available a year from now.

In James lab, research on the genetics of adenoviruses, together with their related adeno-SV40 hybrids, is being vigorously pushed by Terri Grodzicker and Joe Sambrook. They have the general objective of mapping the adeno genome to the ground and are also proceeding to use their recently discovered chain-terminating mutants to look for cell lines that possess suppressor genes. Also most exciting has been the discovery by Mike Matthews and Ulf Pettersson of several forms of the small (5S) adeno-specific RNAs which accumulate late in infection. They are present in very large amounts in the cytoplasm and must play some important control function. But what it is remains tantalizingly unclear.

Another key problem is the form of the SV40 genome within SV40-transformed cells. Hopefully, we shall soon have a definite answer from the work of Mike Botchan, who is engaged in using restriction enzymes to dissect the SV40 DNA sequences from transformed cells to see if the integrated form of SV40 is similar in a variety of independently transformed cells.

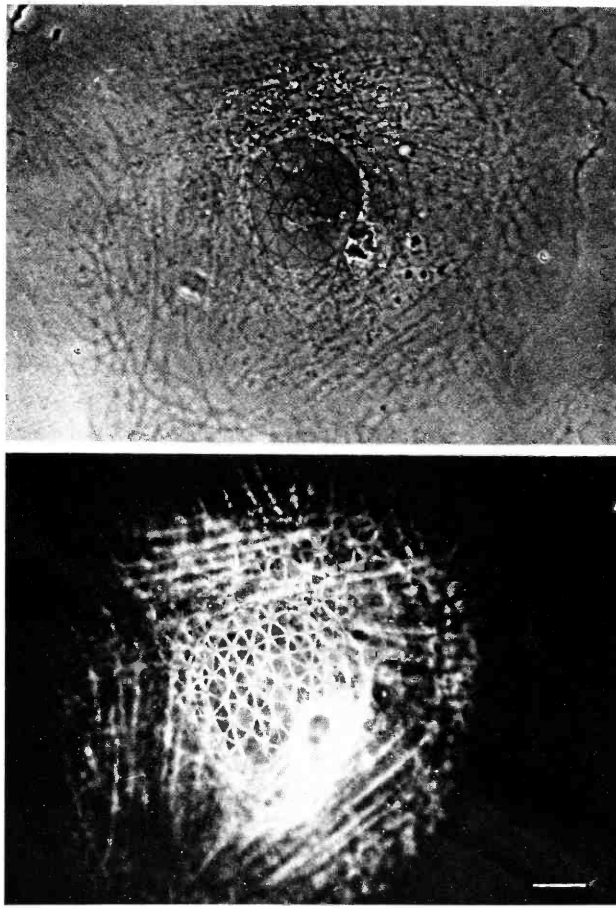
Discovery of a Polygonal Lattice of Muscle Proteins in Spreading Cells

In last year's report we noted the development by Elias Lazarides and Klaus Weber of a powerful immunofluorescent method for probing the location of actin within "non-muscle" cells growing in culture. Most of the actin within flattened growing cells was seen to exist in filamentous form within very thin (0.5 μ–2.0 μ diameter) bundles that can be up to 50 μ in length. Over the past year Lazarides has greatly extended this methodology by utilizing antibodies directed against tropomyosin and α-actinin to show that these "muscle" proteins are also present within the actin-containing bundles. He also found that the rounded cells present after trypsinization develop a highly regular polygonal lattice of these muscle proteins as they begin to reattach and flatten down. The discovery of this lattice represents a major advance in our thinking about cellular organization, revealing an unexpected high degree of organized complexity. We suspect this lattice plays a major role in the shape changes a cell undergoes as it passes through various stages of the cell cycle. We are therefore greatly expanding our efforts in this direction, feeling that these immunofluorescent methods should provide a major tool in understanding how the various muscle proteins interact to bring about amoeboidlike cellular movements.

Major Changes in Our Scientific Staff

With great regret we witnessed the departure of Bob Pollack for a tenured position in the Microbiology Department at Stony Brook. During his four years here, Bob and his colleagues carried out a major series of investigations on revertants of SV40-transformed cells, which also provided important new knowledge as to the SV40 transformation process itself. We were thus most fortunate in having Bob on hand to establish our Mammalian Cell Genetics lab. Bob and his many coworkers always ran a most congenial lab, which they made readily available to others in need of facilities for growing cells in culture. Fortunately, the huge gap left by their departure has now been filled by the recent arrival of Jim McDougall, who brings a group from Birmingham, England, to work on the process of adeno- and herpes-induced cell transformation.

Also a great loss was the departure of Klaus and Mary Weber for the Max-Planck-Institute in Göttigen, where Klaus will head a new section on cell biochemistry. During the two and a half years the Webers were with us, they gave the Lab great strength in protein chemistry, which they utilized most effectively in the study of the SV40 "T"



Indirect immunofluorescence on a population of fully spread out rat embryo cells 24 hours after plating. The cells were reacted with the alpha-actinin antibody and photographed with phase contrast (*top*) and epifluorescence optics (bottom). Note the final fluorescent striations that become apparent with this antibody. Bar = 10 μm. (*Photo by Elias Lazarides*)

antigen as well as in the working out of immunofluorescent techniques for the localization of cellular proteins.

We also already miss Carl Anderson, who is now at the Brookhaven National Laboratory, John Atkins, who has joined Ken Murray in Edinburgh, Ellen Daniell, now

an Assistant Professor of Molecular Biology at Berkeley, Carel Mulder, now at the University of Massachusetts in Worcester, John and Janet Arrand, who have returned to work in London, and Rex Risser, who now works with Wally Rowe at the NIH.

Arriving early this year to lead our Electron Microscopy facility was Tom Broker, who came to us from a postdoctoral period in Norman Davidson's Caltech lab. Coming with Tom was his new wife Louise Chow, who also was a postdoc of Norman Davidson at Caltech. So in making one strong appointment we have in effect made two, and we look foward to much activity both in their lab in Demerec and from the EM facility in Davenport lab. Also new to our staff is Guenter Albrecht-Buehler, who initially came here from Basel to be with Bob Pollack. Guenter now works in McClintock lab, where he is helping create our new Cell Biology section.

We are also happy to note the awarding of Ph.D. degrees to Elias Lazarides from Harvard and to Brad Ozanne from NYU School of Medicine. Both carried out much of their research here at Cold Spring Harbor, and we are much the richer for their respective Institutions having allowed them to do so. Elias is now a postdoc with Keith Porter at Boulder, while Brad is in London working at the ICRF with Robin Weiss.

A Most Exciting Symposium

Our annual June Symposium this year was on "The Synapse," a subject now at the center of research on both the cellular and integrative levels of neurobiology. There were formal presentations by some sixty-four speakers, with the total attendance climbing to two hundred and forty-three. Again we served food in two locations, thus avoiding the long food lines that plagued so many of our earlier meetings. Even though we had fewer formal papers than during last year's very large Symposium, the sessions themselves were equally long and strenuous. Fortunately, the uniformly high quality of the papers kept everyone in good morale and allowed them to leave with the feeling that this Symposium was an event not to be missed.

A most needed break in the meeting was provided by the dinner parties given for the speakers by many of our neighbors. For these most relaxed occasions we wish to thank Mr. and Mrs. Ward C. Campbell, Mr. and Mrs. Robert Cummings, Mr. and Mrs. Norris W. Darrell, Jr., Mr. and Mrs. James A. Eisenman, Mrs. William A. Flanigan, Jr., Mr. and Mrs. Clarence E. Galston, Mr. and Mrs. Angus P. McIntyre, Mr. and Mrs. Walter H. Page, Mr. and Mrs. Samuel D. Parkinson, Mr. and Mrs. James J. Pirtle, Jr., Mr. and Mrs. Walter N. Rothschild, Jr., Mr. and Mrs. Franz Schneider and Mr. and Mrs. Richard J. Weghorn.

Equally pleasant was a most enjoyable afternoon garden party given by Mr. and Mrs. Charles S. Robertson at their home on Banbury Lane. For a while we feared we might be subjected to an afternoon of rain, but the clouds cleared off, and our guests were given the happy opportunity to witness once again the most unique support given to this Lab and its visiting scientists by the many members of the Cold Spring Harbor community.

An Even More Crowded Schedule of Summer Meetings and Courses

Our summer program of meetings and courses ran about four months, bringing altogether more than 1600 people to the Lab for periods of from several days to several months. This was by far our most crowded summer, and our hotel business could boast of essentially one hundred percent occupancy. While this was efficient from a business viewpoint, it also meant that too many of our visitors had to find accommodations in nearby motels, never an ideal situation despite our attempts to run efficient shuttle cars at all hours of the day and night. So the acquisition of more housing remains a major objective. And since it now appears that the 1976 summer program will bring even greater congestion, we cannot fail to act much longer. To this end, plans have been drawn up for the replacement of our most antiquated and unheated Williams House by an entirely new year-round structure consisting of five apartments, each with two bedrooms. We shall attempt to duplicate almost completely the exterior appearance of

Williams while providing inside functionally efficient and esthetically interesting living areas. We plan to start construction late this coming summer, aiming for completion by the start of the 1977 summer.

Spirited Conference on Cell Motility

Our summer ended with the third in our series of meetings on topics related to cell proliferation. This year we focused our attention on cell motility, emphasizing, in so far as possible, the changes in motile properties accompanying both cell division and cell transformation. Our organizers, Bob Goldman, Tom Pollard and Joel Rosenbaum, initially attempted to hold the gathering to a modest size, but as the meeting date drew near we realized that we were about to hold the most concentrated meeting ever held at Cold Spring Harbor. Over 130 different talks were given in the five allotted days, and formal presentations occupied most afternoons as well as the mornings and evenings. Nonetheless, it was a remarkably good meeting, thanks both to shrewd program choices by the organizers and to the enthusiasm of a youthful audience which contained a large contingent of graduate students. So perhaps we should have anticipated that the Saturday evening banquet could last until the predawn hour of 5 a.m. with nonetheless a full audience in attendance at the morning session some four hours later.

We are presently in the process of preparing the resulting manuscripts for publication, hopefully by late August. It appears that it best be printed in a two-volume set totaling some 1200 pages. We can see no way to price it inexpensively and can only hope that due to its high quality and timelessness it will nevertheless find a wide audience.

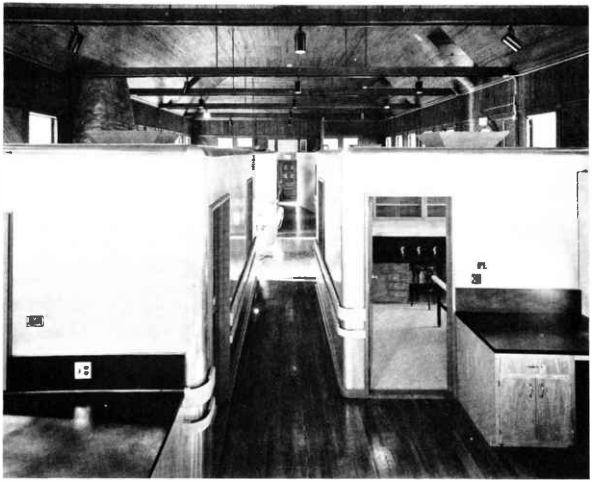
Our Publications Division Continues Very Busy

By now we run a mini-university press type operation, publishing three to four books per year in addition to the annual Symposium volume. The mere number of titles, however, hardly gives a true picture of the magnitude of our work. We seldom publish a book with less than 600 pages, and all too frequently our books climb to the 900-1100-page level. Under ordinary circumstances, production costs for such large books would dictate selling prices between thirty-five and fifty dollars. But most institutional librarians are loath to spend such sums unless strongly coerced by their respective scientific clientele. So we hope that the high quality of our books demands their purchase not only by libraries but also by many individual scientists. In any case we try to see how low we can set the price and still break even. But this is a tricky matter, and our publishing friends tell us that we badly underprice our books. An obvious way out of our dilemma would be to hold smaller meetings so that we could produce 200-300-page books. But to do so would mean that we could no longer allow all serious workers in a given field to participate and publish. Since we are not willing to move in this direction, we are likely to continue to appear naive to our commercially oriented publishing equivalents.

Besides the massive two-volume Symposium on "Tumor Viruses," this year saw the appearance of "RNA Phages" edited by Norton Zinder, a revised edition of "Readings in Mammalian Cell Culture" edited by Bob Pollack, and "Proteases and Biological Control" edited by Ed Reich, Dan Rifkin, and Elliott Shaw. Like its predecessor in the "Conferences on Cell Proliferation" series, this latter volume is over 1000 pages long. Because it contains a most comprehensive overview of the rapidly expanding protease field, we suspect that its publication will be regarded as a major event.

Jones Laboratory Restored

There is no more beautiful building at Cold Spring Harbor than the John D. Jones laboratory. Its construction in 1892 marked the initiation of Cold Spring Harbor as a site for the serious study of biology. For almost fifty years it functioned as a lab for the study of zoology and ecology. When M. Demerec became Director in 1940 and the interests of the Biological Labs became totally centered on genetics, Jones lab was reequipped for summer research in microbial genetics, a function it served well for some thirty m



Interior view of newly completed neurobiology facilities in Jones Lab. (*Photo by Norman McGrath*)

years. I fondly remember the many summers I spent engaged in phage experiments within it. All through these periods it could be occupied only in the summer since the only source of heat was its large fireplace. But now, thanks to the imaginative talents of the architects, Charles Moore and Bill Grover of Essex, Connecticut, the existence of our Robertson Research Fund, and the skilled construction abilities of Jack Richards and his buildings staff, Jones lab has undergone a most miraculous renovation into a year-round laboratory for research in neurobiology. Our architects did not follow the common convention of building research modules against the walls. Instead, they placed them inside the superstructure, covering them with aluminum sheets so as to maximize the contrast with the surrounding wainscotted exterior. I suspect we now possess the esthetically most interesting scientific lab yet to be built anywhere, and I shall be surprised if pictures of it do not begin to grace books on modern architecture.

Its first extended use begins this June, when it will serve as the site of our course on the Vertebrate Central Nervous System. Then for the remainder of the summer and into the fall it will be used for research on the synapse.

A Second Large Grant from the Sloan Foundation

This past summer marked the fifth anniversary of the neurobiology teaching courses that we started using support provided by a $450,000 grant from the Alfred E. Sloan Foundation. This large grant enabled us to renovate completely much of McClintock laboratory for neurobiology as well as to finance much of the equipment and personnel costs of the first years of this program. Since then we have been further helped by a sizable training grant from the National Institutes of Mental Health, as well as by much needed scholarship and equipment support given generously by the Robertson Research Fund, the Grass Foundation and the Eppley Foundation. From the moment the teaching courses began we hoped that means would be found to start up a parallel summer

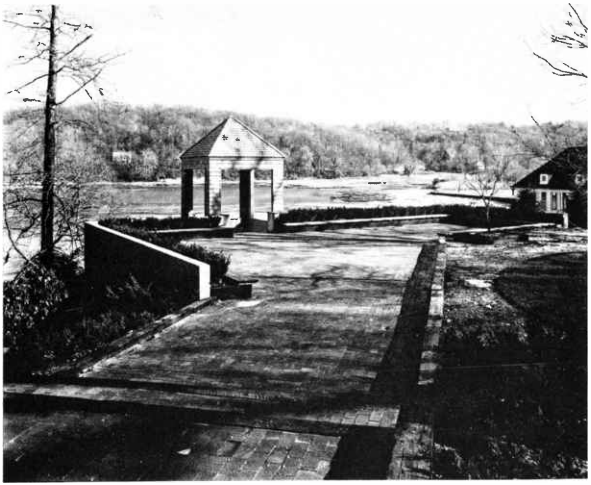
program of research in neurobiology. This objective became a possibility when funds from the Robertson Research Fund allowed us to remodel Jones lab for neurobiology. All then that was lacking was funds to support a contingent of neurobiologists to gather there each summer. So we applied again to the Sloan Foundation citing the many advantages that would emerge were we to be able to have a modest number of neurobiologists in residence every summer. To our delight we have learned that we have been granted $165,000 to cover both equipment and personnel costs over the next years. Our 1976 summer program is almost planned, and already we know that Dr. R. Rahamimoff of the Hebrew University, Jerusalem, will be in residence with several of his coworkers.

Use of Davenport as a Sabbatical Center for Yeast Research

Upon completion of the Davenport lab renovation two years ago, we realized that in addition to its role as a summer teaching lab, it was ideally suited for winter utilization by molecular geneticists on sabbatical leave from their respective universities. The first such use, for a collaborative venture in yeast genetics, brought together David Botstein from MIT, Jerry Fink from Cornell and John Roth from Berkeley. For almost a year they engaged in nonstop experimentation and conversation that centered on the phenomena of suppression. Their joint presence greatly raised the level of intelligent discourse on the Lab grounds, and their departure leaves a gap that proclaims their stay a resounding success.

A Gazebo for the Top of Our New Waste-water Treatment Plant

Work has almost been completed on our new waste-water treatment plant built into the hill in the region between Hooper House and Davenport lab. It should handle all our foreseeable needs over the next several decades. While at present it is equipped only to



The gazebo atop the new waste-water treatment plant. *(Photo by Norman McGrath)*

handle secondary treatment, it has been designed to accommodate both tertiary and quaternary treatment equipment should they someday be deemed necessary. Initially we were greatly concerned that its concrete hulk would detract from one of our most beautiful harbor views. But we almost stopped worrying when we received Charles Moore's plans showing a gazebo on top. Now the gazebo is finished, and we find that we have in fact strengthened the view as one walks down Bungtown Road. Soon we shall remove the obsolescent sand filter and landscape the lower portion. When that happens, the view of the Lab from across the harbor should again verge on the perfect.

An Increasing Ability to Act Quickly Because of the Robertson Research Fund

With the establishment of the Robertson Research Fund some three years ago we acquired a capacity for innovation matched by only a few other sites of biological research. For example, we can now bring visiting scientists to Cold Spring Harbor at very short notice. Thus we were able recently to bring Neil Wilkie here from Glasgow to work for some seven months with Joe Sambrook on the structure of the herpes virus DNA molecule. Robertson support was likewise the crucial factor in allowing Ulf Pettersson to return here last summer to work with Mike Matthews on the adenovirus virus-associated RNA. Equally important, it can provide funds to support scientists who already have had several postdoctoral years but whose future careers would be enhanced by even further postdoctoral experience. For example, Sayeeda Zain, after a postdoctoral period at Yale, wished to come here to do pure research rather than immediately take a university position in Pakistan. And it has allowed Bill Topp to make the transition from being a chemical physicist at Princeton to a cell biologist.

We have also been able to introduce new summer courses in areas where federal monies do not easily flow. Our Advanced Bacterial Genetics course could be given last year only with Robertson support because many of our government's science advisors believe that *E. coli* and its phages are no longer at the forefront of science. Hopefully they have already seen their mistake, and soon we can count on federal money to expand the number of scientists capable of intelligently manipulating the bacterial chromosome.

Equally important, it is only with Robertson money that we have been able to continue our program of summer research by talented college undergraduates. This has been one of our most productive enterprises, providing young students with the opportunity to see if they are really cut out for a scientific career. The answer in most cases has been a resounding "Yes," and both we and the students equally benefit by their presence in our labs. For example, Marianne Wolfner, while working two summers ago in Ray Gesteland's lab, was instrumental in the development of a very efficient in vitro yeast protein synthesis system. Yet two years ago the NSF decided to restrict their URP (Undergraduate Research Program) monies to degree-granting institutions, thereby disallowing any possible governmental support for our program. So here again we can act intelligently only through the possession of Robertson money.

Our Financial Picture Remains Sound

The ever-growing pace of our cancer-related activities more than consumes the funds directly provided by the NCI for cancer research. We were able last year to maintain a balanced research budget only with the additional help provided by our General Medical Research Grant. This year, as in the past several years, the conflicting medical objectives of the Congressional and Executive branches will leave us long uncertain whether we will receive the $140,000 that we are entitled to receive under this program. Yet when I am approached by our staff for new funds, I realize I can say yes only by assuming we shall get this year's General Medical Research monies. Fortunately, the recent Congressional overriding of the HEW veto improves the odds that such funds will again materialize.

If so, we shall again be able to use most of the unrestricted money given to us this year to continue the mammoth task of upgrading all our buildings for modern use. Our

facilities, like those of any other academic institution, steadily depreciate in value, and unless we make substantial capital expenditures we are in fact not breaking even. Happily over the past eight years, some 10–20% of each year's budget could be assigned to capital improvements. Initially, most of that money went to renovate our older buildings, which had steadily depreciated in value over the past 20–50 years. Now we are about eighty percent finished with the job of making up for the past indifference, and the next 2–3 years should see the completion of this task. But even then we shall still have to make sizable transfers to the capital budget to remain even.

Thus the contributions of unrestricted monies that we receive each year from our participating institutions, private foundations, industry and private sources remain an indispensable part of our existence. Without the promise of their further help we would wither, and so we must strive to remain worthy of their continued help.

Our LIBA Support Remains One of Our Greatest Assets

The question of how we can have existed for over eighty years in semirural isolation without the encouragement provided by the presence of other academic institutions has a simple answer. We thrive here because of the long, faithful support given by our local community through its own organization, The Long Island Biological Association. Its members, acting through their directors, have long been an invaluable asset in our quest to do and to teach high-powered science. A recent change in their by-laws now brings about the retirement of Sally Campbell, Bill Rothschild, Betty Schneider and Dudley Stoddard. All have served with distinction, and we now take comfort that they remain in our midst. Happily, Hoyt Ammidon, Jr., David C. Clark, Roderick Cushman and Ted Wickersham join the Board in their places.

The 1975 annual meeting of LIBA was held on December 7 and was presided over by LIBA Chairman, Mr. Edward Pulling. Following the annual election of officers, Mr. George Nicholson, the Director of the American Museum of Natural History, presented a richly illustrated lecture on the "Functions of a Museum."

A Very Strong Board of Trustees

The sane existence of a busy Director demands intelligent help from many quarters, in particular from the Board of Trustees, to whom he is directly responsible. I have been most fortunate in reporting to a group of Trustees, headed by Harry Eagle, that everyone agrees is uniquely effective. It is thus with great regret that I must report that both Art Trottenberg and Posey White, having served two full terms, under our by-laws had to leave the Board as of our November 1, 1975 meeting. Art was long an indispensable member of both the Finance and Executive Committees, while Posey has helped us on innumerable occasions, among which we remember well her invaluable help on our Building Committee. Luckily, Bayard Clarkson of the Sloan-Kettering Institute is again eligible for Board membership, and he joins us this time as an Individual Trustee. We also welcome to the Board Norris W. Darrell, Jr., who practices law with Sullivan and Cromwell in New York and who resides across the harbor from us in Cold Spring Harbor.

Mention must also be made of the long, arduous service of our Finance Committee this fall. The time had come to acquire new investment counselors for the Robertson Research Fund, and we are most indebted to Angus McIntyre and Bud Galston for their help in choosing the firm of Miller, Anderson and Sherrerd.

We Must Never Forget the Joy of Discovery

With our various courses and meetings already spanning some four months and growing in length with each successive year, we are becoming more and more like a research-oriented university rather than a traditional research institute. As a result, we have fewer reasons for going intellectually stale than most research institutes. But at the same time, we must be aware of the danger of being so preoccupied with the hosting of our meetings and courses, or with the running of our publications program, that we lose

sight of the fact that the most worthwhile aspect of science is the joy of discovery; in particular, the establishment of laws of nature that have broad applicability to a variety of natural phenomena. So we must take care always to work toward optimizing the chance that one of us or our visitors can soon see an important scientific problem through to its logical conclusion. If so, we shall remain alive.

February 26, 1976 *J. D. Watson*

Airslie House. *(Photo by Norman McGrath)*

Photo by Alfred Eisenstaedt, courtesy of Time-Life, Inc.

Robert H. P. Olney
(1928 — 1975)

It still remains hard to realize that Bob Olney is no longer with us. Long a prominent member of the Cold Spring Harbor community, he came on our Board of Trustees in 1968 just as I was becoming Director. Almost immediately he became our Treasurer, giving much of his time and thoughtful advice, helping greatly to change our financial outlook from the seemingly hopeless to the guarded optimism that now characterizes our existence. He always came when needed, and many of the important decisions that followed were made because of his wise counsel. Particularly important was his leadership of the 1972 LIBA Fund Drive, which brought to the Lab the funds necessary for the purchase of the Takami property, for the new addition to James lab, and for the renovation of Blackford Hall into a year-round facility. And when Bentley Glass retired, Bob served with great dedication as the Chairman of our Board of Trustees.

And then with cruel suddenness he became gravely ill last summer, to die most prematurely at the age of forty-seven. He leaves behind his wife Joan and their children, Bernie, Linda, Pam, Bobby and Katrina. At their suggestion, Bob's many friends have given gifts to the Laboratory to create a Robert H. P. Olney Fund for Cancer Research. Already some two hundred and fourteen individuals have contributed in the amount of $18,498. It is our intention to use the income generated by this fund to bring to the Laboratory each summer a young scientist to study the cancer cell. We do so hoping that their research can help to cure, if not prevent, further examples of this awful disease.

And further to insure that Bob's memory stays firm in our minds, the fine Queen Ann style residence purchased from the Takami family shall henceforth be known as Olney House.

—J. D. Watson

Clockwise from top left: Richard Roberts; Ray Gesteland; electron microscope; Terri Grodzicker; Robert Pollack; Walter Keller; Paula Grisafi; David Zipser; Guenther Albrecht-Buehler. *(Photos by Ross Meurer; center photo by Robert Yaffe)*

YEAR-ROUND RESEARCH

MOLECULAR BIOLOGY OF TUMOR VIRUS

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The last year has seen a continuation of work on seven major topics: (1) the analysis of defective adenovirus genomes; (2) the purification of and elucidation of the mechanism by which a protein isolated from mammalian cells unwinds turns from superhelical DNA; (3) the analysis of a class of adenovirus mutants that may well contain nonsense mutations; (4) the location of genes coding for several adenovirus proteins, for certain small species of virus-specific RNA (VA RNAs), and the physical mapping of adenovirus mutations; (5) the role of posttranscriptional processing in the control of adenovirus and SV40 gene expression; (6) the analysis and characterization of the transforming regions of adenovirus and SV40 DNAs; and (7) the organization of mammalian chromosomes.

Defective Adenovirus Genomes

It has been known for some time that infection with adenovirus of cells permissive for virus growth yields a variety of incomplete (defective) particles in addition to the maximally infectious complete virions. These incomplete particles have gross differences in the amounts of certain virion proteins and are separable from complete particles by virtue of density differences. The distribution of incomplete particles (as determined by the banding pattern of total particle yield in cesium gradients) varies from serotype to serotype; a characteristic pattern for each serotype is seen regardless of multiplicity of infection or number of passages after plaque purification.

In studying in the past year the origin, structure and biological function of these particles we have concentrated primarily on the DNA contained in the particles and on the ability of these "defective" pools of particles to transform primary cells. It is possible to isolate a class of incomplete particles that contains much less DNA than do complete virions. When such DNA, purified from incomplete particles of adenovirus 2, 3, 12 and Ad2^{+}ND1, is subjected to restriction enzyme analysis, a most intriguing result is observed: although the fragmentation pattern is the same as that of the complete viral DNA, certain fragments appear in as much as tenfold molar excess over others (Fig. 1). The patterns result from an overrepresentation in incomplete particles of one end of the viral genome. In the case of Ad2 and Ad12, where virus-specific sequences in transformed cells have been characterized in detail in this laboratory, we have established that the sequences present in incomplete particles are derived from the end of the viral molecule integrated into the genome of all adenovirus-transformed cells (other parts of the genome being present in some, but not all, lines).

Heteroduplex analysis shows that the DNA species found in incomplete particles are not simply truncated pieces of the normal genome. Upon denaturing the "defective" DNA and allowing it to renature under conditions where only internal duplexes could be expected to form, several unusual structures with duplex regions are formed. The type of structure most easily interpreted is the "extended" panhandle shown in Figure 2. This type of internal duplex in a single DNA molecule indicates that the double-stranded molecule from which it was originally derived contained inverted-terminal repeated sequences. Such structures are seen in all size classes of the DNA, with many different ratios of loop to panhandle length. Likewise, single-stranded circles (characteristic of normal virion DNA and arising from an inverted duplication too short to visualize in the electron microscope) are seen in DNA molecules of all size classes. Reannealing of defective viral DNA for longer periods of time allows inter- as well as intramolecular hybridization to occur and results in the formation of compli-

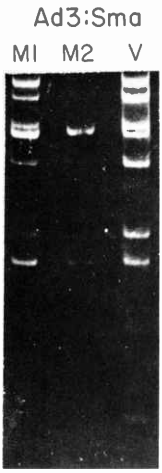

Figure 1

Adenovirus 3 DNA digested with restriction enzyme and electrophoresed on a 1.4% agarose gel. The samples labeled M1 and M2 are from two different preparations of incomplete particles, that labeled V is from complete virion. Fragment D (most prominent in slot M2) is the leftmost fragment on the restriction map.

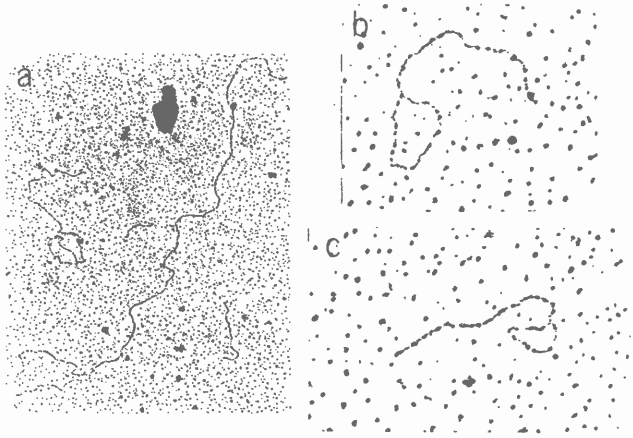

Figure 2

Three examples of panhandle structure resulting from reannealing within a single strand of DNA from adenovirus incomplete particles. The total single-strand length of molecule in *a* is that of complete DNA. Molecules in *b* and *c*, shown here at a higher magnification, are 22 and 15%, respectively, of total length.

cated duplex structures, the analysis of which, along with that of heteroduplexes between defective and nondefective DNA, is yielding a catalog of possible structures of aberrant viral genomes. As these probably have arisen from errors in the replication process, we hope that analysis of their structures will shed light on the mechanism of viral DNA synthesis.

Biological studies on the transforming efficiency of virus particles of various density classes show that the "defective" particles have at least as great a transforming capability per particle as complete particles. DNA extracted from defective particles has been effective in a DNA transformation system.

It is therefore tempting to believe that much, if not all, of the transforming activity of adenovirus may reside in incomplete particles. Perhaps we shall find that the arrangement of viral sequences in DNA molecules extracted from defective particles is the same as that present in adenovirus-transformed cells.

Characterization of DNA-relaxing Enzyme

Work on a protein capable of removing superhelical turns from closed circular, duplex DNA was continued and expanded through the last year. The relaxing protein was purified from human tissue culture cells by conventional biochemical methods. The purified protein has a molecular weight of approximately 70,000 daltons. Under standard reaction conditions (10 mM Tris-HCl pH 7.9; 0.2 M NaCl; 0.2 mM EDTA; 37°C), one molecule of protein completely relaxes 8–15 molecules of superhelical SV40 DNA. Therefore the relaxation of DNA occurs in a catalytic, not stoichiometric, fashion, and the protein can be called DNA-relaxing *enzyme*. The reaction proceeds in the absence of an external energy donor and is completely inhibited by *p*-chloromercuribenzoate and *N*-ethylmaleimide, reagents that specifically block sulfhydryl groups on proteins.

The Number of Superhelical Turns in SV40 DNA

The purified DNA-relaxing enzyme was used to measure the number of superhelical turns in SV40 DNA via two experimental approaches:

(1) When the reaction was performed at 0°C, relaxation proceeds in a stepwise manner and intermediate forms of DNA are generated that contain decreasingly smaller numbers of superhelical turns. By adding small amounts of ethidium bromide into the gel matrix and the electrophoresis buffer, conditions of agarose gel electrophoresis were developed that allowed resolution of all DNA intermediates obtained during a time course of relaxation. By counting the total number of DNA bands, the number of superhelical turns for SV40 DNA was found to be $\bar{\tau} = -30 \pm 3$ (at 0°C, 0.2 M NaCl).

(2) In a second series of experiments, closed circular, superhelical SV40 DNA was treated at 37°C with an excess of DNA-relaxing enzyme in the presence of increasing amounts of ethidium bromide. After removal of the drug each DNA sample consisted of a group of closed circular DNA molecules differing in their number of superhelical turns around a mean value $\bar{\tau}$ in a Gaussian-like distribution. The DNA samples were analyzed by electrophoresis in agarose gels under conditions where the electrophoretic mobility was a function of the number of superhelical turns. Since the distributions around $\bar{\tau}$ of DNA molecules of different samples overlapped, the difference in the mean number of superhelical turns from sample to sample, $\Delta\bar{\tau}$, could be determined and used to measure the mean number $\bar{\tau}$ for untreated SV40 DNA I. By this criterion, SV40 DNA I contains a Gaussian-like distribution of molecules differing by integral numbers around a mean value of $\bar{\tau} = -24 \pm 2$ at 37°C, 0.2 M NaCl. When corrected for the effect of temperature on superhelicity, this value closely corresponds to the number $\tau = -30 \pm 3$ obtained at 0°C. The heterogeneity in τ in SV40 DNA I is probably a consequence of thermal fluctuations in the DNA helix at the time when the last phosphodiester bond is closed in vivo.

The Unwinding Angle of Ethidium Bromide

The buoyant density difference in cesium chloride-propidium diiodide gradients between SV40 DNA I and completely relaxed SV40 DNA I^0 was correlated to the difference in the mean number of superhelical turns between these two DNA samples. The observed buoyant density shift is directly related to the difference in the molar binding ratio (per DNA nucleotide) of ethidium bromide. $\Delta\nu$ for SV40 DNA I and DNA I^0 (at 37°C) was found to be 0.03 ± 0.003. From this, the unwinding angle (ϕe, in degrees) for ethidium bromide was obtained with the following equation:

$$\phi e = 360\,\bar{\tau} / 2\,N\,\Delta\nu,$$

where N is the number of nucleotide pairs in the DNA. Taking $\bar{\tau}$ as -24 ± 2, as determined by electrophoresis, and N as 5200, ϕe was calculated to be $-27.7 \pm 5°$. This is in good agreement with the value $\phi e = -26 \pm 2$ measured by Dr. J. Wang using other methods.

Host-range Mutants of Ad2$^+$ND1

We have continued to isolate and characterize absolute-defective mutants of the nondefective adenovirus 2–SV40 hybrid virus Ad2$^+$ND1. Ad2$^+$ND1 grows efficiently in both monkey and human cells, and this extended host-range property is presumably due to the insertion of SV40 sequences into the Ad2 genome. We have now analyzed seven mutants that behave as though they have all lost the enhancement function provided by SV40. These mutants all grow and plaque poorly on monkey cells, although on human cells they grow as well as their Ad2$^+$ND1 parent. Furthermore, all of the mutants, like Ad2, can be complemented for growth in monkey cells by SV40 but not by Ad2. The mutants also all fail to complement each other. The patterns of protein synthesis of wild-type Ad2$^+$ND1 and the host-range mutants in monkey cells are different. The mutants are defective in the synthesis of several late proteins and thus behave like Ad2 in these nonpermissive conditions. In human cells, the proteins synthesized by Ad2$^+$ND1 and the mutants are similar. However, Ad2$^+$ND1-infected cells contain a 30,000 MW protein that is probably coded for, at least in part, by the SV40 sequences in Ad2$^+$ND1. The host-range mutants fall into two categories with respect to their synthesis of the 30K ND1 protein. Class I mutants make a 30K protein in infected human cells, whereas in cells infected with the second group of mutants (class II), no 30K protein is detectable. In collaboration with Jim Lewis and Carl Anderson in Ray Gesteland's laboratory, we have looked at in vitro synthesis of the 30K protein directed by mRNA that was isolated from mutant-infected human cells and selected by hybridization to SV40 DNA. We have found that the class I mutants synthesize a 30K protein in vitro as well as in vivo. The class II mutants make no 30K protein in vitro; instead, each of them makes a polypeptide fragment of unique size. Thus the host-range mutant *140* directs the synthesis of a 19K fragment, whereas the mutant *71* makes a 10K fragment. None of these mutants have lost the SV40 sequences present in Ad2$^+$ND1 or contain deletions large enough to account for the size of the fragments they produce. All of the host-range mutants have been analyzed with restriction enzymes and show no differences with respect to Ad2$^+$ND1, although a deletion larger than 20–30 base pairs would have been detected by this analysis. However, we have isolated revertants from both a class I and a class II mutant. These revertants grow equally well on human and monkey cells. In infected human cells, the revertant of the class II mutant now produces a 30,000 MW ND1 protein. Thus these mutants probably carry point mutations or at most very small deletions. It is possible that the class II mutants that synthesize no 30K protein, but instead make fragments, contain nonsense mutations or frameshift mutations. Based on the size of the fragments produced by the mutants and assuming that the carboxy terminus of the 30K protein is coded by a DNA sequence lying within 100–200 nucleotides of the SV40 *Hin* G-B junction, we can estimate the position of the hypothetical nonsense codon. The position of the base

change in the mutants relative to Ad2+ND1 is being examined by direct sequence analysis of the DNA in the regions of interest.

We are further characterizing the host-range mutants by comparing the tryptic peptides of wild-type and mutant 30K proteins as well as analyzing their behavior in the in vitro suppressor system developed in Ray Gesteland's laboratory. Since some host-range mutants may be nonsense mutants in the enhancement function provided by the SV40 sequences in Ad2+ND1, they may prove useful in isolating suppressor cells that can support their growth.

Physical Mapping of *ts* Mutations and Template Functions of Adenoviruses

ts *Mutations*

Since *ts* mutations can affect many functions and thus be located in many different genes, it is important to be able to map the sites of the mutations on the viral genome. The method we have developed for mapping mutants involves isolating interserotypic recombinants from crosses of Ad5 and Ad2+ND1 *ts* mutants. Since the parental viruses differ in their cleavage pattern with several restriction enzymes, the recombinants can be analyzed to determine which region of the genome contains Ad5 sequences and which region contains Ad2+ND1 sequences. We have analyzed about 75 recombinants with five restriction enzymes, *Eco*RI, *Hpa*I, *Hin*dIII, *Bam*I and *Sma*I. For each recombinant, the junctions between Ad5 and Ad2+ND1 sequences have been mapped. From these data one can deduce the position of the *ts* mutation in the parental *ts* mutants that were used to generate the recombinants. So far, we have mapped the sites of four Ad2+ND1 *ts* mutants and seven Ad5 *ts* mutants. The assignment of map positions are consistent with each other and with the genetic map.

We are also investigating other methods of positioning mutations and regulatory sequences on the adenovirus genome by marker rescue and of constructing deletion mutants of this virus. Deletion mutants can be generated by ligation of defined fragments of viral DNA obtained by cleavage with restriction endonucleases having cohesive termini, whereas marker rescue depends on the capacity of a specific fragment of wild-type DNA to correct a defect in mutant DNA, thereby localizing the mutation to a segment of DNA corresponding to that fragment. The success of both of these approaches to gene mapping relies heavily on the infectivity of adenovirus DNA, which is low (3 pfu/μg of DNA; Graham and Van der Eb, *Virology* 52:546 [1973]). We have therefore sought conditions for improving the infectivity of adenovirus DNA and are now able to obtain values of 20–30 pfu/μg of DNA. Using these improvements we have performed experiments designed to test the feasibility of these approaches to gene mapping.

Denatured and renatured adenovirus DNA, but not denatured DNA, is capable of plaque formation on human cell monolayers. Moreover, when Ad5 DNA is cut by *Bam*I (Ad5 DNA is cleaved once by this enzyme, which leaves cohesive ends) and the resultant fragments then ligated, the infectivity of this DNA is restored. These experiments offer hope that we may soon be able to map mutations by marker rescue and construct deletion mutants of adenoviruses.

Proteins

Recombinants between Ad5 and Ad2+ND1 must contain both Ad5 genes and Ad2+ND1 genes and therefore synthesize both Ad5 and Ad2+ND1 proteins. Several proteins of Ad5 and Ad2+ND1 can be distinguished by their differing mobilities on SDS-polyacrylamide gels. In collaboration with Carl Anderson, we have compared the proteins synthesized by recombinants and the parental Ad5 and Ad2+ND1 viruses. By analyzing the polypeptides expressed by recombinants of known genetic constitution, we have been able to locate the structural genes for several proteins on the physical map and to assign *ts* mutants to specific genes. The positions of *ts* mutations relative to the map positions of the genes for hexon, 100K protein, fiber, the ND1 protein and the adenovirus-specific single-strand DNA binding protein have been determined. The

recombinants have also been analyzed, in collaboration with Vivien Mautner, with antisera specific for Ad2 and Ad5 hexon and Ad2 and Ad5 fiber. The results of this analysis give the same locations for the hexon and fiber genes as those derived from the analysis of recombinants for the serotype-specific electrophoretic mobility of the hexon and fiber polypeptides that they synthesize. The map positions derived from this study are in agreement with the map locations derived from studies in Ray Gesteland's laboratory using in vitro translation of adenovirus mRNA selected by hybridization to Ad2 restriction fragments.

Virus-associated RNAs

In addition to virus messenger RNA, cells lytically infected with adenoviruses produce a low molecular weight RNA, originally termed virus-associated RNA (VA RNA). Work in several laboratories, including this one, has shown that VA RNA is virus coded and that it is synthesized late in the infectious cycle in extraordinarily large amounts. It exhibits a number of unusual properties and is made more interesting because its function in the cell is presently unknown.

As a beginning in attempting to elucidate its role, we have determined the location of the VA RNA gene on the adenovirus genome. These mapping experiments employed a novel combination of three standard techniques: restriction enzyme cleavage, gel electrophoresis and filter hybridization. The method, developed by Dr. E. M. Southern in Edinburgh and further refined here, should find applications in a number of related areas of research. In the course of the mapping experiments it became clear that the preparations of VA RNA contained two species: a major one, christened VA RNA_I, whose T_1-ribonuclease fingerprint identified it as the other species previously studied by other workers; and a newly discovered minor species, designated VA RNA_{II}, which amounts to only a few percent of the VA RNA preparations and has a characteristic and distinct fingerprint. In their other properties, such as size and cytoplasmic localization, the two species are very similar.

The gene for VA RNA_I surrounds position 30 on the adenovirus genetic map—that is, it lies 30% of the genome length from the left-hand end as the map is conventionally oriented. Fortuitously, the gene is divided in two by cleavage of Ad2 DNA at position 30 with the *Bam* restriction enzyme. The DNA segment lying to the left of this cut codes for the 5′ portion of VA RNA_I, and that on the right encodes the 3′ portion of the RNA. Thus the gene is transcribed from left to right. The gene for VA RNA_{II} has not been mapped with the same degree of precision. It lies within the 700 base pairs, or about 2% of the total genome length, to the right of the VA RNA_I gene.

Infected cells contain several other molecules that appear to be related to VA RNA. At least one of these, much smaller than VA RNA_I itself, gives a fingerprint almost identical to that of VA RNA_I. Another species, separable from VA RNA_I only under special conditions of electrophoresis, has been studied in collaboration with Dr. Ulf Pettersson from Sweden. Like VA RNA_{II}, it maps within the 700 base pairs to the right of VA RNA_I. Its fingerprint shows similarities with that of VA RNA_{II}, but the two RNA species are clearly not identical.

These results show that at least three species of small, cytoplasmic RNAs are generated by a restricted region of the adenovirus genome. This region is in immediate proximity to the 5′ end of a long tract of "late" mRNAs stretching from positions 30–60 on the map. Whether this position is functional or merely coincidental remains to be seen.

Posttranscriptional Processing of Virus-specific RNA

The aim of this research is a detailed study of posttranscriptional control mechanisms in the generation of functional Ad2 or SV40 mRNAs. The occurrence of symmetric transcription of these viral genomes suggests that correct processing of large RNA transcripts may be a very significant part of a productive virus infection. Consequently, we have begun to develop systems capable of detecting in cells enzymes that

cleave or modify RNA. In one type of approach we are isolating large RNA molecules that could serve as templates for such enzymes. In the case of adenoviruses, the obvious choice is the transcript that spans the region of the viral genome coding for the major coat protein, hexon. We are currently engaged in a determination of the exact 5′ and 3′ termini of the message that codes for hexon and of the exact location of its template in the viral genome. Putative precursors of cytoplasmic RNAs that could be used as substrates for in vitro studies of mRNA isolation may then be isolated from the nucleus.

In another method of attack we are mapping on the viral genome the positions that correspond to the sites complementary to the 5′ ends of stable cytoplasmic messengers. For this purpose we are using viral RNA labeled specifically in the "cap," which is known to be added to the 5′ end of messenger RNAs in a posttranscriptional event. By comparing the fine structure of the DNAs from several such regions (using restriction enzymes or direct sequence analysis of specific fragments of DNA), we hope to discover whether there are "leader" sequences that are common to different segments of the viral genome. The existence of such sequences would have important implications for the mechanism by which virus gene expression is controlled.

Transforming Functions of Adenoviruses and SV40

Use of Restriction Enzymes and DNA Infection Techniques to Investigate Size and Location of Transforming Regions of Adenoviruses and SV40

As carried out in this laboratory, the study of restriction enzymes and their modes of action has led to the construction of detailed physical maps of some adenoviruses and SV40. Human adenoviruses and SV40 are known to transform nonpermissive or semipermissive cells in vitro, and the development of the calcium phosphate technique (Graham and Van der Eb, *Virology 54*:536 [1973]) has facilitated the use of the purified DNAs from these viruses as transforming agents. Furthermore, it has been shown by Graham et al. (*Cold Spring Harbor Symp. Quant. Biol. 39*:637 [1974]), and independently in this laboratory, that specific, purified restriction enzyme fragments of adenoviruses 2 and 5 and of SV40 are capable of transforming cells.

In this laboratory we have used primary rat cells and have transformed these with fragments of the adenovirus 2 and 5 genomes ranging in size from 13.5×10^6 daltons (Ad5, R1 A) to 2×10^6 daltons (Ad2, *Hin*dIII F). All the restriction enzyme fragments capable of transformation were found in the left-hand end (7–8%) of the genome. No fragment lacking this region was capable of producing a transformation event. It would seem, therefore, that the genes responsible for adenovirus 2 and 5 transformation lie in the left-hand 8% of the genome (2×10^6 daltons). This portion also codes for T antigen, which is found in all the cell lines transformed by fragments. The adenovirus transformation experiments are being extended to type 12, which is currently being mapped with restriction enzymes. It is known that 17% of the left-hand end of the DNA of this virus contains the genes necessary for establishment and maintenance of transformation.

Primary rat cells have also been transformed with SV40 DNA fragments, and the transforming region of the genome has been located between 0.15 (*Bam*I site) and 0.72 (*Hpa*II and *Hha*I sites) map units. This region is approximately the same size as the adenovirus transforming segment and contains the SV40 early genes, including T antigen. Smaller fragments containing only part of the early region are nontransforming.

These studies are currently being extended to the transformation of permissive cells by DNA fragments (e.g., primary monkey cells have been transformed with SV40 fragments) in the hope of producing genetically useful cell lines. All established cell lines are being analyzed for their viral DNA content, and a set of lines containing well-defined, specific viral DNA sequences will shortly be available for both genetic and cytogenetic experiments.

Naturally occurring defective DNAs and DNA and fragments from mutant virus stocks are also being investigated with the view to defining not only the size, but also the functions, of the transforming regions.

Cataloging Viral DNA Sequences in Transformed Cells

As in previous years, a large effort has been made to analyze the viral DNA sequences present in transformed cells. In collaboration with Dr. A. J. Van der Eb, we have examined a large number of cell lines that have been transformed by specific segments of adenovirus or SV40 DNA. Reassuringly, all of these cell lines contain no DNA sequences from any portion of the viral genome other than that to which they were exposed. Interestingly enough, and by contrast to transformed cell lines established after exposure to adenovirus, the cells transformed by fragments of viral DNA contain few copies of viral sequences.

We had previously found that mouse cells transformed by SV40 can contain quite complicated sets of viral sequences. Recently, however, we have found that many of the lines of SV40-transformed rat cells that were developed by Rex Risser during his years at Cold Spring Harbor contain single copies (or close to it) of all regions of the viral DNA.

Thus we now have available sets of adenovirus- and SV40-transformed cells, which, since they contain simple sets of viral sequences, should be useful material for determining the arrangement and orientation of the integrated viral DNA.

The State of Viral Genes in Transformed Cells

We are using two approaches to determine the arrangement and orientation of virus DNA sequences in adenovirus- and SV40-transformed cells. First we are measuring the distribution of viral sequences among the different sizes of fragments obtained by cleavage of transformed cell DNA by restriction enzymes. We have again used the procedure developed by Dr. E. M. Southern: in our experiments, high molecular weight transformed cell DNA is cleaved with various restriction endonucleases, fractionated by electrophoresis through agarose gels, denatured in situ, and transferred directly to a cellulose nitrate filter for hybridization to virus-specific nucleic acid labeled to high specific activity in vitro. The method is capable of detecting the presence of as little as 0.1 copies of SV40 DNA per diploid quantity of cell DNA. We have found that before cleavage of several SV40-transformed rat cell DNAs with restriction enzymes, viral DNA sequences comigrate with high molecular weight cell DNA; after treatment with restriction endonucleases, such as *Bal*I, that cleave cellular DNA to small fragments but do not attack SV40 DNA, the viral sequences migrate as one band with a molecular weight greater than unit-length viral DNA. The simplest interpretation of this result is that the SV40 DNA is integrated at only one specific site in each of the cell lines. Further analysis with other restriction enzymes is in progress.

In a parallel approach, a method is being devised for obtaining large quantities of integrated viral sequences contiguous with their host-cell attachment regions. The method involves forming recombinant molecules by mixing restriction endonuclease-treated, transformed cell DNA with the DNA of a vector, in this case lambda, which has been treated with the same restriction endonuclease and using polynucleotide ligase to join the free ends, following the procedures described by Morrow et al. (*Proc. Nat. Acad. Sci. 71*:1743 [1974]). The recombinant molecules can be amplified by infection and multiplication in *E. coli*. The recombinant molecules containing the viral sequences can be selected by in situ hybridization of the phage plaques to a highly radioactive viral DNA or RNA.

There are many questions crucial to the understanding of maintenance of the transformed state that can be investigated using the isolated material. Among them are: (a) Are all the viral sequences in tandem array? (b) What are the relative orientations of the viral DNA fragment? (c) Is there a unique host-cell attachment

site? And (d) what is the relationship between the organization of viral genes and their patterns of transcription in transformed cells?

The recognition of these recombinant molecules as potential biohazards has not been overlooked. To ensure the safe handling of all materials, both biological and physical containments are being used. The main virtue in using λ DNA as a vector is that it can be easily manipulated to provide a safe vector, one that is sufficiently enfeebled as to be able only to exist in laboratory conditions.

A physical containment facility conforming to the standards set by NCI and NIH for a "moderate risk" facility is being constructed in James Laboratory. The safeguards include negative air pressure, sterilization of outflow air, laminar flow hoods, UV lights, autoclaving of material before washing or disposal, and air-tight centrifuges.

Development of Cell Lines Containing Viral DNA with Known Flanking Sequences

For several reasons it would be highly desirable to have available cell lines containing viral DNA that is linked to a sequence whose restriction pattern is thoroughly elucidated. We are using several methods to isolate such cell lines. In all cases we are using as the transforming agent SV40 DNA, or segments of it, that has been linked to other DNA sequences. For example, there is available a population of defective Ad2–SV40 hybrids (Ad2–HEY) which contain different amounts of SV40 inserted into specific sites on adenovirus DNA. The structures of these hybrids have been precisely determined by Kelly et al. (*J. Mol. Biol. 89*:113 [1974]), and the virus stock has been shown to consist of three specific types of SV40–adenovirus hybrids as well as nondefective adenovirus 2 and free SV40. As a first step in the experiment we have developed ways to separate all of these viruses or their DNAs. For separation of the viruses we have used density gradients containing sodium iodothalamate. In this system it is possible to separate easily the hybrid viruses from nondefective adenovirus 2 and SV40. The DNAs of the hybrid viruses can then be extracted and separated by conventional agarose gel electrophoresis. DNA of each of the three hybrid species has been used to transform rat kidney cells. The viral DNA sequences in 15 separate clones is currently being analyzed.

Sequence Organization of Chromosomal DNA

Satellite DNA sequences are ubiquitous in the genomes of eukaryote organisms and are loosely defined as those sequences that are tandem arrays of simple, highly repetitive DNA. Until very recently, satellite DNAs were thought to be polymers of short units (roughly 5–30 nucleotides long) with little constraint upon either their sequence or long-range organization. Last year we reported that the bovine genome contained a satellite DNA (satellite I) composed of tandem arrays of a sequence whose repeat length is 1400 base pairs long. The repeat unit itself is composed of smaller internal repeats. Because this DNA represents 7% of the total bovine genome and in situ hybridization indicates that this DNA is present on all of the autosomal chromosomes, we estimate that each chromosome contains 2–3000 copies of the 1400 base pair repeat. This hierarchical system of sequence organization seems to be a general pattern for satellite DNA, since this scheme has been found in *Drosophila*, mouse and monkey satellites.

As we described last year, it was by analyzing the products of the digestion of satellite DNA with restriction endonucleases that the higher order repeat unit was detected. However, the fragments generated were too long for additional sequence analysis. A further physical characterization of the bovine satellite I DNA has been pursued in collaboration with David Goldberg, a participant in our undergraduate research program, and Tom Maniatis. The restriction enzyme *Hae*III cleaves the 1400 base pair repeat into 14 fragments of discrete molecular weights, many of which are small enough to sequence directly. The pyrimidine tracts from several of these fragments appear to be almost identical to each other, with the tract T_3C_7 being present in greater than molar yields in most; on the other hand, other fragments seem to have

pyrimidine tract fingerprints that are unique. Another finding that may be related to the special organization within the satellite repeat is the uneven distribution of G-C pairs within the restriction fragments.

From cross-hybridization experiments it appears that other repetitive DNA sequences within the bovine genome are related. When total bovine DNA is treated with the enzyme *Eco*RI and the fragments separated by gel electrophoresis, five discrete bands can be recognized within the heterogeneous population of size classes. One of these bands corresponds to the satellite I DNA repeat (see Cold Spring Harbor Laboratory's Annual Report 1974). When the total *Eco*RI-cleaved DNA is transferred to nitrocellulose and hybridized with radioactive DNA from each one of the 14 *Hae*III fragments of satellite I, all of the bands seen by the ethidium fluorescence are visible to variable extents in the autoradiograph of the nitrocellulose. Whether or not this cross hybridization is merely a result of a common origin for these repetitive sequences or reflects some structural or functional requirement for satellite DNA remains to be elucidated.

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The problem we have posed for ourselves is: By what mechanism does the cancer cell grow where normal cells will not? Our approach, outlined in the 1974 Annual Report, has been to isolate transformed and revertant clonal lines from SV40-infected normal rat cells and 3T3 mouse cells and by characterizing them for their ability to grow in a large number of restrictive conditions to reveal the hierarchical interaction of these different restrictions.

Recently, faced with the fact that the many cell lines used differed from normal and from each other in too many ways for all differences to be of equal significance, we have begun to determine which differences are most closely related to tumorigenicity.

Our results to date suggest that there is a syndrome of in vitro changes tightly associated with in vivo tumorigenicity. The changes form a syndrome, in that they are themselves tightly linked. The syndrome is (1) secretion of activator(s) of the serum protease plasmin, (2) growth from single cells into spherical colonies despite the absence of a substrate wettable by cell membrane, and (3) loss of intracellular organization of actin as thick cables. Within the syndrome we have found that both proteolytic enzymes and tumor viruses can profoundly disrupt the internal actin-dependent architecture of mammalian cells, and that the disruptions are quite similar.

We may now expect genetics and biochemistry to be applied to a specific problem in viral oncology: How do the viral gene products responsible for maintenance of the transformed state, such as the A gene product of SV40, eventually cause the intracellular collapse of actin-containing structures which we mimic with serum proteases?

In this year of transition from Cold Spring Harbor to Stony Brook, we have benefitted as before from collaborations with friends at other institutions, in particular Dan Rifkin at The Rockefeller University and Seung-Il Shin and Vicki Freedman at Albert Einstein College of Medicine.

Tumorigenicity and the Anchorage Requirement

A series of random clones isolated nonselectively from SV40-infected mouse 3T3 cells has previously been shown to express a spectrum of transformed phenotypes. These include insensitivity to low serum, loss of contact inhibition, and the ability to grow in the absence of anchorage. Clones representative of each transformed phenotype were injected into immune-deficient *nude* mice to identify the virus-induced cellular growth property best correlated with tumor formation. The results indicate that the single in vitro correlate of tumorigenicity in *nude* mice is anchorage-independent growth, as measured by colony-forming ability in methylcellulose.

Tumorigenicity of SV40-transformed cells is not correlated with either the loss of serum sensitivity or resistance to density-dependent growth inhibition. It was also found that a single step selection in vitro for cells that form clones in methylcellulose from nontumorigenic cells results in the selection of highly tumorigenic cells. Conversely, a single step selection in vivo for tumorigenic cells from an anchorage-dependent mouse 3T3 clone results in the rescue of anchorage-independent cells. An analogous study with a set of rat embryo cells transformed with SV40, as well as with mouse 3T3 cells transformed with murine sarcoma virus (Kirsten strain), yielded essentially identical results.

In addition, transformed cells can be selected directly by injecting primary rat embryo cells, newly infected with SV40, into *nude* mice. This observation suggests that by using *nude* mice it should be possible to select transformed descendants from animal cells that are difficult to grow in culture.

Transformation of Rat Embryo Cells

The transformation of primary rat cells into established cell lines by simian virus 40 has been monitored using the different restrictive assays of colony formation in sparse

culture, dense colony or focus formation on a confluent cell sheet, and colony formation in semisolid medium. Primary embryonic rat cell cultures are considerably less susceptible to infection and subsequent transformation than the established mouse 3T3 cell line or later in vitro passages of rat cells. These embryonic cells show a stage-specific susceptibility to transformation but not to infection, with a maximum susceptibility achieved at the 15th to 16th days of gestation. Transformed cell lines selected as isolated colonies or foci following SV40 infection of primary rat cells uniformly synthesize viral T-antigen, whereas they differ greatly in their plating efficiency in semisolid medium containing methylcellulose. Only the assay of colony formation in semisolid medium selects directly for transformants that plate well in that medium and have a high level of plasminogen activator production.

Intracellular Distribution of Actin

We have used an antibody to actin to examine the pattern of distribution of actin within intact, anchorage-dependent and anchorage-transformed cells. With this technique, thick cables are resolved, but structures thinner than 0.1 μ are indistinguishable from isotropic matrix. Immunofluorescence studies of normal cells showed that anchorage-dependent cells were full of large (>0.1 μ diameter) cables. Often these cables extended for many microns, or even the length of the cell. A diffuse fluorescence also filled these cells, indicating the location of the diffuse matrix.

However, anchorage-independent cells were unable to form cables. Lines that were transformed by other criteria, while remaining anchorage-dependent, retained the ability to form large, actin-containing cables. A viral gene must in some way play a role in maintaining this anchorage-related change in intracellular actin distribution, since an active SV40 gene A product is required to maintain the cable-free state. The same gene product may also be required to maintain growth without anchorage (Brugge and Butel, *J. Virol. 15*:619 [1975]).

Plasmin and the Intracellular Distribution of Actin

Treatment with plasmin, but not urokinase or plasminogen, reversibly removes the actin-containing cables from normal rat embryo fibroblasts, and similar results are obtained with trypsin. Thrombin and chymotrypsin are relatively ineffective in causing the disappearance of the cables.

Sera such as dog or monkey that permit high levels of plasmin formation and activity support cell growth in semisolid media better than sera in which plasminogen is activated poorly or that are plasminogen deficient; concomitantly, cables appear in the former but not in the latter sera. The addition of a plasmin inhibitor prevents the disappearance of actin-containing cables.

Teratocarcinoma Studies

Our work on primary cultures of teratocarcinomas has centered around the phenomenon of cellular attachment. Using the appearance of the "differentiated" enzyme creatine kinase as an assay, we find that cells plating on a denatured collagen substratum differentiate far more quickly than those plated on bare glass or plastic. Moreover, the denatured collagen is rapidly digested in the area directly underlying the primitive tumor but in all cases remains intact under well-spread and differentiated cells. This proteolytic effect is in agreement with recent results of D. Rifkin and A. Levine showing that the primitive tumor exhibits high levels of plasminogen activation that decrease nearly exponentially with time in culture. Undenatured collagen substrates are not digested, again indicating a plasmin-related effect.

Carrying this further, we have looked into the effect of substances known to modulate collagen biosynthesis and secretion. Preliminary results indicate that ascorbic acid added daily to the medium greatly increases the rate of attachment and morphologically alters the pattern of differentiation as well. In addition, hydroxy-

proline analogs or molecules that chelate iron (a necessary cofactor in collagen secretion) strictly prevent cellular attachment.

In the absence of anchorage, the teratocarcinoma cell grows in vivo as an ascitic suspension of embryoid bodies. These consist of a single endodermal layer surrounding a core of undifferentiated embryonal cells. We have cultured these embryoid bodies in the absence of anchorage in vitro and have found their course of development to be dependent upon serum species in a manner strikingly reminiscent of the serum-species dependence of plasmin activity and of intracellular actin distribution seen in SV40-transformed rat embryo cell lines. In particular, the endodermal layer, which is a necessary precursor to later differentiation, disappears in cultures of embryoid bodies maintained in dog serum.

Microtubule Visualization

Cytoplasmic microtubules in tissue culture cells can be directly visualized by immunofluorescence microscopy. Antibody against tubulin from the outer doublets of sea urchin sperm flagella decorates a network of fine cytoplasmic fibers in a variety of cell lines of human, monkey, rat, mouse and chicken origin. These fibers are separate, of uniform thickness, and are seen throughout the cytoplasm. The fibers disappear either in a medium containing colchicine or after subjection of the cells to low temperature. The same treatments do not destroy the microfilamentous structures visualized by means of antibody against actin.

Enucleated cells

BHK21 cells enucleated with cytochalasin B were trypsinized and replated. Subsequent spreading and shape formation were monitored with light and transmission electron microscopy. Assembly and changes in distribution of microtubules, microfilaments and 100-Å filaments were related to these processes. These results indicate that cytoplasm contains the information necessary for the normal assembly and distribution of cytoplasmic fibers.

Identification of Biological Molecules by Intrinsic Fluorescence in an SEM Electron Beam

Proteins, nucleic acids and fluorescein-conjugated antibody are shown to be identifiable in situ via the fluorescence excited by the focused electron beam of a scanning electron microscope. A molecular species is identified by its characteristic fluorescence spectrum and by a characteristic alteration of the spectrum with time under the electron beam. Primary protein fluorescence is relatively rapidly destroyed by the beam, but protein photoproduct fluorescence is more rugged and will in some cases permit detection of small numbers of protein molecules. Nucleic acid fluorescence is extremely long lived and will permit detection of small numbers of nucleic acid residues. The theoretical resolution limit for localization of a particular molecular species—about 20 Å—is determined by the known maximum distance for molecular excitation by fast electrons. Direct extrapolation from an observed resolution of 900 Å in the localization of nucleic acid using a low-efficiency detector leads to an experimental resolution limit of less than 60 Å. Fluorescence is strongly quenched by residual water in the specimen. Similar quenching is produced by some macromolecular associations and so may serve to localize such associations.

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Cell-free Translation of Adeno and SV40 Virus mRNAs

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Infection of rodent cells with human adenoviruses can lead to transformation of certain cells to growth properties more characteristic of tumor cells than normal cells. The work of Sambrook et al. (*Cold Spring Harbor Symp. Quant. Biol. 39*:615 [1974]) and Graham et al. (*Cold Spring Harbor Symp. Quant. Biol. 39*:637 [1974]) has demonstrated that only a small fragment of viral DNA, toward the left end of the genome, is required for this transformation, implying that only a very small number of Ad gene products are involved. We have identified the Ad2-coded polypeptides most likely to be involved in this process by exploiting the technique of cell-free translation of mRNAs that have been specifically selected by hybridization to DNA.

As we reported last year, if a specific fragment of viral DNA is used to select mRNA for translation in vitro, the polypeptide products of the translation can be identified as the gene product of that segment of DNA. In this way we have mapped the order of the genes for the Ad2 late polypeptides to be, from left to right: (IVa_2, IX), IIIa, (III, V), P-VII, (II, P-VI), (100K, P-VIII), IV. Further experiments to make this map of the late products more precise are currently in progress. This group of late polypeptides includes the structural components of the virion as well as several nonvirion polypeptides that are synthesized in infected cells.

However, the gene product(s) involved in transformation is known to be that synthesized early in the infectious cycle (Sharp et al., *Cold Spring Harbor Symp. Quant. Biol. 39*:457 [1974]). Thus to identify the early polypeptides, we selected Ad2-specific mRNA from early in infection and translated this RNA. Analysis of the low levels of adeno-specific RNA present early in infection was made possible by the addition of polyamines to the cell-free system, which greatly increases the efficiency of translation. The early mRNAs were mapped by selection with fragments of Ad2 DNA to give the following order: 44K, 50K (0–4.1 pmu Ad2 DNA), 15K (4.1–16.7 pmu), 72K (58.5–70.7 pmu), 15.5K (75.9–83.4 pmu), 19K, 11K (89.7–98.6 pmu). We suspect that 44K and 50K are related polypeptide sequences. Thus 44K (50K) and 15K are the only early polypeptides whose genes are mapped on the region of the genome known to be required for transformation. Furthermore, mRNAs for 15K, and to a lesser extent 44K (50K), are present in three lines of transformed cells we examined. We plan further studies of these two polypeptides to find where they are located in the infected cell and to elaborate various of their biochemical properties in the hope that this information will provide insight into how tumor viruses transform the cells they infect.

Another application of this hybridization-selection, cell-free translation technique is afforded by the adeno-SV40 hybrid viruses Ad2$^+$ND1 and Ad2$^+$ND4 (the latter in collaboration with C. W. Anderson and A. Lewis). In each of these hybrids, a fraction of the SV40 early region is inserted into the Ad2 genome, conferring upon the hybrid the ability to grow on monkey cells. Translation of SV40-specific mRNA from cells infected with each of these viruses identifies the polypeptide gained by the insertion of SV40 genetic information. These polypeptides are 30,000 MW for Ad2$^+$ND1 and 92,000 MW for Ad2$^+$ND4. The latter is very similar in electrophoretic mobility to the translation product of an SV40-specific mRNA found in SV40-infected monkey cells and most likely corresponds to the product of the SV40 early region.

A fruitful extension of this work has been to examine the host-range mutants of Ad2$^+$ND1 isolated by T. Grodzicker. Translation of SV40-specific mRNA isolated from cells infected with these mutants yields in the cases of mutants H39, (H140 and H162) and H71 polypeptide products of 29,000, 19,000 and 10,000 MW, respectively. Another mutant, H124, yields a 30,000 MW product indistinguishable from wild-type product on SDS-polyacrylamide gels. The nature of these mutants is under further investigation (see below) to determine if these mutant polypeptides might be fragments resulting from translation termination mutations.

We have collaborated with B. Rosenwirth and A. Levine in comparing DNA

binding proteins found in adenovirus-infected cells. Tryptic peptide analyses of 50K and 72K proteins in Ad2-infected cells show that 50K is related to 72K, probably by breakdown. The 72K peptide pattern is essentially identical to that of the 72K protein made in cell-free extracts by translation of adenovirus mRNA.

Abortive Infection of Monkey Cells by Ad2

We are studying Ad2 infection of monkey cells to determine the block to its multiplication. Several late Ad2 proteins show varying degrees of reduction in their level of synthesis in Ad2 infections of monkey cells, in comparison to the normal lytic infection of human cells or the Ad2 plus SV40-enhanced infection of monkey cells. We find a similar specific reduction in the synthesis of these proteins when their messages are assayed by cell-free translation (in contrast to results of Eron, Westphal and Khoury, *J. Virol. 15*:1256 [1975]). This finding suggests that the block to Ad2 protein synthesis is at the RNA level rather than in the translational machinery of monkey cells. Analysis of the complexity and concentration of Ad2-specific RNAs, using hybridization of restriction endonuclease fragments of the Ad2 genome to increasing concentrations of RNA, shows that although all species of late Ad2 mRNA are present, the concentration of several species is reduced sevenfold or more in Ad2-infected monkey cells as compared to the enhanced infection of monkey cells with Ad2 plus SV40. These species come from regions of the genome known to encode the deficient proteins.

We are currently trying to investigate whether the specific mRNA deficiencies are a primary result of transcription rates or a secondary effect of transport or translation defects.

Cell-free Synthesis of β-Galactosidase

We have observed that *lac* Z^- amber mutations are suppressed very efficiently (in an extract containing no suppressor tRNA) simply by lowering the incubation temperature from 37°C to 25°C. A *lac* Z^- ochre mutation and an amber mutation in the synthetase gene of the RNA phage Qβ were not suppressed by this technique. This temperature-dependent suppression, as well as normal Su^+ suppression, and termination are intimately linked with the concentration of release factors in the reaction. This was determined by experiments in which either purified release factors (kindly provided by Dr. T. Caskey) or purified antibodies against these factors were added to the in vitro protein synthesis reaction. Addition of the former increased termination, whereas addition of the latter increased suppression. It was also observed that when the protein synthesis reaction was performed at 25°C, reinitiation polypeptides were detected in good yield. This is most likely due to the fact that these polypeptides are insoluble at 37°C but soluble at 25°C. Only soluble polypeptides can be detected by the method used to analyze the reaction product. Whether some degradation of these polypeptides occurs in vitro or whether their in vitro insolubility is related to their observed in vivo instability is not known. However, the fact that the decay of reinitiation polypeptides in vitro follows the same kinetics as in vivo indicates that the two processes may be related.

Yeast Nonsense Suppressors

Three geneticists, John Roth, Gerry Fink and David Botstein, arranged to spend concurrent sabbatical years at Cold Spring Harbor so that they could work together on yeast genetic problems. Their presence and that of Mariana Wolfner provided a strong stimulus for attempting to characterize yeast nonsense suppressors by means of cell-free protein synthesis techniques available in this laboratory. We have collaborated closely with them in developing a system for this purpose.

It had been our experience, as well as others', that extracts from yeast could not yet be induced to synthesize proteins on addition of mRNA. However, we found that if

mammalian ribosomal subunits (mouse ascites) and ribosome initiation factors (rabbit reticulocytes) are added, then protein synthesis can be stimulated by a variety of mRNAs, including yeast cytoplasmic RNA, adenovirus mRNA, globin mRNA and Qβ bacteriophage RNA. When Qβ RNA is used as template, synthesis of the Qβ synthetase protein of MW 66,000 is readily detected, but when RNA from a synthetase amber mutant (*am1* from K. Horiuchi) is used, this 66,000 protein is not made, and a fragment of MW 55,000 is found. Synthesis of this fragment is presumably terminated by the UAG amber codon. Hence we can investigate whether amber suppression can be detected in the cell-free system by looking for elongation of the fragment into a full-length molecule when the yeast crude extract is made from strains carrying amber suppressor mutations.

Extracts from a strain carrying the tyrosine-inserting amber suppressor *sup*6-2 do suppress the Qβ amber mutation in vitro, with a maximum efficiency of about 16%. The recessive lethal amber suppressor RL1 found by Brandriss, Sofi and Botstein (*Genetics 79*:551 [1973]) also suppresses the Qβ amber mutant, and a maximum efficiency of 50% is seen when we use extracts from the aneuploid strain that is haploid except for chromosome III, which specifies the suppressor.

If extracts from a UAA (ochre)-suppressing yeast strain are used to translate Qβ RNA, a read-through protein from the synthetase gene can be seen, implying that UAA is the normal termination codon for this gene. The tyrosine-inserting ochre suppressor *sup4-1* gives up to 65% suppression in this in vitro assay. The ochre suppressors have no effect on amber termination, and vice versa, as expected from the genetic analyses of these suppressors.

In each of the above cases we have shown that the suppression activity is in the tRNA fraction of the yeast extracts by adding purified total tRNA from the suppressor strains to an otherwise nonsuppressing cell-free system. These tRNAs will also give suppression when added to an otherwise mammalian cell-free system, but the observed levels are considerably lower.

We are using this suppressor assay for the following purposes: (1) to purify suppressing tRNA species, in the hope of demonstrating that the suppressor mutation is in the primary structure of the tRNA; (2) to study temperature-sensitive suppressors from yeast; and (3) to try to identify nonsense mutants in specific mammalian cell genes. Dr. David Secher, from the MRC in Cambridge, has collaborated with us to show that one of the heavy chain immunoglobin mutants that makes a fragment of the heavy chain is apparently a frameshift mutation, since ochre suppression of cell-free translation of the mutant mRNA results in synthesis of some longer, but not full-length, product.

Yeast Histidinol Dehydrogenase

The genetics of the *his4* region has been extensively studied by G. Fink and colleagues. However, the nature of the gene products produced by the *hisA,B,C* gene cluster remains to be clearly demonstrated. Efforts to purify histidinol dehydrogenase, an enzyme produced by the *his4C* gene, have employed salt-mediated hydrophobic chromatography and electrophoretic techniques. The process of conversion of the dehydrogenase to a second active form, differing in various properties from the original after DEAE-cellulose chromatography, remains to be clarified. Recent experiments with the affinity matrix N^6-AMP Sepharose 4B have met with a great deal of success. The purified protein preparations now available should yield antibody to histidinol dehydrogenase. Such antibody preparations should be useful in detecting the in vitro synthesized enzyme. Indeed, mRNA from wild-type yeast and yeast derepressed for the histidine biosynthetic enzymes is a good template for cell-free protein synthesis, such that protein products up to 150,000 daltons MW can be detected. The purification of the gene products of the genetically defined *his4* cluster, in conjunction with available techniques for the synthesis of proteins in vitro, should be a step toward elucidating the basic regulatory processes in eukaryotic organisms.

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YEAST GENETICS

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While on sabbatical leave from our respective laboratories (D. Botstein, Massachusetts Institute of Technology; G. Fink, Cornell University; J. Roth, University of California at Berkeley) we spent this past year at Cold Spring Harbor working on a number of problems concerning yeast.

Suppression

In collaboration with Ray Gesteland, from whose earlier work the suppressor project stemmed, an in vitro protein-synthesizing system was developed that uses yeast tRNAs and activating enzymes. This makes it possible to demonstrate in vitro suppression of amber and ochre mutations in any eukaryotic messenger RNA whose information can be expressed in vitro. No suppressors of the UGA codon were available, and we set out to make these suppressors so as to complete the set of three termination signals (UGA, UAA, UAG) which could be assayed in vitro. Likely, but unproven, UGA mutants of the *his4* cluster of yeast were used initially. This mutant looked promising as a UGA since it (1) is strongly polar, (2) is not UAG or UAA, and (3) is induced to revert by mutagens that cause base substitutions. Two types of suppressors were demonstrated, recessive suppressors and haplo-lethal suppressors. These suppressors looked promising, but attempts to interconvert the original mutation with UAA, which should have worked, were unsuccessful. Similarly, attempts to interconvert the new suppressors and ochre suppressor did not succeed. These results suggested that the new suppressors are not the UGA suppressors sought but rather are either missense or frameshift suppressors. This possibility is interesting and will be pursued in the future, but a second approach was required for obtaining the desired UGA suppressors. This involved constructing an "omnipotent suppressor," a new type of suppressor, described by D. C. Hawthorne (pers. comm.), that weakly suppresses

amber, ochre and UGA mutations. The entire collection of polar *his4* mutants was screened using this suppressor, and all polar mutations not of the amber or ochre type are being screened for suppressibility by the omnipotent suppressor. This should permit identification of UGA mutants. At present several promising candidates are on hand, and the study of these is being actively pursued.

A search for temperature-sensitive nonsense mutants was carried out in a general way that we hoped would demonstrate the feasibility of genetic analysis of a variety of essential proteins. The method involves using a temperature-sensitive amber suppressor isolated by G. Fink. This suppressor corrects amber mutations at 25°C but not at 37°C. In strains carrying this suppressor we looked for temperature-sensitive lethal mutations which were amber mutants that owed their temperature sensitivity to the suppressor. Several hundred temperature-sensitive mutants were isolated in this background. These are now being screened for dependence on the suppressor. Ultimately, this approach should be valuable for work on actin and for analysis of a variety of other structural proteins in yeast. Use of such ambers is valuable because only a fragment of the protein is made at the restrictive temperature. This may make it possible to use SDS gel electrophoresis to screen for mutations affecting essential proteins.

Phage Morphogenesis

Using temperature shifts and double mutants carrying both cold-sensitive and temperature-sensitive mutations, D. Botstein has developed a genetic technique which makes it possible to determine the sequence of action of various genes involved in phage morphogenesis. In theory, this technique should be applicable to the cell-cycle mutants of yeast. Before this can be tried, the avilable cell-cycle mutants must be genetically removed from the heavily mutagenized backgrounds in which they are isolated. This was done for a series of 30 cell-cycle mutations obtained from Lee Hartwell. During the year, we ran these strains through two successive back crosses with unmutagenized parents. The resulting collection of "cleaned" cell-cycle mutants should be suitable for use in the functional sequencing of cell-cycle steps.

Actinlike Proteins

Actin is a protein involved in muscle contraction, yet it has now been found in a variety of eukaryotic cell types. Its function in non-muscle cells is not understood. Elias Lazarides and Uno Lindberg (*Proc. Nat. Acad. Sci. 71*:4742 [1974]) have demonstrated that a tight protein complex is formed between actin from mammalian cells and DNase I from bovine pancreas. This surprising finding permitted them to make an affinity column that selectively absorbs actin from crude extracts of cultured cells using commercially available DNase I.

Using such a column we were able to demonstrate that it specifically removed one protein from an extract of yeast cells. Since this protein has the molecular weight characteristic of actin, the column thus provides an assay and purification method for actin. Two attempts at generating antibodies to this protein were unsuccessful, but the biochemical work will be continued. Ultimately, conditional-lethal mutations affecting this protein will be sought, which should then permit its genetic analysis.

MOLECULAR GENETICS

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Some of the problems we have been working on are the following: the genetic basis of specific protein degradation; fine-structure mapping of the λ attachment site with restriction enzymes; restriction enzyme mapping of phage Mu DNA; the analysis of insertion sequences in phage Mu; and the characterization of a mutation in phage Mu which increases its ability to integrate into the host chromosome. We are also continuing our efforts to develop an in vitro system for Mu integration.

In addition to our regular staff, we were fortunate in having A. L. Taylor from Colorado working in our laboratory for several months this year.

The Genetic Basis of Specific Protein Degradation

When a nonsense mutation occurs in the structural gene for β-galactosidase, two kinds of polypeptide chains result. One is the termination or T fragment, extending from the normal N-terminal end to the site of the nonsense mutation; the other is the reinitiation or R fragment, initiating operator distal to the site of the nonsense mutation and extending to the normal C-terminal end. Both the T and the R fragment are highly unstable and rapidly degraded, contrasting strongly with the wild-type β-galactosidase, which is completely stable. The instability of these mutant polypeptides implies the existence of proteolytic systems that can specifically distinguish aberrant proteins from the normal enzymes of the cell. One of the approaches our laboratory has taken to the study of degradation systems has been to isolate mutants that lead to the stabilization of T and R fragments. The first such mutation was isolated by A. Bukhari. It is called *deg*-T and stabilizes all T fragments. This year we have confirmed that *deg*-T mutations do not stabilize R fragments. There must be another system responsible for the degradation of R fragments. This was confirmed by modifying the selection procedure originally used for *deg*-T in such a way as to bias it toward the selection of mutations that stabilize R fragments. The resulting mutation, which stabilizes R fragments against degradation but has no effect on *deg*-T fragments, has been characterized further. We call it *deg*-R. We have continued our studies of *deg*-R and *deg*-T in an attempt to determine the mechanism of their specificity and action, but so far we have been completely unable to obtain good experimental evidence for any of our theories. In particular, T fragments, although extremely unstable in vivo, are stable as soon as the cell is opened and are stable when made in vitro. Thus we are unable to carry out the degradation of T fragments outside the cell. The situation with R fragments may be similar, but it has not yet been completely characterized.

Fine-structure Mapping of the λ Attachment Site with DNA Restriction Endonucleases

A detailed restriction enzyme mapping of the λ attachment site is in progress. This work is a necessary prerequisite to the sequencing of the attachment site. At present, about 26 restriction enzymes have been used. These have yielded 89 pieces of DNA from a large fragment, about 5000 bases long, that is known by genetic criteria to contain the λ attachment site. These pieces range in size from about 10 to about 300 nucleotides and should serve for the beginnings of our sequencing project.

Restriction Enzyme Mapping of Phage Mu DNA

We are continuing our extensive project to map phage Mu DNA by restriction enzymes. Many features of Mu DNA are beginning to emerge from this study. One of the findings of interest is that the C end of Mu–i.e., the end opposite to the split end–has a short region of random bacterial DNA attached to it. Thus phage Mu DNA, as it comes from the virion, has bacterial DNA at both ends. One end has about 1000–2000 nucleotides and the other about 100 nucleotides. Both these bacterial sequences seem to represent a random population of host DNA. As will be seen below, Mu DNA is also attached to bacterial DNA when it is packaged. This means that Mu DNA may be attached to bacterial DNA at both ends during all or most of its life cycle.

Analysis of Insertion Sequences in Mu DNA

There exists in prokaryotes an interesting class of DNA called insertion sequences. These are pieces of DNA that can insert and delete themselves at low but measurable frequencies in many sites on the DNA of bacterial plasmids, chromosomes and phages. This year we have been able to study the effect of these sequences when they insert themselves into phage Mu. In particular, an insertion sequence of about 800 base pairs enters a region of Mu near the C end of the phage, which causes the virus to become completely defective. However, the viral DNA can be rescued by growing wild-type Mu in the strain. When this is done, a mixed population consisting of equal amounts of DNA from the insertion-carrying and the wild-type phage can be isolated. Examination of heteroduplexes between wild-type Mu DNA and Mu DNA with insertion sequences has made it possible to unambiguously map the position of the insertion sequence. The electron microscope studies have confirmed previous work with restriction enzymes. The Mu DNA with the insertion has shorter split ends, that is, less bacterial DNA on the S end but the same amount on the C end. This strongly suggests that Mu is packaged by a mechanism that starts near the C end and packages an amount of DNA about 5% larger than that required for the Mu genome. Mu DNA must be attached to bacterial DNA during this packaging procedure to explain the change in size.

Characterization of a Mutation of Mu That Over Integrates

In the course of a search for a Mu mutation that prevents integration, D. Kamp found one, which he called cII, that actually increases integration. This is demonstrated in an experiment in which Mu phage is integrated on the chromosome of a host carrying a *pro lac* episome. The phage is induced by heat, and before the induced cells have lysed, the episome is rescued by mating to a proline-requiring, Mu-immune recipient strain. By further analysis of the exconjugants it is possible to determine how often a Mu from the original host has integrated into the episome and been transferred out before lysis. When the cII mutation is compared with wild type in this test, it is found on the episome five times more often. cII is also found to lack a cut site of the restriction enzyme *Hin*d III near the C end. It will be interesting to see if this change in restriction enzyme pattern is correlated with the mutant phenotype.

Hybrid Phages

Last year, in collaboration with B. Allet, we described the isolation and analysis of plaque-forming λ-Mu hybrids. These hybrids are turning out to be very useful for identifying Mu genes and their functions, for studying the inversion of the *G* region of Mu DNA, and for developing techniques for in vitro excision of Mu DNA. Furthermore, by growing phage P1 and Mu together we have obtained some strange "P1" particles, which are being characterized.

Mu as a Tool for Genetic Engineering

We have described a rapid method for isolating Mu insertions in transmissible plasmids and episomes. This method, based on the integration of Mu during the lytic cycle, is being used in many laboratories. The λ-Mu hybrids are being used to move *E. coli* genes around and to isolate new λ-transducing particles with interesting bacterial genes.

Polypeptide Splicing?

Attempts to reproduce the genetic results that led us to propose polypeptide splicing two years ago have been completely negative. Many of the heterodiploid *z*⁻ strains which were originally claimed to complement strongly and produce wild-type protomers have been remade and have all been shown not to complement at a significant

level. Correcting these erroneous results has led us to the sad conclusion that splicing almost certainly does not exist in the *lac* operon of *Escherichia coli*.

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ELECTRON MICROSCOPY

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Renovation

Our major effort since arriving at Cold Spring Harbor has been directed toward the design and renovation of both transmission electron microscopy facilities and their accompanying darkrooms—the research facility in Demerec and the general use and teaching facility in Davenport. In Demerec, the usable workbench surface and storage space was doubled with our new floor plan, enabling us to accommodate two additional persons and to perform basic biological and chemical experiments as well as to prepare samples for electron microscopy.

Phage Mu Mutants Containing the Chloramphenicol Resistance Marker

Six different isolates of phage Mu containing the chloramphenicol resistance marker (Mu:Xcam) were isolated and prepared by A. Bukhari. Heteroduplexes were made between these mutant DNAs and wild-type Mu DNA and between combinations of the mutant DNAs themselves. The results are the following:

(1) The chloramphenicol resistance marker in all six mutants is present as a simple insertion of DNA 2800 ± 200 base pairs in length. There is no observable deletion of Mu DNA at the site of *cam* insertion (resolution of EM = 50 bases).

(2) In Mu^+/Mu:Xcam or Mu:Xcam/Mu:Xcam heteroduplexes, the noncomplementary split ends composed of bacterial host DNA in self-renatured wild-type Mu (Mu^+) are seen at extremely low frequency (less than 0.1%). The reverse is also true: whenever there are split ends in the heteroduplexes, there is almost never a *cam* insertion loop present (these are Mu^+/Mu^+). In addition, variable amounts of β-region DNA adjacent to the split ends are also lost in these Mu:Xcam DNAs.

This result is strong evidence for the packaging model proposed by A. L. Taylor and A. I. Bukhari (*Proc. Nat. Acad. Sci: 72*:4399 [1975]). The model states that Mu DNA in a state of covalent linkage to heterogeneous host DNA is packaged by a

headful mechanism from the left or *c* end. The capacity of the phage head is larger than the Mu genome. As a result, some covalently linked bacterial DNA is normally packaged in the phage in wild-type Mu. If there is an insertion of DNA into the Mu genome, the model predicts that the split end will be shortened to accommodate for the gain in the inserted DNA. In the heteroduplexes of Mu$^+$/Mu:X (Mu:X contains an insertion of 720 base pairs on the *c* end), the split ends are indeed somewhat shortened. In L. Chow's electron microscope studies, the loss of variable amounts of β sequences and of DNA split ends entirely in Mu:Xcam DNA is an unequivocal proof of this packaging model.

(3) The β sequences still present in these Mu:Xcam mutant DNAs vary in length from 500–1600 base pairs (29–94% of the total β-region of 1700 base pairs). This is an indication of an imprecise cutting of the DNA on the right end (as well as on the left end). This observation is in agreement with the variable length in the split ends for wild-type Mu DNA. Since the *cam* insertion measures 2800 ± 200 base pairs and the split ends are rarely seen, the maximum capacity of the phage head is probably the complete Mu$^+$ genome plus 2800 ± 200 base pairs.

(4) The *cam* insertion in the six Mu mutants maps at three different locations: 3.3, 3.9 and 4.4 kilobases from the left end. The *cam* insertion is found at one, two or all three locations within any one preparation obtained by induction of Mu:Xcam/Mu cts double lysogens grown from one single clone. However, on any one DNA molecule there is only one *cam* insertion sequence.

(5) In some Mu:Xcam/Mu:Xcam heteroduplexes, two *cam* insertion loops are seen at different locations; there is no indication of pairing between the two single-stranded *cam* loops (Fig. 1). Therefore, at least in some cases, the two *cam* insertions have different orientations at different locations. Since there are three locations but only two orientations, some of the insertions must have the same orientation at different locations.

The *cam* insertion can also be of the same or different orientations at the same location. This is concluded from Mu:Xcam/Mu:Xcam heteroduplexes in which the two *cam* insertions form either a homoduplex region, lengthening the duplex segment between the *c* end and the *G* loop (the two *cam* insertions of the same orientation), or a single-stranded substitution loop (the two *cam* insertions of different orientations). Both Mu:X and Mu:Xcam either do not replicate or do so very inefficiently. These phages are recovered at extremely low frequency after coinfection of sensitive cells or induced lysogens with Mu.

Work is in progress to determine whether there is any sequence homology between the *cam* insertion and the small *x* insertion and between the *cam* insertion and other known insertion sequences in bacteria.

λ-Mu Hybrid Phages Containing the Right End of Prophage Mu DNA

λ-Mu hybrid phages containing fractions of Mu DNA are useful in mapping Mu genes and/or functions. In this study, special interest was focused on λ-Mu phages that contain, within a predominantly λ genome, only small functions of Mu DNA from either the left or the right end of the Mu prophage DNA. Since Mu prophage DNA and mature phage DNA are colinear, the attachment sites of Mu are presumably located at the ends of the prophage DNA or at the ends of mature Mu DNA (as opposed to the heterogeneous host DNA at the physical ends of phage DNA). Therefore, λ-Mu phages with ends of Mu prophage DNA contain the putative attachment sites of Mu. Genetic and physical studies of such phage may provide information on the mechanism of Mu integration.

Many such hybrid phages have been isolated by Regina Kahmann and Dietmar Kamp by induction of a λp*lac* lysogen containing phage Mu integrated in the *lac* operon. Two of them have been studied extensively by the electron microscope-heteroduplex method. λ-Mu1, λ-Mu2 and other λ-Mu's express genetic properties both observed and not observed in either the parental λp*lac* or Mu.

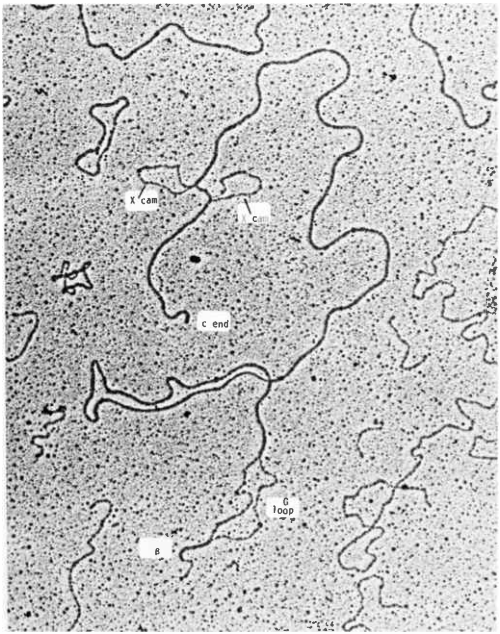

Figure 1
A heteroduplex between Mu:Xcam and Mu:Xcam DNAs. The molecule shows the two *cam* insertions at different locations. The insertions may have the same or different orientations. It also shows a shortened *β* sequence with a complete loss of the split ends. ϕX174 single- and double-strand circles are included as length standards.

Suppression of λ Interference

Upon induction of double lysogens of λcI857 and Mu cIts62, Mu phage is produced at 10^{-5} relative to Mu after induction of a single lysogen. However, induction of double lysogens of Mu and λ-Mu produces Mu phage at 0.2 relative to Mu. The production of λ or λ-Mu is not affected in either case. In comparison, when the double lysogen λ p*lac*:Mu (with Mu integrated in the *lac* operon) is induced, λ is produced at 10^{-5} (relative to λ), whereas Mu is produced at 0.3 (relative to Mu). The result with λ-Mu phages is intermediate to the other cases. A possible explanation for the suppression of interference is that some of the Mu DNA might have integrated into some of the λ-Mu DNA and is transcribed or replicated when λ-Mu is induced.

Expression of Mu gov *Function*

There are two classes of λ-Mu phages—one expresses Mu immunity, and the other expresses the *gov* function limiting the growth of Mu virulent phage, as does the

wild-type Mu lysogen. That is, Mu virulent phage produces turbid plaques on these lysogens. λ-Mu1 and λ-Mu2 belong to the second class and probably contain Mu DNA that harbors the *gov* function.

Killing Effect of λ-Mu2

All λ-Mu hybrid phages except λ-Mu2 can lysogenize sensitive bacteria. λ-Mu2 does not contain a λ *c*-type mutation because it is not complemented during coinfection with wild-type λ. The killing effect is dominant and since the original λ-Mu2 does lysogenize, is acquired after the original isolation. Neither is the killing effect due to a λ mutation, since λ-Mu2 does not kill preestablished λ lysogens.

EM-Heteroduplex Mapping of λ-Mu Phages

Due to the small size of the Mu DNA in the λ-Mu phages, restriction enzyme digestion analyses are not adequate to map the Mu DNA present. Heteroduplexes were therefore made between λ-Mu2/Mu; λ-Mu2/λ*imm*^{434}/Mu; λ-Mu2/λ*plac*, λ-Mu1 or a λ-Mu restriction fragment/Mu; λ-Mu1/λ*imm*^{434}; λ-Mu1/λ-Mu2. The results are the following:

(1) λ-*Mu2*: The only Mu DNA present is the rightmost 1090 base pairs of the Mu β sequence (the right end of Mu lysogen) with, in addition, an insertion of 2600 base pairs of unknown origin at 830 base pairs from the right end of the β sequence (Fig. 2). On EM-heteroduplex examination, the original isolate of nonkiller λ-Mu2 shows no observable difference from the killer λ-Mu2; i.e., the two λ-Mu2's make only homoduplexes. The mutation acquired by the killer λ-Mu2 in the process of induction of a lysogen resulting from the infection of the original nonkiller λ-Mu2 must be small (less than 50–100 bases).

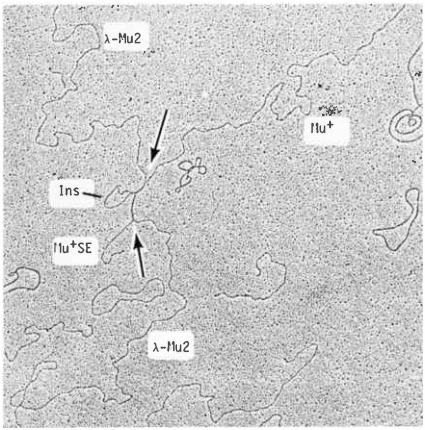

Figure 2

A heteroduplex between Mu+ and λ-Mu2 DNAs. Solid arrowheads mark the two forks of the heteroduplex. The unknown insertion in the β sequence is marked Ins; the split end DNA sequence from the Mu+, Mu+SE. ϕX174 single- and double-strand circles are included as length standards.

(2) λ-*Mu1*: The Mu DNA present is the rightmost 830 base pairs of the Mu β sequence. However, the heteroduplexes of λ-Mu1/λ-Mu2 show that λ-Mu1 contains at least 95%, if not all, of the unknown insertion sequence of 2600 base pairs on the left end of the Mu DNA of 830 base pairs (limit of EM resolution is about 100 base pairs). It does not contain the 260 base pairs of Mu DNA to the left of the insertion as in λ-Mu2.

At the present time, no information is available on the origin of the insertion DNA of 2600 base pairs in both λ-Mu1 and λ-Mu2. It is not known whether the "killing" function of λ-Mu can be attributed to this insertion DNA. On the other hand, the *gov* function of λ-Mu is definitely due to the Mu DNA present, since all λ-Mu's express the *gov* function yet not all of them have the insertion DNA of 2600 base pairs. Therefore the Mu *gov* function is either a protein specified by the rightmost 830 base pairs of the β sequence or a site contained in this segment of DNA, e.g., the putative attachment site which is of Mu prophage in the case of λ-Mu1 and λ-Mu2.

Further EM studies are in progress to examine other λ-Mu hybrid phage DNAs which may map a definitive position of the *gov* function and may give clues concerning the killing property of λ-Mu2 as well as to the origin and function, if any, of the insertion DNA of 2600 base pairs in the middle of the β sequence.

EM work is also in progress to map λ-Mu phages that contain the left end (and the immunity region) of the Mu lysogen.

EM-Heteroduplex Analyses of Yeast Killer Factor RNA

Yeast killer strains contain two species of double-stranded RNAs: one species of 2.5×10^6 daltons MW (large) specifies functions yet unknown; the other, of $1\text{–}1.2 \times 10^6$ daltons MW (medium), coexists with the killing property of the strain and presumably is responsible for the production of the toxin that kills sensitive strains. Yeast nonkiller mutants that still contain the large RNA have been isolated, but the medium species has been replaced by smaller RNA species of different sizes. Preliminary liquid hybridization results indicate that the small RNAs are deletion mutants of the medium RNA.

Electron microscope-heteroduplex studies are in progress to study the sequence relationships among the medium and the various small RNA species. These studies necessarily include identification of conditions under which double-stranded RNA of high G:C content can be denatured and reannealed without introducing strand breakage.

Equipment Redesign

With the assistance of Bill Flanigan of the mechanical shop, J. Scott and T. Broker have made numerous modifications of the two Denton vacuum evaporators to improve the operating characteristics of the machines and to provide increased safety for both the users and the instruments. These modifications include (a) a solenoid-activated shutter to increase the fine-grain quality of platinum metal evaporated from a filament and deposited as a shadow on samples for electron microscopy; (b) a quartz-crystal frequency monitor coupled to the shutter, thereby regulating the amount of platinum deposited on the specimens; (c) disposable shielding around the platinum source to minimize cleaning of the instrument and thus reduce human exposure to platinum; (d) a self-pumping liquid nitrogen reservoir to decrease handling of the coolant and to greatly reduce the pumping time of the vacuum system (Scott, Flanigan and Broker, in prep.) An auxiliary vacuum system has been assembled to allow glow discharge ionization of EM grids in atmospheres of various vapors. In addition, electrical modifications of the Philips 201 electron microscope were made, including the design and installation of a Sonalert alarm to warn of improper positioning of valves in the vacuum system and of a timer to allow records of instrument use.

Publications

An extensive and detailed laboratory manual (*Electron Microscopy of Nucleic Acids*) was prepared to fill a void in the literature and for specific use in instruction in electron microscopy during summer courses and for laboratory personnel. An expanded second edition is nearing completion. Subjects treated include sources of supplies and chemicals, preparation of substrate-covered grids, manipulations of nucleic acid samples (partial denaturation, stabilization against base-pairing, renaturation kinetics and heteroduplex formation), mounting RNA and DNA on EM grids, positive staining and shadowing, negative staining of particles, operation of vacuum evaporators and electron microscopes, darkroom techniques, interpretation of observed structures, data gathering and analysis, and selected references.

In collaboration with A. H. Doermann of the Department of Genetics, University of Washington, and with extensive assistance from Louise Chow, T. Broker wrote a review on the physical, enzymatic, physiologic and genetic requirements for and characteristics of bacteriophage T4 DNA recombination. A major conclusion was that for T4 there is no single "mechanism of recombination," but rather that recombination represents a broad class of events. Recombination is an integral part of both DNA replication and DNA repair, partly because these aspects of DNA metabolism generate and utilize common structures and rely upon common enzymes. Several genetic properties of recombination (e.g., high negative interference, and effects and polarity) were given molecular interpretations.

T4 DNA Replication

With John Chin, an undergraduate research participant, T. Broker began a preliminary electron microscopic investigation of the consequences of UV damages in DNA on T4 replication. T4 was chosen as a model system for this EM approach to studying radiation (and eventually other mutagen) effects on replication because of the large number of different radiation-sensitive mutants, in particular, and replication-defective mutants in general, that have been described in T4. Furthermore, T4 is basically a linear replicon, at times with multiple replication loops on each unit equivalent. At present very little firm information is available on the physical structures of linear replicons throughout the replication cycle.

We have also constructed several multiple mutants of bacteriophage T4 that result in a substantial overproduction of the gene 32 protein, which binds coöperatively to single-stranded DNA and RNA and, as such, is a particularly useful reagent for the electron microscopy of nucleic acids.

Electron Microscopic Labels

As a postdoctoral fellow in N. Davidson's laboratory at Cal Tech, T. Broker developed an affinity labeling method by which various types of macromolecules (DNA, RNA, proteins and other polymers) could be specifically coupled pairwise to each other or to electron-dense labels such as the iron-storage protein ferritin. The purpose was to allow EM mapping of genes and other genetic sequences. The method relies on chemically coupling the vitamin biotin to one molecule (e.g., tRNA) and the egg white protein avidin to another (e.g., ferritin). The affinity of avidin for biotin is the strongest biological affinity known, about one million times stronger than typical antibody-antigen associations. tRNA-biotin can be hybridized to complementary DNA without a significant decrease in the rate of association due to the biotin. Addition of ferritin-avidin results in 70% labeling efficiency of the tRNA gene, as determined by EM observation.

At Cold Spring Harbor, numerous applications of this basic labeling scheme will be explored, including mapping short DNA restriction fragments, visualizing proteins (e.g., polymerases, repressors) bound to DNA, and labeling antibodies, which in a two-step affinity association could direct EM labels to antigens. Some labels visible in

the scanning electron microscope have been used to map *Drosophila melanogaster* ribosomal RNA in nucleoli following in situ hybridization (contribution from Cal Tech). A related application of the biotin-avidin labeling scheme to be tested is gene enrichment of DNA:RNA-biotin complexes on avidin-Sepharose affinity columns. A potential advantage of such a protocol is the unimpaired hybridization of the RNA-biotin to DNA in solution relative to hybridization in which either the RNA or DNA is prefixed on filters or another solid support. Current research is centered on overcoming two technical limitations of the labeling method: (1) biotin must be attached to the target macromolecule via a longer "spacer arm" to allow more efficient interaction with avidin; and (2) the exceptionally positive charge on avidin must be reduced (by acylation) to minimize electrostatic interactions with other macromolecules.

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NUCLEIC ACID CHEMISTRY

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J. Bonventri
B. Doretsky
S. A. Endow
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S. Zain

New Restriction Endonucleases

Seventeen new restriction enzymes have been isolated in this laboratory during the last year, of which six show new specificities. The total number of restriction enzymes now known is 75, representing a total of 37 different specificities. Despite the fact that the bacteria screened were selected on a random basis, one in three of all examined were found to contain restriction enzymes. This points to the very wide distribution of these enzymes, but it has not been possible so far to find any common features among the bacteria possessing them.

Among the enzymes isolated recently, *Blu*I from *Brevibacterium luteum* seems useful for mapping, as it makes relatively few cuts on most DNAs examined. A number of eukaryotic organisms have been examined, but so far only *Chlamydomonas* appears to possess a restriction enzyme. This enzyme has been studied in collaboration with W. Burton and R. Sagar and shows many of the characteristics of a type II restriction endonuclease; however, its purification is difficult, and further work is required to fully characterize the products of digestion. Recognition sequences have been deduced, in collaboration with K. Murray and G. Wilson, for the specific endonucleases *Hha*I (GCG$^{\downarrow}$C) from *Haemophilus haemolyticus*, *Alu*I (AG$^{\downarrow}$CT) from *Arthrobacter luteus*, *Bam*H-I (G$^{\downarrow}$GATCC) from *Bacillus amyloliquefaciens* H, and *Bal*I (CGG$^{\downarrow}$CCG) from *Brevibacterium albidum*.

Mapping

The *Hin*dIII map of the adenovirus 2 genome is now complete and has been related to maps previously deduced for *Eco*RI, *Hpa*I, *Sal*I and *Bam*H-I. Further mapping with the specific endonucleases *Bal*I and *Bum*I is underway, and new, fast methods for mapping are being explored. One promising method involves degradation with exonuclease III to varying extents followed by resynthesis with a DNA polymerase and

^{32}P-labeled deoxynucleotide triphosphates. Subsequent cleavage with a restriction endonuclease and analysis of labeled fragments permits the relative location of the fragment within the genome to be correlated with the extent of exonuclease III degradation.

A fine-structure map of the left-hand 4.4% of the Ad2 genome (the *Hpa*I "e" fragment) is now available, and more than 40 cleavage sites have been mapped within this 1600-nucleotide-long fragment. These data are being used for direct DNA sequence analysis and also for defining the start of the in vivo transcript from this region (see below).

Fine-structure maps of (a) the nondefective hybrid virus Ad2$^+$ND1 in the region of the SV40 insertion and (b) the corresponding region in Ad2 have been obtained. Comparison with the map of the SV40 genome has allowed the identification of restricted fragments containing the integration site. Sequence analyses of these fragments and the corresponding fragments of Ad2 are in progress.

In Vivo Transcripts

mRNA, present on polysomes late after Ad2 infection, has been hybridized to the 1600-nucleotide-long *Hpa*I "e" fragment. Following treatment with RNase T_1, the RNA present as hybrid was recovered and fingerprinted. Fingerprints have also been obtained for RNA hybridized to shorter terminal fragments of 990 and 650 nucleotides in length. As expected from the known direction of transcription in this region, the RNA fingerprints became less complex with decreasing fragment size. Analysis of individual oligonucleotides eluted from these fingerprints showed that only a single oligonucleotide was present which could not be completely digested with ribonuclease T_2, indicative of a "capped" structure from the 5′ end of the mRNA. This same oligonucleotide is present in all three fingerprints and is being sequenced. From the complexity of the RNA fingerprint obtained after hybridization to the 650-nucleotide fragment, it is possible to estimate that the 5′ end of the mRNA lies some 300–400 nucleotides in from the extreme end of the Ad2 genome. Further hybridization/fingerprint studies are in progress to enable that position to be defined exactly and to allow DNA sequence analysis of the region immediately preceding the start of the mRNA.

Adenovirus Termini

Direct DNA sequence analysis of the inverted terminal repetition is in progress. During the course of this work several puzzling observations have emerged, and their significance is being explored. Polynucleotide kinase fails to label the 5′ ends of Ad2 DNA. They are also resistant to the action of λ exonuclease. Sequences from the 3′ strand have been determined and shown to be 3′ TAG .... T4 polymerase and DNA polymerase I are both able to add a single G residue; however, AMV reverse transcriptase cannot. These observations are consistent with the terminal structure 5′ *CATC ..., where the 5′-terminal C is modified in such a way that it is not 3′ TAG ....
recognized as a C by AMV reverse transcriptase and is resistant to both polynucleotide kinase and λ exonuclease. One possibility is that the modification is a peptide resulting from the degradation of the terminal protein described by Bellett (*Virology* *56*:54 [1973]). Experiments are in progress to test this hypothesis and also to characterize the terminal protein. Preliminary results, obtained using ^{35}S-labeled virions, suggest that this protein has a molecular weight of 50–55K and may be covalently bound within 70 nucleotides of the end of the genome.

Publications

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Roberts, R. J., P. A. Myers, A. Morrison and K. Murray. A new specific endonuclease from *Arthrobacter luteus*. (Submitted to *J. Mol. Biol.*)
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DNA-PROTEIN INTERACTIONS

T. MANIATIS
Sim Gek Kee

Molecular Cloning of Purified Eukaryotic Genes

We have begun studies on the organization of eukaryotic transcriptional units. Our approach is to map and study DNA sequences surrounding various eukaryotic structural genes. In collaboration with Argiris Efstradiadis and Fotis Kafatos of Harvard University, we have developed methods for cloning and amplifying eukaryotic genes starting with purified messenger RNA. A full-length reverse DNA transcript of rabbit globin mRNA was produced using reverse transcriptase isolated from avian myeloblastosis virus (Efstradiadis et al., *Cell 4*:367 [1975]). The size of the DNA transcript was determined by electrophoresis through denaturing polyacrylamide gels calibrated with DNA molecules of known molecular weight (Maniatis et al., *Biochemistry 14*:3787 [1975]). We found that reverse transcriptase generates a small hair-pin loop at the 3' end of the cDNA, which can serve as a primer for the synthesis of a complementary DNA chain by *E. coli* DNA polymerase I. Thus it was possible to obtain a double-stranded DNA molecule containing all of the sequences present in globin messenger RNA. The single-stranded loop present at one end of the DNA can be removed by digestion with the single-strand-specific endonuclease S1.

With Allan Maxam of Harvard, various restriction endonuclease cleavage sites within globin DNA have been mapped relative to the hair-pin loop by examining restriction endonuclease digestion products of the DNA on denaturing polyacrylamide gels before and after exposure to S1 endonuclease. As expected, the size of the 3'-terminal DNA fragment containing the hair-pin loop is reduced by a factor of two upon treatment with S1.

In order to amplify this DNA for further studies, the dA:dT joining procedure (Jackson et al., *Proc. Nat. Acad. Sci. 69*:2904 [1972]; Lobban and Kaiser, *J. Mol. Biol. 78*:453 [1973]; Wensink et al., *Cell 3*:315 [1974]) has been used to construct hybrid DNA molecules containing globin DNA linked to the DNA of an *E. coli* plasmid carrying a tetracycline drug resistance marker. Bacteria were transformed with hybrid DNA and selected by their ability to grow in the presence of tetracycline. Globin DNA-containing cells were identified by their ability to hybridize globin messenger RNA, using in situ hybridization procedures developed by Grunstein and Hogness of Stanford University. By mapping restriction endonuclease cleavage sites in the plasmid DNA near the site of the globin DNA insertion it was possible to determine the amount of globin DNA in each hybrid. We have isolated hybrid DNA containing globin sequences approximately the size expected for full-length globin genes. Two of these DNA molecules exhibit different endonuclease cleavage patterns, suggesting that one of the molecules contains globin α-chain sequences, whereas the other contains β-chain sequences. Both plasmid DNA molecules hybridize efficiently to globin mRNA and both contain restriction endonuclease cleavage sites within the globin DNA segment, which are also found in the purified globin gene, indicating that no extraneous, non-globin sequences have been introduced into the bacterial plasmid DNA. We are presently using hybrid DNA as a probe to map DNA sequences surrounding the genes for the α- and β-chain structural genes within rabbit chromosomes.

SV40 Chromatin

With Mike Botchan and Walter Keller we are studying the structure and transcription of SV40 nucleoprotein complexes isolated from the nuclei of infected cells and from purified virus. Using in vitro protein labeling procedures we have detected non-histone proteins associated with purified viral DNA–protein complexes. We have shown that one of these proteins remains bound to DNA after extensive purification and have identified it as a major viral capsid protein, VP1. Using restriction endonucleases and in vitro DNA labeling methods (Maniatis et al., *Proc. Nat. Acad. Sci. 72*:1184 [1975]) we are presently attempting to determine if this protein is bound to a specific site on SV40 DNA.

To study the possible role of histone and non-histone proteins in the selective transcription of SV40 DNA we are examining the in vitro transcription products produced by an endogenous RNA polymerase activity associated with the complex, by *E. coli* RNA polymerase, and by mammalian RNA polymerase II. The origin of these transcripts are localized on the SV40 physical map using a hybridization procedure developed by Southern (*J. Mol. Biol. 98*:503 [1975]).

Publications

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Maniatis, T., A. Jeffrey and D. Kleid. 1975. Nucleotide sequence of the rightward operator of bacteriophage lambda. *Proc. Nat. Acad. Sci.* 72:1184.

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CELL BIOLOGY

G. ALBRECHT-BUEHLE
K. Burridge
S. Chait
W. Gordon
R. Lancaster
E. Lazarides

We can now distinguish between two main ways of checking the phenotypic discrepancies between normal and transformed cells. One way attempts to find new, transformation-specific molecular components in transformed cells or cellular phenomena on a molecular level that are changed upon cell transformation. The other attempts to understand motility phenomena and related processes in normal and transformed cells. Both ways of proceeding lead to the same central questions: "What is the role of the plasma membrane in cell transformation?" and "What is the role of microfilament bundles in cell morphology and motility?"

We have chosen two different approaches to tackle these questions. One approach is the study, by indirect immunofluorescence, of the localization of structural muscle proteins in non-muscle cells. The other approach is the study of the function of filopodia (microspikes), since they appear to be just a single microfilament bundle, 0.2 μm in diameter and 5–20 μm in length, which is coated by plasma membrane and extends into the environment of the cell.

The study of the major structural muscle proteins in non-muscle cells was begun last year (see Annual Report 1974, Proteins in SV40 Infected and Transformed Cells), whereas the study of filopodia and the quantitation of their actions was initiated this year. Therefore the latter will be described in more detail. Although presently we are concentrating on filopodia, we have in mind a more general study of the motile activities of the surface of animal cells and their relationship with microfilaments.

Filopodia have been observed for more than 30 years, both in vivo and in vitro. Until now, however, their actions have had to be described in a largely anecdotal way. Last year we found an assay which offered the first quantitative approach to the analysis of filopodia function.

Furthermore, experiments described below suggest that filopodia are cellular organs that explore the nonfluid environment of a cell and mediate the extension of the cell into areas where a certain as yet unknown quality of the substrate is found. Therefore one may conjecture that filopodia are generally involved in the recognition by one cell of the close proximity of another. If one target of transforming agents is this exploratory mechanism of filopodia, then one may expect a reduction, or even the loss, in transformed cells of the capacity to recognize a neighbor cell. Thus transformed cells may not receive the "signals" from filopodia that in normal cells would produce the extension of a cell process or the initiation of mitosis.

We began to investigate the mechanism of filopodia action in normal 3T3 mouse fibroblasts in order to establish a frame of reference for "normal" filopodia function. We also compared the behavior of filopodia of SV40 virus-transformed mouse and rat embryo fibroblasts with that of their normal counterparts. The results of the latter study are preliminary and not included in this report. As to the ultrastructure of filopodia, we collaborated with Robert D. Goldman from the Mellon Institute in Pittsburgh. Recently, William Gordon, a graduate student from the University of California at Berkeley, joined the group. He is trying to localize actin, myosin, α-actinin and tropomyosin in filopodia by using indirect immunofluorescence techniques in light microscopy and peroxidase staining in electron microscopy.

Particle Removal by Filopodia

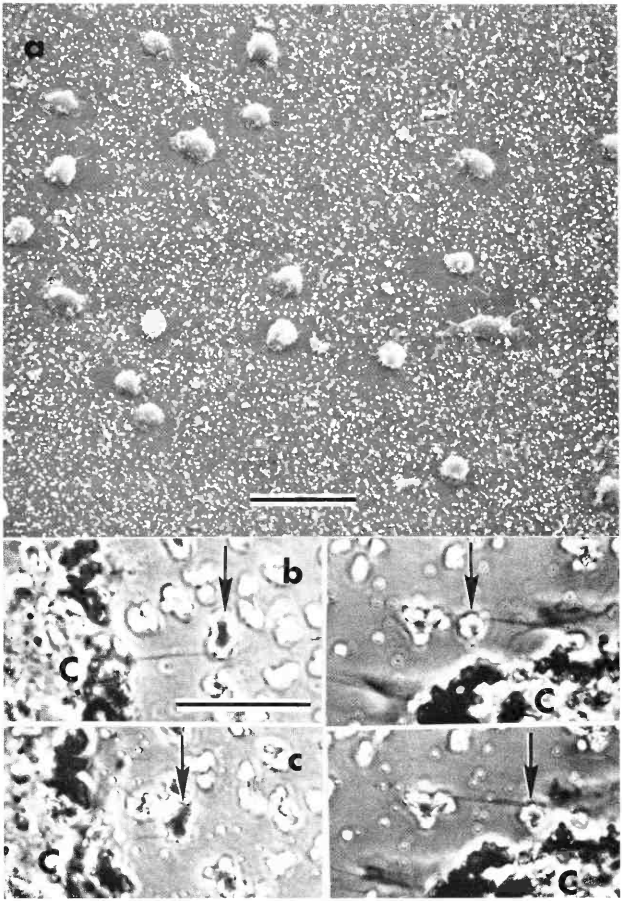
If colloidal gold particles of 0.2–0.4 μm diameter are densely and evenly deposited on a glass substratum and freshly suspended 3T3 cells plated on top, 95–98% of the cells remove all particles within a radius of 20 μm in 30–50 minutes. Observations of live cells show that the particles are removed by filopodia that grow in large numbers out of the cell body about 15–20 minutes after plating. The removals occur at speeds of 0.2 μm/sec and essentially in two ways: either the filopodium retracts as a whole, together with the particle, or the particle flows along a stretched out and attached filopodia towards the cell body. Retraction of filopodia without contact to a particle is very rare. Cells that removed the surrounding particles could be easily distinguished from cells that did not. By counting the percentage of cells capable of particle removal, one can obtain a quantitative measure of the function of filopodia under various conditions. Using this assay, we found, for example, that the retraction of filopodia, but not the outgrowth, was reversibly inhibited by temperatures below 26°C, by pH below 6.7, and by cytochalasin B in concentrations higher than 2 μg/ml.

The idea that particle removal might express an exploratory function of filopodia came from the suspicion that the filopodia possibly mistook the loose particles for an extended solid substratum. They might have removed the particles simply because they always produce a retraction force upon contact, as if testing its firmness.

The Exploratory Function of Filopodia

In order to test this hypothesis, we plated 3T3 cells on a glass substratum partially coated with gold particles too firmly attached to be removed by filopodia. Observing cells that had settled on the glass near the borderline to a gold area, we observed a preferential extension of lamellipodia towards the gold area, provided it had been contacted previously by filopodia. This preferential extension occurred even though other filopodia had also contacted the glass surface and although 3T3 cells are able to spread on both glass and gold substrata.

In another type of experiment we observed freshly plated 3T3 cells near another already spread 3T3 cell. If both cell and glass had been contacted by filopodia, the first lamellipodia extended away from the spread-out cell.



Gold particle removal by filopodia during early spreading of 3T3 cells. (*a*) Scanning electron micrograph of a whole field of cells 50 minutes after plating. Bar = 50 μm. (Tilt angle: 45°.) (*b,c*) Retraction of a particle-attached filopodium as a whole. C indicates the cell body. Arrow points to the moving cluster of gold particles. Time difference between *b* and *c* is 1 minute. Bar in *b* = 10 μm. (*d,e*) Centripetal flow of a particle cluster along a stretched-out filopodium. Time difference between *d* and *e* is 2 minutes.

Both phenomena are reversibly inhibited by cytochalasin B, although filopodia grow out and apparently adhere to their respective targets in the presence of the drug.

Both types of preferential lamellar extensions occur towards the area of higher adhesion while the cell is in contact with these areas only by filopodia. Therefore we take these findings as strong support for the interpretation of filopodia as organs of single cells which detect an as yet unknown quality of the substrate.

Localization of Structural Muscle Proteins in Non-muscle Cells

During the past year we have been engaged in determining the structural organization of actin filaments in non-muscle cells grown in tissue culture. During the course of our studies it became apparent that antibodies raised against well-defined and well-characterized major structural proteins of skeletal muscle, in particular actin, α-actinin and tropomyosin, could be used to localize these proteins in a variety of non-muscle cells grown in tissue culture. Most of the epithelioid or fibroblastic cell types that are grown in tissue culture have the remarkable ability to adhere strongly to glass or plastic substrata and spread out, frequently assuming lengths of more than 100 μm. Such fully spread-out cells are characterized by phase-contrast-dense fibers which frequently span the long axis of the cell and are known as "stress fibers." Immunofluorescence studies with antibodies to actin, α-actinin and tropomyosin have indicated that all three proteins are localized within these filamentous bundles. The actin antibody exhibits a continuous fluorescence along the stress fibers, whereas both the tropomyosin and α-actinin antibodies exhibit a type of periodic fluorescence. These latter two types of periodic fluorescence are usually variable, but reaction of the cells with both antibodies simultaneously or sequentially suggests that these two types of fluorescence are complementary. These results indicate that in non-muscle tissue-culture cells, α-actinin and tropomyosin interact with the actin filaments in a highly regular manner, and that the structure of the actin filament bundles in the less differentiated non-muscle cells is closely similar to the structure of these filaments in skeletal muscle. However, unlike skeletal muscle, the highly organized structure of actin filaments in non-muscle cells is dynamic, and changes in cell shape can easily result in the disaggregation of these filaments. Thus when a fully spread-out cell is exposed to proteolytic enzymes, such as trypsin, or when it goes into mitosis, the cell looses its flattened phenotype and rounds up. Concomitant with this rounding up, the cytoplasmic actin filament bundles disaggregate. When the cells are allowed to flatten out again, they slowly resume firm adhesion to the substratum, with a concomitant slow reorganization of their lost actin filament bundles. This process of spreading has been used as a model system to study, by indirect immunofluorescence, the interaction of actin, α-actinin and tropomyosin with the newly reorganizing stress fibers. One of the most characteristic ways in which these fibers re-form is via a transiently formed, highly regular network of actin filaments. The vertices of the network contain α-actinin but no tropomyosin, whereas the connecting filament bundles between the vertices contain tropomyosin but no α-actinin. Filaments containing all three proteins are also seen to span from the vertices of the network to the edges of the cell. This latter class of filaments appears indistinguishable from the actin filament bundles normally seen in fully spread-out cells. Thus the vetices of the network may function as nucleation sites and later as organization sites for the formation of actin filament bundles. We have also undertaken a more detailed study of the localization of myosin in non-muscle cells and are investigating the role that this molecule might play in the organization of actin filaments and in the locomotory activity of tissue-culture cells.

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———. 1976. Aspects of the structural organization of actin filaments in tissue culture cells. In *Cell motility* (ed. R. D. Goldman, T. Pollard and J. Rosenbaum). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. (In press.)

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Leech Course

1975

Photo by Seymour Benzer

POST GRADUATE TRAINING PROGRAMS

SUMMER 1975

Since its inception, the postgraduate program at Cold Spring Harbor Laboratory has been aimed toward meeting the rather special need for training in new interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. The intention is to provide intensive training in the most recent developments and techniques in each of the subjects so that it should be possible for the students to enter directly into research in the particular area.

To do this, we bring together a workshop staff from many laboratories around the world. These instructors direct intensive laboratory and lecture programs supplemented with a continuous series of seminar speakers which insures up-to-date coverage of current research work.

This year the program included a new course, Immunogenetics in Relation to Experimental Tumor Immunology.

ANIMAL CELL CULTURE WORKSHOP — June 13–July 3

The course consisted of a series of laboratory exercises and lectures related to laboratory procedures. Initial exercises covered such basic culture techniques as propagation of established cell lines, initiation of primary cultures, growth of differentiated cells from various embryonic tissues, cloning, mycoplasma assays, and karyotype analysis using quinacrine fluorescence or trypsin banding. Experiments utilizing more specialized techniques were also performed. These experiments included cell synchronization, autoradiography, induction and selection of HGPRT mutations, enucleation of chloramphenicol-resistant cells and formation of cytoplasmic hybrids, and the preparation and use of liposomes to effect cell fusion. Sendai virus-mediated cell fusion was used to study complementation of Gly^- mutants in CHO cells and to microinject proteins into culture cells by fusion with protein-loaded, red blood cells. Cell surface antigens on human–mouse hybrid cells were studied using the mixed hemadsorption assay. The induction of hemoglobin synthesis in erythroleukemia cells and immunoglobulin production in mouse myeloma cells were also studied. Each evening a guest lecturer spoke on recent developments in cell biology.

INSTRUCTORS

Coon, Hayden, Ph.D., National Institutes of Health, Bethesda, Maryland
Rechsteiner, Martin, Ph.D., University of Utah, Salt Lake City, Utah

ASSISTANTS

Ambesi, Severio, National Institutes of Health, Bethesda, Maryland

PARTICIPANTS

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Axelrod, David E., Rutgers University, New Brunswick, New Jersey
Brown, Roger F., Southwest Texas State University, San Marcos, Texas
Bustamante, Ernesto D., Johns Hopkins University, Baltimore, Maryland
Calhoun, David H., Mt. Sinai School of Medicine, New York, New York
Carpenter, Graham F., Vanderbilt University, Nashville, Tennessee
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Hopkins, Thomas J., State University of New York, Stony Brook, New York
Inselburg, Joseph W., Dartmouth Medical School, Hanover, New Hampshire

Manley, James L., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Martinell, James, Wesleyan University, Middletown, Connecticut
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Peters, Reiner A., University of California Medical Center, San Francisco, California
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Ray, Dan S., University of California, Los Angeles, California
Schlessinger, Joseph, Cornell University, Ithaca, New York
Thiery, Jean Paul, Rockefeller University, New York, New York
Thon, Wolfgang, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin

SEMINARS

Eagle, H., Albert Einstein College of Medicine. *Animal cell culture. A perspective.*
Pollack, R., State University of New York, Stony Brook. *Viral transformed cells.*
Poste, G., Roswell Park Memorial Institute. *On the mechanism of cell fusion.*
Papahadjapoulos, D., Roswell Park Memorial Institute. *Liposomes.*
Hsie, A., Oak Ridge National Laboratory. *Quantitative analysis of mutation at the HGPRT locus in CHO cells.*
Orkin, S., National Institutes of Health. *Studies on differentiation in erythroleukemia cells.*
Donady, J., Wesleyan University. *Genetic approach to cell differentiation using* Drosophila *embryonic tissues.*
Pardee, A., Princeton University. *Events in the animal cell cycle.*
Sato, G., University of California, La Jolla. *The role of hormones in the growth of animal cells.*
Neufeld, E., National Institutes of Health. *Contributions of cell culture to lysosomal storage disease.*
Gospodarowicz, D., Salk Institute. *Control of division of mammalian cells in vitro.*
Rutishauser, U., Rockefeller University. *Specific interactions and modulation at the cell surface.*
Eisenstadt, J., Yale University. *Cytoplasmic inheritance in animal cells.*
Green, H., Massachusetts Institute of Technology. *Adipose conversion in the mammalian cell: A problem in regulation and cell heredity.*
Trisler, D., National Institutes of Health. *Thymidine kinase activity in hybrids between mammalian cells and chick red blood cells.*
Roth, S., Johns Hopkins University. *Analysis of growth control in cultured fibroblasts.*
Cohn, Z., Rockefeller University. *Endocytosis and the vacuolar apparatus.*
Basilico, C., New York University. *Temperature-sensitive mutations in animal cells.*
Rao, P., M.D. Anderson Hospital. *Premature chromosome condensation: Use in the analysis of chromatid breaks.*
Scharff, M., Albert Einstein College of Medicine. *Genetic studies of myeloma proteins in cell culture.*

MOLECULAR CYTOGENETICS WORKSHOP — June 13–July 3

This year's cytogenetics course integrated classical and molecular approaches to analysis of chromosomes. As in the past several years we utilized chromosome rearrangements, renaturation kinetics, in situ hybridization, visualization of transcription, heteroduplex mapping and chromosome banding to study specific problems of chromosome structure and function.

New this year was the introduction into the course of restriction enzymes, DNA sequencing and isolation of chromatic subunits. Richard Roberts and Ken van Holde aided us in these areas. We also benefited from the contributions of Barbara McClintock, both in assisting with a field trip and for discussions on plant chromosomes and pattern formation.

Students were primarily from biochemical backgrounds and included representatives from France, Germany, Spain and Russia. Because the previous research experience of the students ranged from studies on bacterial chromosome structure to am-

phibian developmental biology, student seminars were an important part of the course.

An increasing number of techniques for the study of chromosome structure make use of the electron microscope. The course particularly benefited from the fine EM facility being organized by T. Broker, L. Chow and J. Scott.

INSTRUCTORS

Pardue, Mary Lou, Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts
Laird, Charles, Ph.D., University of Washington, Seattle, Washington

PARTICIPANTS

Celma, Maria Luisa, Yale University, New Haven, Connecticut
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Ryder, Oliver A., University of California at San Diego, La Jolla, California

SEMINARS

Judd, B., University of Texas at Austin. *Polytene chromosomes.*
Pardue, M. L., Massachusetts Institute of Technology. *Organization of DNA sequences in* Drosophila *chromosomes.*
Sambrook, J., Cold Spring Harbor Laboratory. *Virus genomes in cells.*
Laird, C., University of Washington. *Transcription units of* Drosophila.
Bakken, A., University of Washington. *Studies on lampbrush chromosomes in mammalian and amphibian oocytes.*
Davis, R., Stanford University. *In vitro constructed hybrid DNA molecules with bacteriophage lambda.*
Roberts, R., Cold Spring Harbor Laboratory. *Restriction enzymes.*
———*Nucleic acid sequences.*
van Holde, K., Oregon State University. *Subunit organization of chromatin.*
Miller, J., Columbia University. *Applications of chromosome banding.*

NERVOUS SYSTEM OF THE LEECH WORKSHOP — June 13–July 3

The aim of this workshop was to provide ten 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 those techniques for recording from leech cells, now straightforward and relatively easy, but which 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 in order to study their geometry.

The final phase of the course consisted of devising and performing original experiments, some of which proved to be of sufficient interest to be pursued in greater detail.

INSTRUCTORS

Nicholls, John, Ph.D., M.D., Stanford University Medical School, Palo Alto, California
Jansen, Jan, Ph.D., M.D., University of Oslo, Oslo, Norway
Van Essen, David, Ph.D., University College, London, England
Stuart, Ann, Ph.D., Harvard Medical School, Boston, Massachusetts

PARTICIPANTS

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Futamachi, Kin J., National Institutes of Health, Bethesda, Maryland
Hui, Chiu Shuen, Yale Medical School, New Haven, Connecticut
Macagno, Eduardo R., Columbia University, New York, New York
Oertel, Donata, University of Wisconsin, Madison, Wisconsin
Sawyer, R. T., University of California, Berkeley, California
Siddiqi, Obaid, Tata Institute, Bombay, India
Singh, Udai, Columbia University, New York, New York

SEMINARS

Nicholls, J., Stanford University Medical School. *Introduction to leech CNS.*
———*Receptive fields of sensory cells.*
———*Changes in synaptic connections after lesions and in cultured ganglia.*
———*Glial cells and K accumulation.*
———*Synaptic connections of sensory and motor nerve cells.*
Sawyer, R., University of California at Berkeley. *Biology of leeches.*
———*Development of leeches.*
Jansen, J., University of Oslo. *Hyperpolarization of sensory cells following impulses.*
———*The S interneuron.*
———*Regeneration in leech CNS.*
Van Essen, D., University College, London. *Regeneration of sensory cells to skin.*
Stuart, A., Harvard Medical School. *Motor cells.*
Muller, K. J., Carnegie Institute. *Fine structure of leech CNS.*
McMahan, U. J., Harvard Medical School. *Peroxidase injection of individual cells.*
Fernandez, J., Case Western Reserve University. *Morphology of regenerating fibers.*
Stent, G., University of California at Berkeley. *Neural control of circulation.*
———*Leech eyes.*
Kristan, W., University of California at Berkeley. *Analysis of swimming.*
Cohen, L., Yale Medical School. *Optical recognition of signaling in leech CNS.*

ANIMAL VIRUS WORKSHOP — July 7–July 26

The workshop consisted of morning lectures and discussion groups covering the basic outlines of eukaryotic molecular biology and a review of the major classes of animal viruses. The laboratory exercises held each afternoon included preparation of primary cell cultures, growth of continuous tissue culture cell lines, preparation and purification of virus stocks, electrophoretic analysis of viral nucleic acids and proteins, restriction enzyme analysis, assay of virion associated enzymes, translation of viral RNA by cell-free extracts, C_0t analysis, and viral mediated oncogenic transformation. A variety of informal evening lectures were held to review the theory and practice of techniques used in modern animal virology.

In addition to the formal laboratory exercises, opportunities were available for carrying out individual research projects.

INSTRUCTORS

Levine, Arnold, Ph.D., Princeton University, Princeton, New Jersey
Neiman, Paul, Ph.D., University of Washington, Seattle, Washington

ASSISTANTS

Linial, Maxine, Ph.D., University of Washington, Seattle, Washington
van der Vliet, Peter, Ph.D., State University of Utrecht, Utrecht, The Netherlands

PARTICIPANTS

Berns, Anton, Salk Institute, San Diego, California
Chattopadhyay, Sisir, National Institutes of Health, Bethesda, Maryland
Chien, Hueh-hsiu, California Institute of Technology, Pasadena, California

Collins, John, Case Western Reserve University, Cleveland, Ohio
Danglot, Claude, Laboratoire du P-Vilagines, Paris, France
DeLarco, Joseph, National Institutes of Health, Bethesda, Maryland
Dumas, Lawrence, Northwestern University, Evanston, Illinois
Goubin, Gerard, Institute de Cancerologie et d'Immunogenetique, Villejuif, France
Heere, Leonard, Medical University of South Carolina, Charleston, South Carolina
Hercules, Kathleen, University of Colorado Medical Center, Denver, Colorado
Mastro, Andrea, Pennsylvania State University, University Park, Pennsylvania
Peters, Gordon, University of Wisconsin, Madison, Wisconsin
Petigrow, Carol, Mt. Sinai School of Medicine, New York, New York
Pipas, James M., Florida State University, Tallahassee, Florida
Rohrlich, Susannah, Rockefeller University, New York, New York
Rutishauser, Urs, Rockefeller University, New York, New York
Sausville, Edward, Albert Einstein College of Medicine, New York, New York
Seidman, Michael, National Institutes of Health, Bethesda, Maryland
Sternglanz, Rolf, State University of New York, Stony Brook, New York
Sumida-Yasumoto, Chikako, Albert Einstein College of Medicine, New York, New York

SEMINARS

Levine, A. J., Princeton University. *Animal cells in culture and the cell cycle.*
Weintraub, H., Princeton University. *Eukaryotic DNA structure, chromatin and DNA replication.*
van der Vliet, P. C., State University of Utrecht. *In vitro DNA synthesis.*
Darnell, J., Rockefeller University. *RNA synthesis in eukaryotic cells.*
Lodish, H., Massachusetts Institute of Technology. *Protein synthesis in eukaryotic cells.*
Gesteland, R., Cold Spring Harbor Laboratory. *In vitro translation viral RNAs.*
Ruddle, F., Yale University. *Somatic cell hybrids and tissue culture.*
Burger, M., Biozentrum der Universitat Basel. *Cell surfaces.*
Joklik, W., Duke University Medical Center. *Introduction to viral pathways and mechanisms.*
Carter, W., Roswell Park Memorial Institute. *Cellular responses to viral infection.*
Neiman, P., University of Washington. *Oncornaviruses.*
Linial, M., University of Washington. *Ts mutants and complementation.*
Levine, A.J., Princeton University. *Adenoviruses.*
Sambrook, J., Cold Spring Harbor Laboratory. *Adenovirus transforming cells.*
Nowinski, R., University of Wisconsin. *Viral immunology.*
Laemmli, U. K., Princeton University. *Gel electrophoresis.*
Baltimore, D., Massachusetts Institute of Technology. *Enteroviruses.*
Gelinas, R., Cold Spring Harbor Laboratory. *Restriction enzymes.*
Huang, A., Harvard Medical School. *Rhabdoviruses.*
Graham, A., McGill University. *Reoviruses.*
Broker, T., Cold Spring Harbor Laboratory. *DNA microscopy.*
Choppin, P., Rockefeller University. *Myxoviruses and paramyxoviruses.*
Astrin, S., Institute for Cancer Research. *Transcription of viral genes in chromatin.*
Tegtmeyer, P., Case-Western Reserve University. *Papovaviruses.*
Kelly, T., The Johns Hopkins University. *Adenovirus-SV40 hybrids.*
Rapp, F., The Pennsylvania State University. *Herpesviruses.*
Khoury, G., National Institutes of Health. *Parvoviruses and human papovaviruses.*
Kates, J., State University of New York at Stony Brook. *Poxviruses.*

MOLECULAR BIOLOGY AND GENETICS OF YEAST — July 7–July 26

The yeast workshop this year focused on a mutational analysis of the early portion of G_1 in the cell cycle. Analysis of these mutants allows the construction of a model for the initiation of cell division which proposes that information concerning the metabolic state of the cell is integrated early in G_1 and that this information forms the signal committing the cell to mitotic division. It was clear to participants that a study of the control of the cell cycle in yeast could have far-reaching implications for the understanding of cellular differentiation and proliferation in mammalian cells.

INSTRUCTORS

Fink, Gerald, Ph.D., Cornell University, Ithaca, New York
Lawrence, Christopher, Ph.D., University of Rochester, Rochester, New York
Sherman, Fred, Ph.D., University of Rochester, Rochester, New York

ASSISTANTS

Greer, Helen, Ph.D., Cornell University, Ithaca, New York

PARTICIPANTS

Bennett, William, University of Texas, Dallas, Texas
Buettner, Michael, University of Wisconsin, Milwaukee, Wisconsin
Cameron, John, Stanford University, Stanford, California
Christensen, Bjorn, Carlsberg Laboratory, Valby, Denmark
Das, Asis, National Institutes of Health, Bethesda, Maryland
Eccleshall, Thomas, Albert Einstein College of Medicine, New York, New York
Fowler, Robert, Princeton University, Princeton, New Jersey
Fujimura, Robert, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Herrmann, Richard, California Institute of Technology, Pasadena, California
Hollis, Richard, University of Iowa, Iowa City, Iowa
Maitra, Pabitra, Tata Institute, Bombay, India
Mohar-Betancourt, Oscar, University of Louvain, Louvain-la-Neuve, Belgium
Malone, Robert, University of Oregon, Eugene, Oregon
Pukkila, Patricia, Yale University, New Haven, Connecticut
Ruis, Helmut, University of Wien, Wien, Austria
Sripati, Conjeevaram, Albert Einstein College of Medicine, New York, New York

SEMINARS

Marmur, J., Albert Einstein College of Medicine. *DNA in yeast.*

Roth, R., Illinois Institute of Technology. *Genetic control of replication during meiosis.*

Gesteland, R. F., Cold Spring Harbor Laboratory. *In vitro suppression.*

Hartwell, L. H., University of Washington. *Genetic control of cell division in yeast.*

———*Integration of the cell cycle with the life cycle.*

Sherman, F., University of Rochester. *Deletions in the iso-1-cytochrome c gene.*

———*Nucleotide sequence governing initiation of translation.*

Prakash, L., University of Rochester. *Control and specificities of chemical mutagenesis.*

Lawrence, C., University of Rochester. *Genetic control of UV mutagenesis in yeast.*

Needleman, R., Albert Einstein College of Medicine. *Mitochondrial mutants of yeast.*

Manney, T., Kansas State University. *Genetic control and related functions of the α factor.*

Mortimer, R. K., University of California at Berkeley. *Genetic mapping in yeast.*

———*Gene conversion and postmeiotic segregation.*

Moens, P. B., York University. *Morphological aspects of mitosis and meiosis in yeast.*

Fink, G. R., Cornell University. *The "killer" phenomenon in yeast.*

———*The regulation of histidine biosynthesis in yeast.*

Ballou, C. E., University of California at Berkeley. *Genetic control and regulation of yeast cell wall mannans.*

EXPERIMENTAL TECHNIQUES IN NEUROBIOLOGY — July 7–July 26

In this Neurobiology II course, the neuromuscular junction of the frog and the central ganglia of the mollusc, *Aplysia,* were used as experimental preparations for training students in basic electrophysiological methods for cellular neurobiology. Examination of certain characteristics of the resting potential, action potential and synaptic potentials of these two preparations served as a framework for introducing the following techniques: microdissection, fabrication of single and multibarrelled capillary microelectrodes, intra- and extracellular recording and stimulating procedures, current clamp and voltage clamp circuitry, extra- and intracellular application of ions and drugs (ionophoresis and pressure injection), and intracellular staining for light and electron microscopy.

The last few days of the course were devoted to individual experimental projects chosen by the students—projects that permitted them to try techniques not taught in the course and to use biological preparations that they intended to study upon returning to their own laboratories.

INSTRUCTORS

Kehoe, JacSue, Ph.D., Ecole Normale Supérieure, Paris, France
Chiarandini, Dante, M.D., New York University School of Medicine, New York, New York
Kunze, Diana, Ph.D., University of Texas Medical Branch, Galveston, Texas
Stefani, Enrique, M.D., Instituto Politecnico Nacional, Mexico City, Mexico

PARTICIPANTS

Byers, A. Duncan, California Institute of Technology, Pasadena, California
Elliott, Ellen, California Institute of Technology, Pasadena, California
Froehner, Stanley, Harvard University, Cambridge, Massachusetts
Ghysen, Alain, California Institute of Technology, Pasadena, California
Hammerschlag, Richard, City of Hope National Medical Center, Duarte, California
Kushner, Pinky, University of Oregon, Eugene, Oregon
Lichtman, Jeff, Washington University, St. Louis, Missouri
Orr, Dominic, California Institute of Technology, Pasadena, California
Reichardt, Louis, Harvard University, Cambridge, Massachusetts
Server, Alfred, Stanford University, Stanford, California

IMMUNOGENETICS IN RELATION TO EXPERIMENTAL TUMOR IMMUNOLOGY — July 29–August 17

The course focused on how selective gene action governs the composition of cell surfaces, the basic methodology in the various systems discussed being the identification of single gene products by immunogenetic techniques. Three broad areas, all centered on the mouse as model, were covered by the seminars: the physiology and function of the immune system, developmental biology, and the expression of type-C viral genetic information.

INSTRUCTORS

Boyse, Edward, M.D., Sloan-Kettering Institute for Cancer Research, New York, New York
Fleissner, Erwin, Ph.D., Sloan-Kettering Institute for Cancer Research, New York, New York

PARTICIPANTS

Axelrod, Helena, Princeton University, Princeton, New Jersey
Burridge, Keith, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Chakraborty, Prasanta, Albert Einstein College of Medicine, New York, New York
Ellis, Ronald, Sloan-Kettering Institute for Cancer Research, New York, New York
Gutzeit, Herwig, Biozentrum, Basel, Switzerland
Kavathas, Paula, Immunobiology Research Center, Madison, Wisconsin
Kirsch, Ilan, Harvard Medical School, Boston, Massachusetts
Krog, Hans-Henrik, Fibiger Laboratory, Copenhagen, Denmark
Ledbetter, Jeff, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin
Ludwig, Hanns Otto, Institut fur Virologie, Giessen, West Germany
Muschel, Ruth, Albert Einstein College of Medicine, New York, New York
O'Brien, Stephen, National Institutes of Health, Bethesda, Maryland
Reinke, Claudia, University of Freiburg, Freiburg, West Germany
Rose, Stephen, University of Virginia, Charlottesville, Virginia
Shen, Fung-Win, Sloan-Kettering Institute for Cancer Research, New York, New York
Swartzendruber, Douglas, Los Alamos Laboratory, University of California, Los Alamos, New Mexico
Tachovsky, Thomas, Wistar Institute, Philadelphia, Pennsylvania
Theze, Jacques, Salk Institute, San Diego, California
Wallenfels, Barbara, Children's Hospital, Boston, Massachusetts
Weber, Jeff, New York University Medical School, New York, New York

SEMINARS

Boyse, E. A., Sloan-Kettering Institute for Cancer Research. *Basic aims and techniques of cell surface immunogenetics (I), (II).*

Gershon, R. K., Yale University. *Introduction to cellular immunology.*

Cantor, H., Harvard University. *Ly differentiation components expressed on functionally different subclasses of T cells.*

Paul, W., National Institutes of Health. *Ir gene products and regulation of the immune response.*

——— *Ia antigens and cell interactions.*

Goldstein, G., Sloan-Kettering Institute for Cancer Research. *The physiology of thymic differentiation.*

Scheid, M., Sloan-Kettering Institute for Cancer Research. *Lymphoid cell differentiation from precursor cells in vitro.*

Artzt, K., Cornell University. *Introduction to the T-locus.*

Spiegelman, M., Cornell University. *The embryology of T-locus mutants.*

Dooher, G., Cornell University. *T-locus effects on sperm.*

Solter, D., Wistar Institute. *Teratomas as a system for studying development.*

Artzt, K., Cornell University. *A T-locus antigen on teratoma cells and normal embryonic cells.*

Flaherty, L., New York State Department of Health. *The* W *and* steel *loci.*

Hyman, R., Salk Institute. *Somatic cell genetic approaches to the analysis of cell surface antigens.*

Rakic, P., Harvard University. *Morphologic evidence for cell surface involvement in development of the nervous system.*

Schachner, M., Harvard University. *Differentiation antigens of the nervous system in mice.*

Pincus, T., Stanford University. *Genetics of host-MuLV interactions (I).*

Lilly, F., Albert Einstein College of Medicine. *Genetics of host-MuLV interactions (II).*

Levy, J., University of California at San Francisco. *Xenotropic viruses.*

Fleissner, E., Sloan-Kettering Institute for Cancer Research. *MuLV-specific proteins and antigens.*

Stephenson, J., National Institutes of Health. *MuLV gene expression in mouse cells.*

Lerner, R., Scripps Clinic and Research Foundation. *Expression of MuLV envelope glycoprotein in differentiated tissues.*

ADVANCED MOLECULAR GENETICS WORKSHOP — July 29–August 17

Students in this course performed the following experiments designed to instruct them in the principles and techniques of using bacteriophage λ as a vehicle for the isolation and manipulation of specific regions of the host chromosome: (1) Bacterial DNA fragments were inserted into the λ chromosome by base-pairing and ligation of the single-stranded ends formed by the action of restriction endonuclease *Eco*R1. Phage particles carrying this DNA were isolated following transfection of $CaCl_2$-treated host cells. (2) Transducing phage lines were isolated by insertion and abnormal excision of the λ chromosome in a host lacking the normal λ attachment site. New hosts lacking the λ attachment site were constructed and characterized. (3) Insertion mutants carrying λ DNA in a specific gene were isolated. Transducing lines carrying the insertion proximal and insertion distal segments of the gene were isolated and crossed to regenerate the mutant gene.

The students also isolated strains carrying mutations in *lacP* that partially relieved catabolite repression. The mutations were then crossed onto a *ϕ*80*plac* phage. In addition, a restriction enzyme analysis of the *lac* region in *ϕ*80*plac* DNA was carried out using the *Hin*II enzyme. A study of RNA polymerase binding to *lacP* mutant DNA was carried out using the *Hin*II digest pattern.

Second-site revertants were selected from *E. coli* strains which carried temperature-sensitive mutations in RNA polymerase. Those that were simultaneously cold-sensitive were selected for analysis. These were mapped and shown to occur in several different regions of the chromosome. Both 2-aminopurine EMS and NG were employed as mutagens.

INSTRUCTORS

Miller, Jeffrey H., Ph.D., University of Geneva, Geneva, Switzerland
Reznikoff, William S., Ph.D., University of Wisconsin, Madison, Wisconsin
Weisberg, Robert, Ph.D., National Institutes of Health, Bethesda, Maryland

PARTICIPANTS

Barkley, Mary, University of California at San Diego, La Jolla, California
Fralick, Joe, Texas Technological University School of Medicine, Lubbock, Texas
Galas, David J., University of California, Livermore, California
Garg, Govind K., University of Illinois, Urbana, Illinois
Haas, Robert A., Columbia University, New York, New York
Mattes, Ralf E., University of Regensburg, Regensberg, West Germany
Oliver, Donald, Tufts University, Boston, Massachusetts
VanVliet, Francoise, University of Brussels, Brussels, Belgium
Winston, Fred, Massachusetts Institute of Technology, Cambridge, Massachusetts
Wood, Janet, Cornell University, Ithaca, New York

SEMINARS

Beckwith, J., Harvard Medical School. *Lac fusions as a tool for genetic analysis.*

Gilbert, W., Biological Laboratories, Harvard University. *Repressor-operator interactions.*

Zipser, D., Cold Spring Harbor Laboratory. *Protein splicing.*

Roberts, R., Cold Spring Harbor Laboratory. *Biochemistry of restriction enzymes.*

Maniatis, T., Harvard University. *Lambda operator sequences.*

Broker, T., Cold Spring Harbor Laboratory. *The DNA sequence organization of F and F-primes, and the sequences involved in Hfr formation.*

NEUROBIOLOGY OF *DROSOPHILA MELANOGASTER* WORKSHOP — August 4–August 24

This course was designed for students with no previous experience in either handling *Drosophila* or in electrophysiological recording techniques. The basic objective of the course was to introduce the students to a broad range of research activities in which *Drosophila* is being used to study the nervous system, with emphasis on those research activities in which the use of mutants appears to be particularly appropriate or fruitful.

The course began with an introduction to *Drosophila* culture techniques, elements of genetics and the techniques of mutagenesis. Approximately half of the course was spent on electrophysiological studies. The following recording techniques were introduced in order of increasing technical difficulty: extracellular ERG recordings, larval muscle recording and indirect flight muscle recording, and intracellular recordings of the photoreceptor potentials. All laboratory work was accompanied by lectures on the *Drosophila* visual and flight motor systems. The last section of the course introduced the students to the vision-mediated behavior of *Drosophila*, including the optomotor response of the flying and walking insects and a study of the pattern-induced orientation behavior of flying insects under simple stimulus conditions.

Three other topics, for which no laboratory experiments were performed, were covered by speakers. These included the application of tissue culture techniques to *Drosophila* neurons, temperature-sensitive neurological mutations, and learning in *Drosophila*.

INSTRUCTORS

Pak, William L., Ph.D., Purdue University, West Lafayette, Indiana
Hartl, Daniel L., Ph.D., Purdue University, West Lafayette, Indiana
Buchner, Erich, Ph.D., Max-Planck-Institut für biologische Kybernetik

ASSISTANTS

Wilcox, Mike, B.S., Purdue University, West Lafayette, Indiana
Conrad, Sherry, Purdue University, West Lafayette, Indiana

PARTICIPANTS

Aceves-Pina, Efrain O., Massachusetts Institute of Technology, Cambridge, Massachusetts
Gepner, Janice, Massachusetts Institute of Technology, Cambridge, Massachusetts

King, Robert, Northeastern University, Boston, Massachusetts
Koerner, James, University of Minnesota, Minneapolis, Minnesota
Larrivee, Denis, Purdue University, West Lafayette, Indiana
Minke, Baruch, Purdue University, West Lafayette, Indiana
Mulrennan, S. Cecilia, Regis College, Weston, Massachusetts
Orr, Dominic, California Institute of Technology, Pasadena, California
Serra, Virginia, University of Calabria, Rende, Italy
Threlkeld, Stephen, McMaster University, Ontario, Canada

SEMINARS

Pak, W., Purdue University. *Use of mutants to study the photoreceptor process.*

Hartl, D., Purdue University. *Chromosome mechanics.*

Donady, J., Connecticut Wesleyan University. *Genetic approach to muscle and nerve differentiation.*

Kankel, D., Yale University. *Genetic mosaics and development of the nervous system.*

Wong, P., City of Hope National Medical Center. *Genetic control of flight motor function in* Drosophila melanogaster (I), (II).

Hall, L., Massachusetts Institute of Technology. *Isolation and use of temperature-sensitive neurological mutants of* Drosophila melanogaster.

——*Localization of acetylcholine receptors in* Drosophila.

Buchner, E., Max-Planck-Institut für biologische Kybernetik. *Movement detection by processing spatially sampled visual information.*

Quinn, C., Princeton University. *Genetic approach to learning in* Drosophila.

BASIC PRINCIPLES OF NEUROBIOLOGY — June 13–July 3

This course was designed for approximately 20 students, who, with little or no previous knowledge, wished to familiarize themselves with the conceptual bases of neurobiology and current research in the area. The course consisted of lectures, discussion sessions and quizzes, and laboratory demonstrations.

Initial lectures focused on the elementary properties of a nerve cell, the resting and action potential mechanisms, excitatory and inhibitory synaptic transmission, cellular integration, and morphology. Later the course considered how the basic properties of the nerve cell could be used to explain the properties of more complex nervous tissue, such as the spinal cord, the retina and the visual cortex. Because of the importance of neuronal development in comprehending nervous system function, several lectures were devoted to this topic.

Besides these areas, the following topics which are presently generating enthusiasm among the neurobiological community were discussed: the nature and regulation of the acetylcholine receptor, oculomotor control, plasticity in both invertebrate and mammalian control nervous systems, and the neurological basis of language.

After each lecture, students were questioned on the major points by the instructors to ensure maximum comprehension and retention. In addition, demonstrations were given of the electrophysiological properties of both the frog neuromuscular junction and the leech CNS.

INSTRUCTORS

Kelly, Regis B., Ph.D., University of California Medical Center, San Francisco, California
Dennis, Michael, Ph.D., University of California Medical Center, San Francisco, California
Frank, Eric, Ph.D., Harvard Medical School, Boston, Massachusetts
Shatz, Carla, Harvard Medical School, Boston, Massachusetts

PARTICIPANTS

Beckman, Jacques S., Edinburgh University, Edinburgh, Scotland
Boulter, James, Dartmouth Medical School, Hanover, New Hampshire
Byers, A. Duncan, California Institute of Technology, Pasadena, California
Eley, Susan, University of Leicester, Leicester, England
Ghysen, Alain, California Institute of Technology, Pasadena, California
Gibbs, Warren, University of Geneva, Geneva, Switzerland

Grunhagen, Hans-Heinrich, Institut Pasteur, Paris, France
Lampert, Murray A., Princeton University, Princeton, New Jersey
Lester, Bruce R., Purdue University, West Lafayette, Indiana
Lichtman, Jeff William, Washington University, St. Louis, Missouri
Mathies, Richard A., Yale University, New Haven, Connecticut
Mullner, Hubert, University of Konstanz, Konstanz, Germany
Murray, Mark J., University of Oregon Health Sciences Center, Portland, Oregon
Orr, Dominic P., California Institute of Technology, Pasadena, California
Paul, Dieter, Salk Institute, San Diego, California
Pellegrini, Marleen J., University of California, Davis, California
Segre, Camilla, University of Milan, Milan, Italy
Singer, Judith, City of Hope National Medical Center, Duarte, California
Strong, Peter N., University of California, San Francisco, California
Symonds, Barbara, Princeton University, Princeton, New Jersey

SEMINARS

Nicholls, J., Stanford University Medical School. *Introduction: Electrical signaling in the nervous system.*
——*Ionic basis of resting and action potentials.*
——*Experimental evidence.*
——*Passive electrical properties.*
——*Chemical and electrical synapses.*
——*Permeability changes produced by transmitters.*
——*Quantal release of transmitter.*
Nauta, W., Massachusetts Institute of Technology. *Overall organization of the brain* (I), (II).
Kuno, M., University of North Carolina. *Synaptic transmission in the spinal cord.*
——*Organization of the stretch reflex.*
——*Sensory transduction and adaptation.*
Dowling, J., Harvard Medical School. *Anatomy of the retina.*
——*Physiology of the retina.*
Hubel, D., Harvard Medical School. *The lateral geniculate.*
——*Anatomical organization of the visual cortex.*
——*Physiological organization of the visual cortex.*
——*Depth perception.*
——*The vestibular system.*
——*Color vision.*
Cowan, M., Washington University School of Medicine. *Embryology of the brain.*
——*Development of synaptic connections* (I), (II).
Dennis, M., University of California at San Francisco. *Formation of neuromuscular junctions.*
Patterson, P., Harvard Medical School. *Tissue culture as a tool for studying the nervous system.*
——*Transmitter synthesis and release by sympathetic ganglion cells in vitro.*
Schachter, M., Boston Children's Hospital. *Cell surface antigens.*
Nicholls, J., Stanford University Medical School. *Plasticity in the leech CNS.*
Frank, E., Harvard Medical School. *Regeneration of neuromuscular junctions.*
Kelly, R., University of California at San Francisco. *Synthesis and release of neurotransmitters.*
Geschwind, N., Boston City Hospital. *Neurological basis of language.*
Hall, Z., Harvard Medical School. *Structure of the acetylcholine receptor.*
——*Regulation of the acetylcholine receptor.*
Shatz, C., Harvard Medical School. *Visual cortex of the Siamese cat.*
LaVail, J., Harvard Medical School. *Tracing pathways in the CNS.*
Robinson, D., Wilmer Institute, Johns Hopkins University. *Oculomotor control in the monkey.*
Wiesel, T., Harvard Medical School. *Modification of the visual cortex by visual experience.*

Some speakers at the Synapse meeting and illustrations from Symposium Volume XL. Clockwise from top: E. Rojas/J. Nicholls; schematic drawing of gap junction (Goodenough); sketches illustrating computational theory of texture vision (Marr); "leeches taking a foothold" (Miyazaki et al.); diagram of components of model for mechanism of axonal transport (Schwartz et al.); R. Guillery/E. Barrett/T. Wiesel/R. Rahamimoff/A. Kelly; neurons in muscle cultures seeded with cells isolated from spinal cords (Fischbach et al.). Center: *(top)* W. Loewenstein/P. Rakic; *(bottom)* S. Kuffler/A. Hodgkin/S. Brenner. *(Photos of speakers by Steve Burden)*

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

The way we perceive bits of information, often to memorize, and later to think with, has long proved a major challenge to the scientific mind. In attacking this problem we assume that no paradox exists in using our brains to tell us what they are and how they function. Instead we largely worry whether we have yet evolved into a life-form intelligent enough to probe the myriad levels of complexity that underly even the simplest forms of rational behavior.

The fundamental units that must be studied are the nerve cells and the synapses which link them together. In doing so we must understand how electrical impulses are transmitted along neurons as well as work on the factors that determine how, when and where the synaptic interconnections are made. By now many of the questions surrounding the nerve impulse itself have been resolved. But the question of what synapses are and how they are made remains a mystery that currently is intriguing to an increasingly large number of the world's better scientists.

The time thus seemed right to choose the synapse as the focus for our 40th Symposium. Helping to arrange the program were Drs. Seymour Benzer, Eric Kandel, Stephen Kuffler, John Nicholls, David Potter and Gunther Stent. Their combined list of suggested speakers revealed such a great diversity of interests and emphasis that decisions regarding the final program at times seemed somewhat capricious. The end result, however, was a most exciting meeting with over two hundred and forty people in attendance. Most pleasantly, many of our guests told us that it was the best gathering of neurobiologists that has occurred in their memories.

Support for this meeting, in particular in the form of travel funds for our speakers from abroad, came from the National Science Foundation, the National Institutes of Health, and the U.S. Energy and Research Development Administration. Their continued support for so many years is most greatly appreciated.

TUESDAY EVENING, June 3

Welcoming Remarks: J.D. Watson, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Opening Address: S.W. Kuffler, Harvard Medical School, Boston, Massachusetts

WEDNESDAY MORNING, June 4

Chairperson: R. Rahamimoff, Hebrew University-Hadassah Medical School, Jerusalem, Israel

S.L. Palay and V. Chan-Palay, Departments of Anatomy and Neurobiology, Harvard Medical School, Boston, Massachusetts: "The fine structural analysis of synapses in the neuropil."

J.E. Heuser, Department of Physiology, University of California, San Francisco: "Evidence for selective retrieval of synaptic vesicle membrane following exocytosis at the frog neuromuscular junction."

M.V.L. Bennett, P.G. Model and S.M. Highstein, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York: "Stimulation induced depletion of vesicles, fatigue of transmission and recovery processes at a vertebrate central synapse."

U.J. McMahan, S. Roper and D. Purves, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Acetylcholine sensitivity of multiply innervated autonomic neurons."

WEDNESDAY EVENING, June 4

Chairperson: P. Greengard, School of Medicine, Yale University, New Haven, Connecticut

T.S. Reese, LNNS, NINCDS, NIH, Bethesda, Maryland: "Transient structural changes accompany transmitter discharge at the frog neuromuscular junction."

R. Rahamimoff, S.D. Erulkar,* E. Alnaes, H. Meiri and S. Rotshenker, Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel; Department of Neurobiology, Harvard Medical School, Boston, Massachusetts; *Fellow of the Guggenheim Memorial Foundation. "Modulation of transmitter release by calcium ions and nerve stimuli at the frog neuromuscular junction."

B. Grafstein, Department of Physiology, Cornell University Medical College, New York: "The role of axonal transport in the function of the nerve terminal."

J.H. Schwartz, J.E. Goldman, R.T. Ambron and D.J. Goldberg, Department of Physiology, Division of Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York: "Transport at an axon bifurcation in an identified serotonergic neuron of *Aplysia*."

V.P. Whittaker, M.J. Dowdall, K. Wächtler and H. Zimmermann, Abteilung für Neurochemie, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, W. Germany: "Biochemical organization of cholinergic neurones."

THURSDAY MORNING, June 5

Chairperson: E.R. Kandel, College of Physicians and Surgeons, Columbia University, New York, New York

S. Benzer, Division of Biology, California Institute of Technology, Pasadena: "Genetic approaches to synaptic problems in *Drosophila*."

O. Siddiqi and S. Benzer,* Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India; *Biology Division, California Institute of Technology, Pasadena: "Neurophysiological defects in temperature-sensitive mutants of *Drosophila*."

C. Levinthal, F. Levinthal, V. LoPresti and E. Macagno, Department of Biological Sciences, Columbia University, New York: "Anatomy and development of identified cells in isogenic organisms."

J. White, J. Sulston, N. Thompson and S. Brenner, MRC Laboratory of Molecular Biology, Cambridge University, England: "Structure and development of ventral cord of C elegans."

P. Rakic, Department of Neuropathology, Harvard Medical School, and Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts: "Failure to bridge a missing synaptic link in mutant cerebellar cortex."

THURSDAY EVENING, June 5

Chairperson: S. Benzer, California Institute of Technology, Pasadena, California

C.F. Stevens, Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle: "Molecular basis for postjunctional conductance increases induced by acetylcholine."

H.C. Hartzell, S.W. Kuffler and D. Yoshikami, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "The number of acetylcholine (ACh) molecules in a quantum and the interaction between quanta at the subsynaptic membrane of the skeletal neuromuscular synapse."

K. Peper and F. Dreyer, Physiologisches Institut, Universität des Saarlandes, Homburg/Saar, Germany: "Quantitative iontophoresis and noise analysis at the voltage clamped frog skeletal muscle fibre."

C.M. Armstrong and F. Bezanilla, University of Rochester, New York; University of Chile, Santiago: "Gating current in axon membrane."

E. Rojas, Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France: "Gating mechanism for the activation of the Na-channels in nerve membranes."

FRIDAY MORNING, June 6

Chairperson: C. Levinthal, Columbia University, New York, New York

J.S. Kelly and F. Dick, MRC Neurochemical Pharmacology Unit, Medical School, Cambridge, England: "Differential labeling of glial cells and GABA-inhibitory interneurons and nerve terminals following the microinjection of [β–^{3}H]alanine, [^{3}H]DABA and [^{3}H]GABA into single folia on the cerebellum."

M.A. Raftery, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena: "Structural and functional characteristics of an acetylcholine receptor."

A. Karlin, M.G. McNamee, C.L. Weill and R. Valderrama, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York: "Facets of acetylcholine receptor structure."

J.P. Changuex, J.P. Bourgeois, A. Brisson, M. Briley, J. Cartaud, P. Devaux, H. Grunhagen, M. Moreau, J.L. Popot, A. Sobel and M. Weber, Institut Pasteur and Université Paris VI and VII, France: "Functional properties of the cholinergic receptor protein from torpedo electric organ in its membrane-bound, purified and reassociated states."

A. Maelicke and E. Reich, The Rockefeller University, New York: "The acetylcholine receptor. II. Ligand binding and agonist-antagonist discrimination by purified receptor."

FRIDAY EVENING, June 6

Chairperson: J.P. Changeux, Institut Pasteur, Paris, France

M.R. Bennett and A.G. Pettigrew, Neurobiology Laboratory, University of Sydney, Australia: "The formation of neuromuscular synapses."

M.J. Dennis, C.A. Ort and J.W. Yip, Department of Physiology, University of California, San Francisco: "Physiology of synapses developing in vivo."

P.N. Devreotes and D.M. Fambrough, Carnegie Institution of Washington, Baltimore, Maryland: "Turnover of ACh receptors in skeletal muscle."

J.P. Brockes and Z.W. Hall, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Acetylcholine receptors in normal and denervated muscle."

E. Frank, K. Gautvik and H. Sommerschild, Institutes of Physiology and Neurophysiology, University of Oslo, Norway: "Persistence of junctional acetylcholine receptors at mammalian neuromuscular synapses following denervation."

SATURDAY MORNING, June 7

Chairperson: D. Potter, Harvard Medical School, Boston, Massachusetts

G. Fischbach, Department of Pharmacology, Harvard Medical School, Boston, Massachusetts: "Nerve-muscle junction formation in vitro."

P.G. Nelson, Behavioral Biology Branch, NICHD, NIH, Bethesda, Maryland: "Factors related to synaptogenesis in tissue culture."

Y. Kidokoro, S. Heinemann and B.L. Brandt, The Salk Institute, La Jolla, California: "Synapse formation and nerve trophic effect on muscle cell lines."

P.H. O'Lague, P.R. MacLeish, C.A. Nurse, P. Claude, E.J. Furshpan and D.D. Potter, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Further observations on the formation of cholinergic synapses by developing sympathetic neurons in dissociated cell culture."

P. Patterson, L. Chun and L. Reichardt, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Biochemical studies on primary sympathetic neurons grown in cell culture."

SATURDAY EVENING, June 7

Chairperson: B. Grafstein, Cornell University Medical College, New York, New York

E.R. Kandel, V. Castellucci and M. Brunelli, Division of Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York: "A common presynaptic locus for the synaptic mechanisms underlying short-term habituation and sensitization of the gill-withdrawal reflex in *Aplysia*."

J.K.S. Jansen, M.C. Brown and D. Van Essen, Institute of Physiology, University of Oslo, Norway: "Formation and elimination of synapses in rat skeletal muscle."

T. Lømo, Department of Neurophysiology, University of Oslo, Norway: "Control of ACh sensitivity in rat muscle fibers."

J.G. Nicholls, S. Miyazaki and B. Wallace, Department of Physiology, Stanford University School of Medicine, California: "Modification and regeneration of synaptic connections in cultured leech ganglia."

D. Van Essen and J.K.S. Jansen, Institute of Physiology, University of Oslo, Norway: "The specificity of reinnervation by identified sensory and motor cells in the leech."

M. Kuno, Department of Physiology, University of North Carolina Medical School, Chapel Hill: "Response of spinal motoneurons to section and restoration of peripheral motor connections."

SUNDAY MORNING, June 8

Chairperson: H.M. Gerschenfeld, Ecole Normale Supérieure, Paris, France

P. Greengard, Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: "Cyclic AMP, protein phosphorylation and synaptic transmission."

J.S. Kehoe, Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France: "Electrogenic effects of neutral amino acids on certain neurons of *Aplysia californica*."

E.A. Kravitz, P.D. Evans, B.R. Talamo, B.G. Wallace and B.A. Battelle, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Octopamine neurons in lobsters: Location, morphology, release of octopamine and possible physiological role."

M. Otsuka and S. Konishi, Department of Pharmacology, Tokyo Medical and Dental University, Japan: "Substance P and excitatory transmitter of primary sensory neurons."

R.B. Kelly, S.G. Oberg and G.M. Wagner, Departments of Biochemistry and Physiology, University of California, San Francisco: "Transmitter release modified by β-bungarotoxin."

SUNDAY AFTERNOON, June 8

Chairperson: S.L. Palay, Harvard Medical School, Boston, Massachusetts

D.A. Goodenough, Department of Anatomy, Harvard Medical School, Boston, Massachusetts: "Structural and permeability studies on isolated hepatocyte gap junctions."

J.P. Revel, California Institute of Technology, Pasadena: "Scanning electron microscope and freeze etch studies of cellular interactions."

W.R. Loewenstein, Department of Physiology and Biophysics, University of Miami School of Medicine, Florida: "Permeable junctions."

S.F. Schaeffer and E. Raviola, Department of Anatomy, Harvard Medical School, Boston, Massachusetts: "Ultrastructural analysis of functional changes in the synaptic endings of turtle photoreceptor cells."

MONDAY MORNING, June 9

Chairperson: A.L. Hodgkin, Cambridge University, Cambridge, England

D.A. Baylor and R. Fettiplace, Department of Physiology, Stanford University, School of Medicine, California: "Transmission of signals from photoreceptors to ganglion cells in a vertebrate retina."

G.H. Gold, G.L. Fain and J.E. Dowling, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: "Coupling between receptors in the marine toad *Bufo marinus*."

S. Ozawa, S. Hagiwara and A.E. Stuart, Department of Physiology, University of California at Los Angeles; Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Transmission of slow potentials from photoreceptor cells to ganglion cells in the giant barnacle."

A. Kaneko and H. Shimazaki, Department of Physiology, Keio University School of Medicine, Tokyo, Japan: "Transmission of signals from photoreceptors to bipolar and horizontal cells in the carp retina."

D.M.K. Lam, Department of Physiology, Harvard Medical School, Boston, Massachusetts: "Synaptic chemistry of horizontal cells in the vertebrate retina."

MONDAY AFTERNOON, June 9

Chairperson: G. Zweig, California Institute of Technology, Pasadena, California

D.H. Hubel and T.N. Wiesel, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Functional architecture of primary visual cortex in the normal monkey."

S.M. Zeki, Department of Anatomy, University College, London, England: "Synaptic specificity in the prestriate cortex of the monkey."

M.G. Yoon, Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada: "On topographic polarity of the optic tectum in adult goldfish."

T.N. Wiesel, D.H. Hubel and S. LeVay, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts: "Changes of ocular dominance columns in monocularly deprived monkeys."

C. Blakemore, R.C. Van Sluyters and J.A. Movshon, Physiological Laboratory, Cambridge, England: "Synaptic competition in the kitten's visual cortex."

R.W. Guillery and V.A. Casagrande, Department of Anatomy, University of Wisconsin, Madison: "Adaptive synaptic connections formed in the visual pathways of Siamese cats in response to aberrant inputs."

TUESDAY MORNING, June 10

Chairperson: D.H. Hubel, Harvard Medical School, Boston, Massachusetts

G.S. Stent and W.B. Kristan, Jr., Department of Molecular Biology, University of California, Berkeley: "Neuronal control of the leech swimming rhythm."

W. Reichardt, Max-Planck-Institut für Biologische Kybernetik, Tübingen, W. Germany: "Specification of nonlinear interactions in the visual system of the fly."

D. Marr, Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts: "Analyzing natural images."

G. Zweig, Department of Physics, California Institute of Technology, Pasadena, California: "Basilar membrane motion."

Summary: C.F. Stevens, University of Washington, Seattle, Washington

RNA TUMOR VIRUSES

Arranged by

J. THOMAS AUGUST, *Albert Einstein College of Medicine*

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WEDNESDAY EVENING, May 28

R.P. Junghans, C.A. Knight and P.H. Duesberg, Molecular Biology and Virus Laboratory, University of California, Berkeley: "Studies of the endogenous DNA polymerase activity of the Rous sarcoma virus."

J.M. Taylor and R, Illmensee, The Institute for Cancer Research, The Fox Chase Center, Philadelphia, Pennsylvania: "The site on the genome of an avian sarcoma virus at which primer is bound."

J.E. Dahlberg, E. Lund, F. Harada and G. Peters, Department of Physiological Chemistry, University of Wisconsin, Madison: "Interaction between primer and template RNAs of avian leukosis-sarcoma viruses."

W.A. Haseltine, A. Panet, D. Baltimore, G. Peters,* F. Harada* and J.E. Dahlberg,* Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; *Department of Physiological Chemistry, University of Wisconsin, Madison: "Specific binding of tryptophan transfer RNA to avian myeloblastosis virus reverse transcriptase."

E. Fritsch, S. Mizutani, G.M. Cooper and H.M. Temin, McArdle Laboratory, University of Wisconsin, Madison: "Formation of the provirus of spleen necrosis virus."

R.C. Gallo, M.S. Reitz and J.W. Abrell, NIH, Bethesda, Maryland: "Transcription of exogenous viral 70S RNA by purified reverse transcriptase from three primate and one murine RNA tumor virus."

E. Rothenberg and D. Baltimore, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: "Studies on reverse transcription in vitro: Improved yield and length of product."

THURSDAY MORNING, May 29

M. Linial and P. Neiman,* Department of Medicine, University of Washington; *Fred Hutchinson Cancer Research Center, Seattle, Washington: "Analysis of subgroup E virus replication in C/EV-chick cells."

A.T. Khoury and H. Hanafusa, The Rockefeller University, New York: "Provirus DNA after single and multiple infections with avian tumor virus."

R.V. Guntaka, O.C. Richards, J. Tal, P. Shank, J.M. Bishop and H.E. Varmus, Department of Microbiology, University of California, San Francisco: "Intermediates in the synthesis of proviral DNA of avian sarcoma virus."

D. Stehelin, H.E. Varmus and J.M. Bishop, Department of Microbiology, University of California Medical Center, San Francisco: "Detection of nucleotide sequences associated with transformation by avian sarcoma viruses."

M. Shoyab and M.A. Baluda, Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles: "Host induced modifications of avian oncornaviruses."

A.M. Gianni, D. Smotkin, S. Rozenblatt and R. Weinberg, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: "Free proviral DNA from Moloney MuLV-infected mouse cells."

G.G. Lovinger, R.V. Gilden and M. Hatanaka, Flow Laboratories, Inc., Rockville, Maryland: "Synthesis of murine virus-specific DNA in newly infected cells."

H. Okabe, R.V. Gilden and M. Hatanaka, Flow Laboratories, Inc., Rockville, Maryland: "Analyses of type C interspecies pseudotype viruses."

D.P. Nayak and A.R. Davis, Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles: "The nature of the endogenous oncornaviral DNA sequences in mammalian cells."

THURSDAY EVENING, May 29

J.N. Ihle, J.G. Farrelly, F.T. Kenney and D. Joseph, Oak Ridge National Laboratory, Tennessee, and Basic Cancer Research Program, Frederick Cancer Research Center, Frederick, Maryland: "Evidence for sequence differences in the 34S RNA subunits of AKR murine leukemia virus."

L.-H. Wang, K. Beemon, P. Vogt and P. Duesberg, Department of Molecular Biology and Virus Laboratory, University of California, Berkeley: "Mapping of RNase T_1-resistant oligonucleotides and biological functions of tumor virus RNAs."

J.M. Coffin, M.A. Billeter and C. Weissmann, Institut für Molekularbiologie, Universität Zürich, Switzerland: "The order of large oligonucleotides in Rous sarcoma virus RNA."

J.P. Bader and D.A. Ray, Chemistry Branch, NCI, NIH, Bethesda, Maryland: "Analysis of oncornavirus RNA."

J.M. Bailey, W.W. Bender, N. Davidson, S. Hu, H.J. Kung, R.M. McAllister and M.O. Nicolson, Department of Chemistry, California Institute of Technology, and Children's Hospital, University of Southern California Medical School, Los Angeles: "Structure of RD-114 and related RNAs."

Vannevar Bush Lecture Hall, the site of our summer meetings (*Photo by Jack Richards*)

J. Maisel, E. Scolnick and P. Duesberg, Department of Molecular Biology, University of California, Berkeley, and NCI, NIH, Bethesda, Maryland: "Differences in size and base sequence between the RNA components of Harvey sarcoma virus."

N. Tsuchida and K. Fujinaga, Department of Molecular Biology, Sapporo Medical College, Cancer Research Institute, Sapporo, Japan: "Viral genome RNA of Harvey sarcoma virus."

P. Duesberg, S. Kawai, L.-H. Wang and H. Hanafusa, Department of Molecular Biology, University of California, Berkeley, and The Rockefeller University, New York: "RNAs of a replication-defective and a corresponding nondefective Rous sarcoma virus."

E. Stavnezer, L. Fanshier and J.M. Bishop, Department of Microbiology, University of California Medical Center, San Francisco: "The synthesis of avian sarcoma virus RNA involves a precursor and symmetric transcription."

FRIDAY MORNING, May 30

D.M. Galehouse and P. Duesberg, Department of Molecular Biology and Virus Laboratory, University of California, Berkeley: "RNA and proteins of Kirsten sarcoma-xenotropic murine leukemia virus propagated in rat and duck cells."

R.A. Lerner, B.C. Del Villano and C.W. Wilson, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: "Anatomy of expression of endogenous gp70 during murine development and differentiation."

R. Eisenman, F. Zucco,* H. Diggelmann* and V.M. Vogt,† Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; *Swiss Institute for Cancer Research, Lausanne, Switzerland; †Institute for General Microbiology, Bern, Switzerland: "Further studies on the biosynthesis of the internal structural proteins of avian RNA tumor viruses."

S.Z. Shapiro and L. Eoyang, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: "High molecular weight precursor proteins of Rauscher murine leukemia virus."

S. Hino, J.R. Stephenson and S.A. Aaronson, NCI, NIH, Bethesda, Maryland: "Analysis of immunologic determinants of mammalian RNA type C viral glycoproteins."

L.F. Velicer, G.F. Okasinski, B.B. Mason and A.J. Conley, Michigan State University, East Lansing: "Feline leukemia virus gene expression: Analysis of newly synthesized polypeptides."

J.A. Bilello, S.Z. Shapiro and M. Strand, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: "Evidence for linked expression of gp69/71 and transformation genes in a defective Kirsten murine sarcoma virus."

W.G. Robey, M.K. Oskarsson, D.K. Haapala,* P.J. Fischinger* and G.F. Vande Woude, Viral Biology Branch and *Viral Leukemia and Lymphoma Branch, NCI, NIH, Bethesda, Maryland: "The identification of cleavage products of FeLV(MSV) virion associated p60 as structural polypeptides."

E. Fleissner, T. Pincus, D. Buchhagen, P. O'Donnell, H. Ikeda and J.-S. Tung, Memorial Sloan-Kettering Cancer Center, New York: "Biochemical and serological evidence for polymorphism of murine leukemia viruses."

M. Strand and J.T. August, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: "Relatedness of mammalian RNA tumor viruses."

FRIDAY EVENING, May 30

T.L. Benjamin and E. Goldman, Department of Pathology, Harvard Medical School, Boston, Massachusetts: "Effect of murine leukemia virus upon infections with a nontransforming mutant of polyoma virus."

N.H. Hopkins and P. Jolicoeur, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Cell to cell heterogeneity of expression of MLV by NIH 3T3 cells infected by horizontal spread of N-tropic virus derived from Balb/c and isolation of three biologically distinguishable N-tropic viruses."

K. Huebner and C.M. Croce, The Wistar Institute, Philadelphia, Pennsylvania: "Segregation of the murine sarcoma virus genome in somatic cell hybrids between normal mouse cells and murine sarcoma virus transformed human cells."

R. Jaenisch, H. Fan and B. Croker, The Salk Institute, San Diego, California: "Infection of preimplantation mouse embryos with leukemia virus: Tissue distribution of viral DNA and RNA and leukemogenesis in the adult animal."

R.W. Tennant, B. Schluter,* W.-K. Yang and A. Brown,* Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory, Tennessee, *Department of Microbiology, University of Tennessee, Knoxville: "Evidence for a product from mouse cells with the *Fv-1* locus which inhibits leukemia virus infection."

R. Soeiro, M. Sveda, U. Ray, T. Krontiris and B.N. Fields, Departments of Cell Biology and Medicine, Albert Einstein College of Medicine, Bronx, New York: "Host restriction of Friend leukemia virus."

S.K. Datta and R.S. Schwartz, Hematology Service, New England Medical Center Hospital, and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts: "Trans-tropism of leukemia viruses in lymphocytes."

R.A. Steeves, K.J. Blank and F. Lilly, Departments of Developmental Biology and Cancer and of Genetics, Albert Einstein College of Medicine, Bronx, New York: "Genetic control of Friend virus-transformed colony-forming cells by the *Fv-2r* gene in mice."

P. Roy-Burman, B.K. Pal, R.M. McAllister and M.B. Gardner, University of Southern California School of Medicine, Los Angeles: Structural phosphoproteins of mammalian RNA tumor viruses."

SATURDAY MORNING, May 31

S.S. Kalter, R.L. Heberling, S.T. Barker, O.S. Weislow and A. Hellman,* Department of Microbiology and Infectious Diseases, Southwest Foundation for Research and Education, San Antonio, Texas; *Office of Biohazards and Environmental Control, NCI, Bethesda, Maryland: "Baboon (*P. cynocephalus*) oncornaviruses."

H.L. Robinson and L.B. Crittenden,* Department of Medicine, Stanford University, California; *Avian Physiology Laboratory, East Beltsville, Maryland: "The existence, expression and biological activity of inducible C-type viruses in chicken cells."

Ch. Moroni and G. Schumann,* Friedrich Miescher-Institut, Basel, Switzerland; *Research Department, Pharma Division, CIBA-GEIGY, Ltd., Basel, Switzerland: "Induction of endogenous C-type viruses in lymphoid cells of mice."

J.R. Stephenson, S.R. Tronick, R.K. Reynolds and S.A. Aaronson, NIH, Bethesda, Maryland: "Distribution of endogenous type C RNA viruses among inbred strains of mice."

U.R. Rapp and R.C. Nowinski, McArdle Laboratory of Cancer Research, University of Wisconsin, Madison: "Induction of XC^- and XC^+ MuLV from mouse cells."

Y. Ikawa, Y. Inoue, M. Aida and K. Miyamoto,* Laboratory of Viral Oncology, Cancer Institute, Tokyo, Japan; *Department of Pathology, National Cancer Center Research Institute, Tokyo, Japan: "Increased release of type C virus during erythrodifferentiation of cultured Friend leukemia cells."

H.A. Young, E. M. Scolnick and W.P. Parks, NCI, NIH, Bethesda, Maryland: "Glucocorticoids and breast cancer viruses in tissue culture."

M.S. Reitz, Jr., F. Wong-Staal and R.C. Gallo, NCI, NIH, Bethesda, Maryland: "A biochemical characterization of murine intracisternal type-A particles and comparison with murine type-C particles."

G. Todaro, R. Beneviste, C. Sherr, M. Lieber and R. Callahan, NIH, Bethesda, Maryland: "Infectious primate type C virus group: Evidence for an origin from an endogenous virus of the rodent, *Mus caroli*."

J.J. Haaijman, J. Brinkof, J. Ouwehand and P. Bentvelzen, REP-Institutes of the Netherlands Organization for Health Research TNO, Rijswijk (ZH), The Netherlands: "Oncornavirus immunoassay with the Sepharose bead immunofluorescence system."

SATURDAY AFTERNOON, May 31

R.R. Friis, D. Becker, R. Kurth and H. Bauer, Institut für Virologie, Justus Liebig Universität, Giessen, W. Germany, and the Imperial Cancer Research Fund Laboratories, London, England: "Two different types of transformation defective mutants of Rous sarcoma virus."

G. Calothy and B. Pessac, Foundation Curie, Institut du Radium, Orsay, France: "Induction of cell proliferation in chick embryo neuroretinal cells infected in vitro with avian tumor viruses: Genetic studies."

C. Metroka, S. Kawai and H. Hanafusa, The Rockefeller University, New York: "Temperature sensitive mutants of Rous sarcoma virus (RSV) in replication."

E. Hunter, M.J. Hayman, R.W. Rongey and P.K. Vogt, Departments of Microbiology and Pathology, University of Southern California, Los Angeles: "A mutant of RSV defective in virus assembly."

H. Yoshikura, Institut du Radium, Orsay, France: "Production of infectious murine sarcoma virus without detectable production of infectious murine leukemia virus from mouse cells."

P. Besmer and D. Baltimore, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: "The role of the envelope in xenotropic restriction and interference among murine RNA tumor viruses."

P.T. Peebles and B.I. Gerwin, Viral Leukemia and Lymphoma Branch, NCI, Bethesda, Maryland: "Viral DNA polymerase and p30 protein expression in murine and nonmurine cells transformed by murine sarcoma virus in the absence of detectable helper virus."

J.S. Rhim and R.J. Huebner, Microbiological Associates, Inc., and NCI, NIH, Bethesda, Maryland: "Characterization of nonproducer human cells induced by Kirsten sarcoma virus."

A.M. Wu, M.S. Reitz and R.C. Gallo,* Bionetics Research Laboratory, and *NCI, Bethesda, Maryland: "Studies on the synthesis of type-C virus and viral components by mouse fibroblasts nonproductively infected with murine sarcoma virus."

SUNDAY MORNING, June 1

N. Rosenberg, D. Baltimore and C.D. Scher, Massachusetts Institute of Technology, Cambridge, and Harvard Medical School, Boston, Massachusetts: "The direct transformation of lymphoid cells by Abelson murine leukemia virus."

D.M. Livingston, C. Ferguson and R. Gollogly, Sidney Farber Cancer Center, and Harvard Medical School, Boston, Massachusetts: "Relationship between the appearance of cysteine auxotropic cells and type C viral lymphomagenesis in the mouse."

L. Chieco-Bianchi, A. Colombatti, D. Collavo and G. Biasi, Laboratory of Experimental Oncology, University of Padova, Italy: "Murine sarcoma virus oncogenesis: Dependency on exogenous and endogenous type C virus expression."

L.R. Rohrschneider, R.R. Friis, R. Kurth, G. Pauli and H. Bauer, Institut für Virologie, Justus Liebig-Universität, Giessen, W. Germany: "Biochemical properties of tumor-specific cell surface antigens on avian oncornavirus transformed cells."

G.F. Rabotti and B. Teutsch, College de France, Laboratoire de Medecine Experimentale, Paris: "Antibody specification during oncogenesis by Rous sarcoma virus in the natural host."

J.N. Ihle, J.C. Lee, J. Longstreth and M.G. Hanna, Jr., Basic Cancer Research Program, Frederick Cancer Research Center, Frederick, Maryland: "Antigenic specificities of natural sera from $B6C3F_1$ mice."

T. Pincus, H. Snyder and E. Fleissner, Memorial Sloan-Kettering Cancer Center, New York: "Reactivities of hyperimmune and naturally occurring sera with type-C viruses and viral proteins."

D.P. Bolognesi, T. Mohanakumar, W. Schafer* and R.S. Metzgar,* Departments of Surgery and Immunology, Duke University Medical Center, Durham, North Carolina; *Max-Planck-Institut, Tubingen, Germany: "Antigenic relationships between Friend leukemia virus and human leukemia associated antigens."

G.R. Dreesman, D.L. Brill, D.L. Bronson, A.Y. Elliott, E.E. Fraley and J.L. Melnick, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas, and Division of Urology, University of Minnesota Medical School, Minneapolis: "Cross-reacting type-C virus p30 antigen in cultures derived from a human transitional cell tumor."

J.C.H. De Man, Chr. Zurcher, J. Brinkhof, P. Bentvelzen and S.O. Warnaar, Pathology Laboratory, State University, Leiden; Institute for Experimental Gerontology TNO; Radiobiological Institute TNO, Rijswijk; Laboratory for Physiological Chemistry, State University, Leiden, The Netherlands: "C-type virus antigens detected by immunofluorescence in human bone tumor cultures."

This meeting was partially funded by a grant from the National Cancer Institute, National Institutes of Health.

SV40, POLYOMA AND ADENOVIRUSES

Arranged by
TERRI GRODZICKER, *Cold Spring Harbor Laboratory*

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WEDNESDAY EVENING, August 13

P. Beard, N.H. Acheson and I.H. Maxwell,* Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland; *University of Geneva, Switzerland: "Strand-specific transcription of polyoma virus DNA early in productive infection and in transformed cells."

R. Kamen and H. Shure, Department of Molecular Virology, Imperial Cancer Research Fund Laboratories, London, England: "Virus-specified RNA in cells productively infected by polyoma virus, counting and mapping of molecular species."

N.H. Acheson and P. Beard, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: "Strand origin of polyoma virus-specific giant nuclear RNA and 16S and 19S messenger RNAs."

D.T. Simmons and M.A. Martin, National Institutes of Health, Bethesda, Maryland: "Synthesis of SV40-specific RNA in isolated nuclei."

Y. Aloni, O. Laub, M. Shani and Y. Reuveni, Department of Genetics, The Weizmann Institute of Science, Rehovot, Israel: "Control mechanisms of SV40 gene expression."

S. Lavi and A.J. Shatkin, Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: "Methylated SV40-specific RNA from nuclei and cytoplasm of infected BSC-1 cells."

M.H. Green and T.L. Brooks, Department of Biology, University of California at San Diego, La Jolla: "Isolation of two forms of SV40 transcriptional complexes from infected monkey cells."

B. Schaffhausen and W.T. Murakami, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: "Histones and histone acetylation in polyoma virus."

B. Schaffhausen and T. Benjamin, Department of Pathology, Harvard Medical School, Boston, Massachusetts: "Comparisons of the virion proteins of wild-type polyoma virus and transformation-defective host range mutants."

E. Frost and P. Bourgaux, Centre Hospitalier Universitaire, Sherbrooke, P.Q., Canada: "Decapsidation of polyoma virus."

T. Friedmann, Department of Pediatrics, School of Medicine, University of California at San Diego, La Jolla: "Polyoma protein processing."

W. Gibson, T. Hunter, M. Vogt and W. Eckhart, Salk Institute, San Diego, California: "Structural protein differences in a late mutant (ts59) of polyoma virus."

THURSDAY MORNING, August 14

T. Spillman, B. Spomer and L. Hager, Biochemistry Department, University of Illinois, Urbana: "Affinity of SV40 tumor antigen for various DNA molecules."

J.K. Collins and P. Tegtmeyer, Department of Microbiology, State University of New York, Stony Brook: "Binding of SV40 *A* protein to DNA."

D.M. Livingston, D. Jessel, T. Landau and D. Tenen, Division of Medical Oncology, Sidney Farber Cancer Center, Boston; Department of Medicine, Peter Bent Brigham Hospital, Boston; and Harvard Medical School, Boston, Massachusetts: "SV40 T antigen-DNA reactions."

K. Rundell and P. Tegtmeyer, Department of Microbiology, State University of New York, Stony Brook: "Biochemical characterization of the SV40 *A* protein."

S.I. Reed, J. Ferguson, J.C. Alwine and G.R. Stark, Department of Biochemistry, Stanford University Medical Center, California: "Different T antigens are induced by two different strains of SV40."

J.C. Alwine, S.I. Reed, J. Ferguson and G.R. Stark, Department of Biochemistry, Stanford University Medical Center, California: "The lability of SV40 T antigen in intact cells and in extracts of cells infected with wild-type or tsA mutant viruses."

D. Paulin, P. Gaudray* and F. Cuzin,* Institut Pasteur, Paris, France; *Centre de Biochimie, Université de Nice, France: "T-antigen synthesis in mouse cells transformed or infected by thermosensitive mutants of polyoma virus."

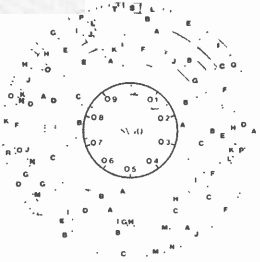
W.F. Mangel, S.T. Bayley,* T. Wheeler* and A.E. Smith,* Department of Cell Regulation and *Department of Molecular Virology, Imperial Cancer Research Fund, London, England: "Cell-free synthesis of SV40 early proteins."

C. Prives, M. Aboud, E. Gilboa, H. Aviv, M. Revel and E. Winocour,* Biochemistry Department and *Virology Section, The Weizmann Institute of Science, Rehovot, Israel: "The cell-free translation of different classes of SV40 mRNA."

S. Rozenblatt, K.J. Danna,* M. Gorecki,* R.C. Mulligan* and B.E. Roberts,* Center for Cancer Research and *Department of Biology, Massachusetts Institute of Technology, Cambridge: "Characterization of SV40-specific polypeptides synthesized in vitro."

S.T. Bayley, W.F. Mangel,* T. Wheeler and A.E. Smith, Department of Molecular Virology and *Department of Cell Regulation, Imperial Cancer Research Fund, London, England: "Cell-free synthesis and gene location of polyoma and SV40 structural proteins."

Adeno — SV40 — Polyoma
TUMOR VIRUS MEETING



COLD SPRING HARBOR
August 13 17 1975

Poster: Map of the positions at which SV40 DNA is cleaved by several restriction enzymes. (*Photo by Christine Healey*)

J.M. England, M.K. Howett and K.B. Tan, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: "The use of hypertonic labeling conditions to analyze protein synthesis in cells lytically infected or transformed by SV40."

THURSDAY EVENING, August 14

E.A. Craig, S.G. Zimmer, D. Carlson and H.J. Raskas, Departments of Pathology and Microbiology, Washington University School of Medicine, St. Louis, Missouri: "Analysis of adenovirus 2 nuclear RNA synthesized early in productive infection."

W.S.M. Wold and M. Green, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: "Adenovirus 2 genome expression and messenger RNA maturation at early and late stages of productive infection."

S.L. Bachenheimer and J.E. Darnell, The Rockefeller University, New York: "Evidence that high molecular weight adenovirus specific nuclear RNA is the precursor of cytoplasmic mRNA."

M. McGrogan, E.A. Craig and H.J. Raskas, Departments of Pathology and Microbiology, Washington University School of Medicine, St. Louis, Missouri: "Genome mapping of adenovirus 2 cytoplasmic RNA species transcribed late in productive infection."

R. Weinmann, J.A. Jaehning, H.J. Raskas and R.G. Roeder, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Missouri: "Function and regulation of RNA polymerases II and III in adenovirus-infected KB cells."

U. Pettersson, L. Philipson, C. Tibbetts and M.B. Mathews,* Department of Microbiology, The Wallenberg Laboratory, Uppsala University, Sweden; *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Mapping of VA RNA on adenovirus 2."

T.H. Carter and H.S. Ginsberg, Columbia University, New York: "Viral transcription in KB cells infected by temperature-sensitive "early" mutants of type 5 adenovirus."

S.J. Flint, Y. Werwerka-Lutz, A.S. Levine, J. Sambrook* and P.A. Sharp, Department of Biology, Center for Cancer Research, Massachusetts Institute of Technology,

Cambridge; *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Transcription of adenovirus 2/SV40 hybrid viruses."

J. Ortin, K.H. Scheidtmann, R. Greenberg and W. Doerfler, Institute of Genetics, University of Cologne, West Germany: "Characterization of viral-specific RNA in cells infected and transformed by adenovirus type 12."

E.L. Winnacker, R. Schilling, K.H. Schüller and B. Weingärtner, Institute of Genetics, University of Cologne, West Germany: "Location of origins and termini of adenovirus type 2 DNA replication."

M.S. Horwitz and J. Pan,* Department of Microbiology, Albert Einstein College of Medicine, New York; *Department of Human Genetics, Yale Medical School, New Haven, Connecticut: "Origin and direction of replication of adenovirus type 2 DNA."

FRIDAY MORNING, August 15

K.N. Subramanian, R. Dhar and S.M. Weissman, Yale University, New Haven, Connecticut: "Nucleotide sequences of a fragment of SV40 DNA spanning the origin of DNA replication."

M.C. Chen, E. Birkenmeier and N.P. Salzman, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: "Simian virus 40 DNA replication: Characterization of a gap at the DNA termination site."

K.K.-Y. Yu, J. Kowalski and W.P. Cheevers, Cancer Research Laboratory, University of Western Ontario, London, Canada: "Requirements for protein synthesis in polyoma virus DNA replication."

J.-E. Germond, B. Hirt, P. Oudet,* M. Gross-Bellard* and P. Chambon,* Swiss Institute for Experimental Cancer Research, Lausanne; *Institut de Chimie Biologique, Strasbourg, France: "Supercoiling of SV40 DNA and chromatin structure."

D.A. Golstein and M.R. Hall, Scripps Clinic and Research Foundation, La Jolla, California: "Generation of supertwists in polyoma and SV40 DNA."

M.A. Martin and P.M. Howley, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: "Synthesis of oligomeric supercoiled SV40 DNA molecules in productively infected monkey cells."

S.P. Goff and P. Berg, Department of Biochemistry, Stanford University Medical Center, California: "The structure and origin of SV40 closed circular dimers made late in lytic infection."

W. Keller, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Interaction of purified DNA-ligase and DNA-relaxing enzyme with SV40 DNA."

R.J. DeLeys and D.A. Jackson, Department of Microbiology, University of Michigan, Ann Arbor: "Structural studies on covalently closed, relaxed SV40 DNA."

D.E. Pulleyblank, M. Shure, D. Tang, J. Vinograd and P. Vosberg, California Institute of Technology, Pasadena: "Supercoiling induced by thermal fluctuations in solutions containing closed circular DNA and a highly purified nicking-closing enzyme from mouse cell nuclei."

D.A. Jackson and D.M. Igdaloff, Department of Microbiology, University of Michigan Medical Center, Ann Arbor: "Hairpins and covalently closed linears: New forms of SV40 DNA."

FRIDAY EVENING, August 15

W.A. Scott, W.W. Brockman, C.-J. Lai and D. Nathans, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: "Biological activities of SV40 deletion mutants."

T. Shenk, J. Carbon* and P. Berg, Department of Biochemistry, Stanford Medical Center, California; *Department of Biological Sciences, University of California, Santa Barbara: "Construction and characterization of viable deletion mutants of SV40."

C. Cole, T. Landers, T. Shenk, S. Manteuil, M. Dieckmann, S. Goff and P. Berg, Department of Biochemistry, Stanford University Medical Center, California: "Characterization of defective deletion mutants of SV40."

J.E. Mertz and P. Berg, Department of Biochemistry, Stanford Medical Center, California: "The complementation groups of SV40."

M. Fluck, R. Staneloni and T. Benjamin, Department of Pathology, Harvard Medical School, Boston, Massachusetts: "Complementation studies with nontransforming mutants of polyoma."

E. Lund, M. Fried and B. Griffin, Department of Tumour Virus Genetics, Imperial Cancer Research Fund, London, England: "Defective polyoma DNAs."

D. Davoli, D. Ganem, A.L. Nussbaum and G.C. Fareed, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: "Reiteration mutants of SV40: Characterization, cloning and biological properties."

G.R.K. Rao and M.F. Singer, Laboratory of Biochemistry, NCI, Bethesda, Maryland: "Studies on defective SV40 containing monkey DNA sequences."

T. Grodzicker, C. Anderson and J. Sambrook, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "The physical locations of ts mutations and structural genes in adenovirus DNA."

T. Harrison, F. Graham* and J. Williams, MRC Virology Unit, Institute of Virology, Glasgow, Scotland: *Department of Biology, McMaster University, Hamilton, Ontario, Canada: "Host-range mutants of human adenovirus type 5."

SATURDAY MORNING, August 16

A.J. Van der Eb, P.J. Abrahams, C. Mulder,* J.H. Lupker, F.L. Graham, A. Van de Voorde† and W. Fiers†, Laboratory for Physiological Chemistry, University of Leiden, The Netherlands; *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; †Laboratory for Molecular Genetics, University of Gent, Belgium: "Identification and isolation of the transforming genes of adenoviruses and SV40."

M.R. Green, G. Chinnadurai and J.K. Mackey, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: "The integration and expression of viral genes in Ad 12 transformed hamster cells."

J.K. McDougall, A.R. Dunn, P.H. Gallimore and F.H. Ruddle, Department of Cancer Studies, University of Birmingham, England, and Department of Biology, Yale University, New Haven, Connecticut: "Localization of integrated adenovirus DNA."

K. Huebner, F.L. Graham, A. Van der Eb and C.M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania, and Department of Physiological Chemistry, State University of Leiden, The Netherlands: "Chromosome assignment of the gene for adenovirus 5 T-antigen in adenovirus 5 transformed human cells."

K. Baczko, E. Fanning, J. Groneberg, J. Schick and W. Doerfler, Institute of Genetics, University of Cologne, West Germany: "Integration of adenovirus 2 DNA in productively infected KB cells."

I. Prasad, D. Zouzias and C. Basilico, Department of Pathology, New York University School of Medicine, New York: "The state of the viral DNA in rat cells transformed by polyoma virus."

W.R. Folk, B.R. Fishel and D.M. Anderson, Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor: "Viral DNA synthesis in (Py)ts-a BHK-CL 3 cells."

L. Bacheler and M. Vogt, Salk Institute, San Diego, California: "Virus-specific transcription in 3T3 cells transformed by a temperature-sensitive mutant, tsA, of polyoma virus."

P.M. Howley and M.A. Martin, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: "Human papovavirus KB: A physical map of the viral genome and quantitation of viral DNA sequences in transformed hamster cells."

A.H. Fried, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Heidelberg, W. Germany: "Demonstration of a homogeneous group of SV40-cellular hybrid DNA molecules in a transformed cell line."

J. Leong, D. Garfin, H. Goodman, H. Smith* and P. Rigby,† Department of Biochemistry and Biophysics, University of California, San Francisco; *School of Public Health, University of California, Berkeley; †Department of Biochemistry, Stanford University, California: "Detection and quantitation of viral RNA in SV40-infected cells."

M. Shani, Y. Aloni, E. Huberman and L. Sachs, Department of Genetics, The Weizmann Institute of Science, Rehovot, Israel: "Gene activation by transfer of mammalian chromosomes: Activation of simian virus 40 by transfer of fractionated chromosomes from transformed cells."

SATURDAY AFTERNOON, August 16

J. Corden, M. Engelking and G. Pearson, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: "The folded chromosome of adenovirus."

D.T. Brown, M. Westphal, B.T. Burlingham, U. Winterhoff and W. Doerfler, Institute of Genetics, University of Cologne, Germany: "The structure and composition of the adenovirus type 2 core."

E. Everitt and A.S. Levine, NCI, NIH, Bethesda, Maryland: "Identification of a precursor DNA-protein complex in adenovirus assembly."

K. Hosokawa and M. Sung,* Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; *Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale: "Characterization and biosynthesis of the third arginine-rich protein of adenovirus type 5."

W.W. Chin and J.V. Maizel, Jr., National Institute of Child Health and Human Development, Bethesda, Maryland: "In vivo identification and characterization of the early proteins in the Ad2-infected HeLa cell."

J.B. Lewis, J.F. Atkins, C.W. Anderson, P.R. Baum, R. Solem and R.F. Gesteland, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Mapping of Ad2 early polypeptides."

C.W. Anderson, J.B. Lewis,* P.R. Baum* and R.F. Gesteland,* Brookhaven National Laboratory, Upton, New York; *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "In vitro synthesis of proteins encoded by SV40 and the SV40 segment inserted into Ad2^{+}ND1 and Ad2^{+}ND4."

H. Westphal, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland: "Adenovirus 2 messenger RNA: Cleavage by RNase III and cell-free translation."

J. Weber, Centre Hospitalier Universitaire, Sherbrooke, P. Q., Canada: "Temperature sensitivity of processing of adenovirus type 2 (Ad 2) proteins."

R.S. Kauffman and H.S. Ginsberg, Columbia University, New York: "Characterization of a mutant of type 5 adenovirus defective in hexon transport."

SUNDAY MORNING, August 17

A.M. Lewis, Jr., Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: "Comparison of the efficiency of transformation of hamster and rat cells by Ad2 and nondefective Ad2-SV40 hybrids."

J. Avila and R.G. Martin, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland: "DNA synthesis in SV40-transformed cells."

J.L. Anderson, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland: "Critical role of SV40 gene A in regulation of transformed growth in mouse brain cells."

J.A. Robb, Department of Pathology, University of California at San Diego, La Jolla: "Regulation of SV40 tumor (T) antigen."

E.H. Postel and A.J. Levine, Department of Biochemical Sciences, Princeton University, New Jersey: "The temperature dependent stimulation of cellular thymidine kinase by SV40 tsA class mutants."

H.L. Ozer and M.L. Slater, The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: "Polyoma infection of temperature-sensitive mutants of 3T3 cells."

M. Graessmann and A. Graessmann, Institut für Molekularbiologie und Biochemie der Universität Berlin, Germany: "'Early' simian virus 40-specific RNA contains information for tumor antigen formation and chromatin replication."

M. Graessmann and A. Graessmann, Instit für Molekularbiologie und Biochemie der Universität Berlin, Germany: "Regulation of simian virus 40 late gene expression in nonpermissive cells."

M. Boccara and F. Kelly, Departement de Biologie Moleculaire, Institut Pasteur, Paris, France: "Susceptibility to SV40 and polyoma of cell lines derived from the mouse teratocarcinoma."

H.S. Smith, S. Turner, J. Leong* and P.W.J. Rigby,† School of Public Health, University of California, Berkeley; *Department of Biochemistry, University of California Medical Center, San Francisco; †Department of Biochemistry, Stanford University School of Medicine, California: "Effect of culture conditions on an SV40-transformed clone of Balb3T3."

E.G. Gurney, Department of Biology, University of Utah, Salt Lake City: "Revertants of SVT2 which show G1 inhibition of growth."

This meeting was partially funded by a grant from the National Cancer Institute, National Institutes of Health.

RNA POLYMERASES

Arranged by
MICHAEL CHAMBERLIN, *University of California at Berkeley*
RICHARD LOSICK, *Harvard University*

167 Participants

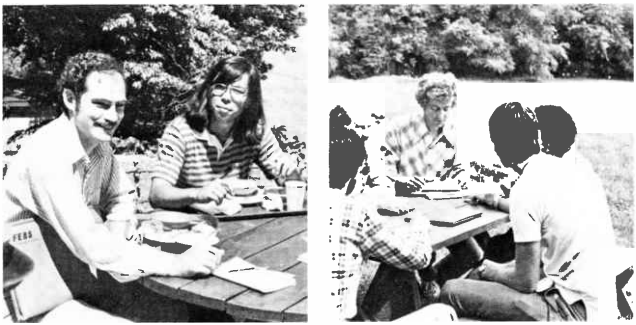
TUESDAY EVENING, August 19

Chairperson: M. Chamberlin, University of California, Berkeley, California

R. Hausmann and C. Tomkiewicz, Institut für Biologie III der Universität Freiburg, W. Germany: "Genetic analysis of template specificity of RNA polymerases (gene 1 products) coded by phage T3 × T7 recombinants within gene 1."

S. Clark, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: "Novel multi-subunit RNA polymerase induced by *Bacillus subtilis* phage PBS2."

S.C. Falco and L.B. Rothman-Denes, Department of Biophysics and Theoretical Biology, University of Chicago, Illinois: "Transcriptional activities induced by bacteriophage N4."



Richard Losick *(left, foreground)* and Michael Chamberlin *(right, center)* enjoying a brief respite from their duties as meeting organizers. *(Photos by Christine Healey)*

WEDNESDAY MORNING, August 20

Chairperson: R. Burgess, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin

P. Palm, A. Heil and W. Zillig, Max-Planck-Institut für Biochemie, Martinsried bei München, W. Germany: "Reconstitution of RNA polymerase from its isolated subunits as a method for the study of the function of single subunits."

J.D. Harding and S. Beychok,* Department of Biochemistry, University of California, San Francisco; *Departments of Biological Sciences and Chemistry, Columbia University, New York: "RNA polymerase assembly in vitro. Reactivation of denatured core enzyme and studies of assembly intermediates."

U.I. Lill, E.M. Behrendt and G.R. Hartmann, Institut für Biochemie der Ludwig-Maximilians-Universität München, W. Germany: "On the intergeneric hybridization of subunits of the DNA-dependent RNA polymerase."

H.R. Lill and G.R. Hartmann, Institut für Biochemie der Ludwig-Maximilians-Universität München, W. Germany: "On the oligomerization of the DNA-dependent RNA polymerase from *E. coli*.

C.-W. Wu, L. Yarbrough, Z. Hillel and F.Y.H. Wu, Department of Biophysics, Albert Einstein College of Medicine, New York: "Fluorescent probe studies on the sigma subunit of *Escherichia coli* RNA polymerase."

W. Stender, A. Stütz and K.H. Scheit, Abteilung Molekulare Biologie, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, W. Germany: "Modification of DNA-dependent RNA polymerase from *E. coli* by an alkylating derivative of rifamycin."

P. Konigsberg and S.R. Simon, Department of Biochemistry, State University of New York, Stony Brook: "MgATP inhibition of *E. coli* RNA polymerase."

L.R. Yarbrough and C.W. Wu, Albert Einstein College of Medicine, New York: "Interaction of *E. coli* RNA polymerase with rifampicin."

WEDNESDAY EVENING, August 20

Chairperson: J. Miller, University of Geneva, Geneva, Switzerland

M. Goman and J. Scaife, Department of Molecular Biology, University of Edinburgh,

Scotland: "Construction in vitro of λ transducing phages containing RNA polymerase genes."

J.B. Kirschbaum, J. Greenblatt, J.-D. Rochaix and B. Allet, Department of Molecular Biology, University of Geneva, Switzerland: "Studies with a λ *rif* transducing phage carrying the genes for the β and β′ subunits of *E. coli* RNA polymerase."

B. Young, S. Guterman, D. Boyd and A. Wright, Department of Molecular Biology, Tufts Medical School, Boston, Massachusetts: "Temperature-sensitive RNA synthesis caused by a mutation in the gene for the β′ subunit of *Salmonella typhimurium* RNA polymerase."

L.R. Brown, Department of Microbiology, Oregon State University, Corvallis: "Properties of the gene region encoding the β subunit of RNA polymerase and other RNA synthesis genes."

S.R. Jaskunas, R. Burgess,* L. Lindahl and M. Nomura, Institute for Enzyme Research and *McArdle Laboratory, University of Wisconsin, Madison: "Clustering of RNA polymerase genes with genes required for translation."

R. Lathe and J.-P. Lecocq, Laboratoire de Genetique, Universite Libre de Bruxelles, Rhode-St.-genese, Belgium: "A putative RNA polymerase mutation with a chromosomal location outside the *rif* gene cluster."

M. Schweiger, M. Hirsch-Kauffmann, W. Zillig, H. Ponta and M. Pfennig-Yeh, Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, Germany: "One RNA polymerase operon? No!"

J.H. Miller, J.B. Kirschbaum, I. Claeys, B. Molholt, S. Nasi, G. Gross, D.A. Fields and E.K.F. Bautz, Department of Molecular Biology, University of Geneva, Switzerland, and Department of Molecular Genetics, University of Heidelberg, Germany: "Mutations affecting *E. coli* RNA polymerase in vivo and in vitro."

R.E. Glass, M. Goman and J. Scaife, MRC Molecular Genetics Unit, Department of Molecular Biology, University of Edinburgh, Scotland: "Regulation of RNA polymerase expression in *E. coli*."

A. Ishihama, M. Taketo, T. Saitoh, R. Fukuda, Y. Iwakura and K. Ito, Institute for Virus Research, Kyoto University, Japan: "Control of the formation of RNA polymerase in *Escherichia coli*."

M.P. Oeschger, Department of Microbiology, Georgetown University, Washington, D.C.: "Effects of the concentration of RNA polymerase on the rates of cell growth and specific gene expression in *E. coli*."

H. Bremer, Department of Molecular Biology, University of Texas at Dallas, Richardson, Texas: "Functioning, functional, and nonfunctional RNA polymerase in *Escherichia coli* B/r."

THURSDAY MORNING, August 21

Chairperson: W. Gilbert, Harvard University, Cambridge, Massachusetts

J. Hirsh and R. Schleif, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: "Electron microscope studies of proteins (repressors, RNA polymerase) specifically bound to DNA."

S. Stahl and M.J. Chamberlin, Department of Biochemistry, University of California, Berkeley: "Groups in the minor groove of the DNA helix affect promoter recognition by T7 RNA polymerase."

J. Jacobsen, T.-S. Hsieh and J.C. Wang, Department of Chemistry, University of California, Berkeley: "Unwinding of the DNA helix by *E. coli* RNA polymerase."

J.P. Richardson, Department of Chemistry, Indiana University, Bloomington: "Transcription of superhelical PM2 DNA."

P. Botchan, D. Court, H. Echols, A. Folkmanis, L. Green and J. Simúth, Department of Molecular Biology, University of California, Berkeley: "Some experiments on regulation of transcription from phage λ DNA."

B.J. Meyer, D.G. Kleid and M. Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: "Interaction of RNA polymerase at the promoters P_R, P_L and PRM."

P.H. Seeburg, and H. Schaller, Mikrobiologie der Universität Heidelberg, Germany: "Interaction of *E. coli* RNA polymerase with promoters from fd RF DNA."

D. Pribnow, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: "Promoter structure."

W.S. Reznikoff, W.M. Barnes, K. Thornton, R. Dickson and J. Abelson, Department of Biochemistry, University of Wisconsin, Madison, and Department of Chemistry, University of California at San Diego, La Jolla: "The *lac* promoter."

D. Steege, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "Isolation and sequence analysis of the 5′ region of the *E. coli* lactose repressor mRNA."

T. Sekiya, T. Takeya, R. Contreras, H. Küpper, A. Landy and H.G. Khorana, Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, and Division of Biomedical Sciences, Brown University, Providence, Rhode Island: "Nucleotide sequences at the two ends of the *E. coli* tyrosine tRNA genes and studies on the promoter region."

H. Küpper, R. Contreras, A. Landy and H.G. Khorana, Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, and Division of Biomedical Sciences, Brown University, Providence, Rhode Island: "Promoter-dependent transcription of the $tRNA_1^{tyr}$ gene in a restriction fragment of $\phi 80psu^+$ III DNA."

THURSDAY EVENING, August 21

Chairperson: W. Zillig, Max-Planck-Institut für Biochemie, Munich, Germany

J.S. Krakow, S.A. Kumar, M.D. Haber and E. Fronk, Department of Biological Sciences, Hunter College, City University of New York: "Studies on pyrophosphate exchange by RNA polymerase."

W. Bähr, W. Stender, K.H. Scheit and T.M. Jovin, Abteilung Molekulare Biologie, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, W. Germany: "Equilibrium and kinetic studies on the interaction of DNA and rifampicin with RNA polymerase from *E. coli*."

W. Wehrli, J. Handschin and W. Wunderli, Biological Research Laboratories, Pharmaceutical Division, Ciba-Geigy, Ldt., Basel, Switzerland: "The interaction between DNA-dependent RNA polymerase of *E. coli* and rifampicin."

G. Rhodes and M. Chamberlin, Departments of Genetics and Biochemistry, University of California, Berkeley: "A kinetic analysis of RNA chain initiation."

D.E. Johnston and W.R. McClure, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: "Abortive initiation on bacteriophage λ DNA."

K. Carlson and W. Szybalski, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: "Mn^{++}-dependent in vitro transcription of the immunity region of coliphage lambda DNA."

J.D. Harris and R. Calendar, Department of Molecular Biology, University of California, Berkeley: "Transcription of phage P4 mRNA in vivo and in vitro."

G. Gussin, W. DeLorbe, J. Dodds, J. Surzycki and S. Surzycki, Department of Botany and Zoology, University of Iowa, Iowa City: "In vitro transcription of adenovirus 2 DNA with *E. coli* RNA polymerase."

P. Weglenski and B. Tyler, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Regulation of transcription by glutamine synthetase."

R. Block, Committee on Higher Degrees in Biophysics, Harvard University, Cambridge, Massachusetts: "Ribosomal RNA synthesis in a partially purified system from *Escherichia coli*."

FRIDAY MORNING, August 22

Chairperson: R. Losick, The Biological Laboratories, Harvard University, Cambridge, Massachusetts

R.L. Petrusek, J.J. Duffy and E.P. Geiduschek, Department of Biology, University of California at San Diego, La Jolla: "Phage SP01-specified determinants of selective transcription."

J. Pero, T. Fox, J. Nelson and R. Tjian, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: "Transcription of phage SP01 middle and late genes in vitro."

H.R. Whiteley, G.B. Spiegelman, J.M. Lawrie and W.R. Hiatt, Department of Microbiology, University of Washington, Seattle: "The in vitro transcriptional specificity of RNA polymerase isolated from SP82-infected *Bacillus subtilis*."

M. Salas, M.R. Inciarte, F. Jiménez, J.M. Sogo, J. Corral and E. Viñuela, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain: "Early transcription of phage ϕ29 DNA by *B. subtilis* RNA polymerase and termination factor."

D. Rothstein, M. Osburne, C. Keeler and A.L. Sonenshein, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: "RNA polymerase mutants of *Bacillus subtilis* with altered sporulation properties."

R. Tjian, T. Linn and R. Losick, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: "Subunit composition of RNA polymerase from sporulating *Bacillus subtilis*."

F.J. Sanchez-Anzaldo, R. Fukuda and R.H. Doi, Department of Biochemistry and Biophysics, University of California, Davis: "Polypeptides associated with RNA polymerase core of *Bacillus subtilis*."

FRIDAY EVENING, August 22

Chairperson: A. Stevens, Oak Ridge National Laboratory, Oak Ridge, Tennessee

R.W. Moyer, Department of Biochemistry, Columbia University, New York: "Synthesis of bacteriophage T5-specific RNA in infected *Escherichia coli* by the host cell RNA polymerase."

L.R. Snyder, D.L. Montgomery and R.J. Frederick, Department of Microbiology and Public Health, Michigan State University, East Lansing: "RNA polymerase mutants and the regulation of transcription by T4 bacteriophage."

A. Stevens, Biology Division, Oak Ridge National Laboratory, Tennessee: "A salt-promoted inhibitor of RNA polymerase isolated from T4 phage-infected *E. coli*."

P.R. Srinivasan and B. Dharmgrongartama, Department of Biochemistry, Columbia University, College of Physicians and Surgeons, New York: "Transcriptional control in T3 phage-infected *E. coli* B."

B.A. Hesselbach and D. Nakada, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: "*E. coli* RNA polymerase and T7 phage 'host shut-off' function."

H. Ponta, M. Pfennig-Yeh, H.J. Rahmsdorf, M. Hirsch-Kauffmann, P. Herrlich, W. Zillig and M. Schweiger, Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, Germany: "Role of phosphorylation of host RNA polymerase after T7 infection."

W. Zillig, H. Rohrer and R. Mailhammer, Max-Planck-Institut für Biochemie, Martinsried bei München, W. Germany: "Structural modifications of DNA-dependent RNA polymerase in *E. coli* and their possible involvement in gross regulation of transcription."

SATURDAY MORNING, August 23

Chairperson: D. Botstein, Massachusetts Institute of Technology, Cambridge, Massachusetts

G. Galluppi, C. Grimley, C. Lowery and J.P. Richardson, Department of Chemistry, Indiana University, Bloomington: "Rho termination factor: ATPase activity and altered presence in *suA* mutants."

D. Ratner, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: "The interaction of proteins with immobilized *E. coli* RNA polymerase (and the genetic mapping of termination factor rho)."

F. Lee, C.L. Squires, C. Squires and C. Yanofsky, Department of Biological Sciences, Stanford University, California: "Transcription termination in the leader region of the tryptophan operon of *E. coli*."

M. Rosenberg, B. de Crombrugghe and R. Musso, Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland: "Termination of transcription in bacteriophage λ."

N.C. Franklin, Department of Biological Sciences, Stanford University, California: "Transcription by *E. coli* RNA polymerase is modified by λ's *N* protein with the effect of preventing certain terminations."

S. Adhya, M. Gottesman, B. de Crombrugghe and D. Court, Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland: "λ *N* function, transcription termination and polarity."

C. Epp and M. Pearson, Department of Medical Genetics, University of Toronto, Canada: "Association of bacteriophage λ proteins with *E. coli* RNA polymerase."

J. Roberts, C. Roberts, S. Hilliker* and D. Botstein,* Department of Biochemistry, Cornell University, Ithaca, New York; *Department of Biology, Massachusetts Institute of Technology, Cambridge: "Transcription termination and positive control in lambdoid phages."

SATURDAY AFTERNOON, August 23

Chairperson: L. Bogorad, Harvard University, Cambridge, Massachusetts

P.S. Fitt, N.N. Barua, B.G. Louis and P.I. Peterkin, Department of Biochemistry, University of Ottawa, Canada: "Studies with *H. cutirubrum* RNA polymerase."

S. Miller, F. Ausubel and L. Bogorad, Department of Biology, Harvard University, Cambridge, Massachusetts: "Characteristics of a blue-green algal RNA polymerase."

A. Hildebrandt, Fachbereich Biologie der Universität, Konstanz, W. Germany: "Differential template activities of RNA polymerases A and B from *Physarum*."

J.M. Buhler, J. Huet, F. Iborra, A. Sentenac and P. Fromageot, Commissariat à l'Energie Atomique, Centre d'Etudes Nucléaires de Saclay, Service de Biochimie, Gif-sur-Yvette, France: "Yeast RNA polymerases. Presence of common subunits in RNA polymerases A and B. Evidence for a new form of enzyme, RNA polymerase A*."

L.D. Schultz and B.D. Hall, Departments of Biochemistry and Genetics, University of Washington, Seattle: "α-Amanitin sensitivity and other properties which distinguish between yeast RNA polymerases IB and III."

M.J. Holland, G.L. Hager and W.J. Rutter, Department of Biochemistry and Biophysics, University of California, San Francisco: "The role of yeast RNA polymerases I and II in specific transcription of homologous DNA."

M.G. Goldberg, P. Valenzuela and W.J. Rutter, Department of Biochemistry and Biophysics, University of California, San Francisco: "Protein cofactors affecting the transcription of DNA by RNA polymerase I."

S.J.Surzycki, S. Ratcliff and D. Shellenbarger, Botany Department, University of Iowa, Iowa City: "Putative sigma factor from chloroplasts of *Chlamydomonas reinhardtii*."

L. Bogorad, Department of Biology, Harvard University, Cambridge, Massachusetts: "Chloroplast and nuclear DNA-dependent RNA polymerases of *Zea mays*."

J.J. Jendrisak and R.R. Burgess, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: "Purification and subunit analysis of wheat germ RNA polymerase II."

SUNDAY MORNING, August 24

Chairperson: E. Bautz, University of Heidelberg, Heidelberg, West Germany

C. Kedinger, G. Krebs and P. Chambon, Institut de Chimie Biologique et U. 44 de l'INSERM, Faculté de Médicine, Strasbourg, France: "Molecular structure of animal RNA polymerases."

V.E. Sklar and R.G. Roeder, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Missouri: "Structure and properties of eukaryotic class III RNA polymerases."

A.L. Greenleaf and E.K.F. Bautz, Molekulare Genetik der Universität Heidelberg, W. Germany: "DNA-dependent RNA polymerases from *Drosophila melanogaster*."

P.H.W. Butterworth and T.J.C. Beebee, Department of Biochemistry, University College London, England: "Functional heterogeneity of eucaryotic DNA-dependent RNA polymerase form A."

C.J. Ingles, M.L. Pearson,* M. Buchwald,† B.G. Beatty, M.M. Crerar,* A. Guialis, P.E. Lobban,* L. Siminovitch* and D.G. Somers,* Banting and Best Department of Medical Research, and *Department of Medical Genetics, University of Toronto, Canada, †Department of Genetics, The Hospital for Sick Children, Toronto, Canada: "Regulation of RNA polymerase II activity in mammalian cell mutants."

J.A. Jaehning, R. Weinmann, T.G. Brendler, H.J. Raskas and R.G. Roeder, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Missouri: "Function of RNA polymerases II and III in adenovirus infected KB cells."

B. Lescure and M. Yaniv, Department of Molecular Biology, Pasteur Institute, Paris, France: "Binding sites of *E. coli* and calf thymus B RNA polymerase on polyoma DNA."

A.G. So, K.M. Downey and J.J. Byrnes, Departments of Medicine and Biochemistry, University of Miami, Florida: "Characterization of RNA product synthesized with reticulocyte RNA-dependent RNA polymerase."

This meeting was partially funded by a grant from the National Cancer Institute, National Institutes of Health.

BACTERIOPHAGE MEETINGS

Arranged by
AHMAD I. BUKHARI, *Cold Spring Harbor Laboratory*
MARTHA HOWE, *University of Wisconsin*

123 Participants

MONDAY EVENING, August 25

D. Barker and N. Kleckner, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Lambda-*tet*^{R} transducing phages."

L.A. MacHattie and J.B. Jackowski, Department of Medical Genetics, University of Toronto, Canada: "Insertion and excision of a chloramphenicol resistance determinant in λ."

A.I. Bukhari and S. Froshauer, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Insertions in bacteriophage Mu DNA."

M.M. Bendig and H. Drexler,* Department of Microbiology, University of Michigan, Ann Arbor; *Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: "Transduction of Mu-pfu by phage T1."

H.I. Miller and E.N. Jackson, Department of Microbiology, University of Michigan, Ann Arbor: "*Eco*RI analysis of the DNA of bacteriophages P22 and P22-lambda hybrids."

Open Discussion: "NIH guidelines on recombinant DNA molecules"

TUESDAY MORNING, August 26

A. Rimon and A.B. Oppenheim, Department of Microbiological Chemistry, The Hebrew University-Hadassah Medical School, Jerusalem, Israel: "Analysis of gene expression of the temperate cyanophage SP1."

L. Souza, E.W. Six and R. Calendar, Molecular Biology Department, University of California, Berkeley, and Microbiology Department, University of Iowa, Iowa City: "A mutant of satellite phage P4 which can only grow with a coinfecting helper."

G. Deho, L. Tinelli and R. Goldstein, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: "Satellite phage P4 inhibits the growth of its helper P2. Isolation and characterization of a P4 mutant affected in this function."

B.W. West, J.R. Scott and J. Laping, Department of Microbiology, Emory University, Atlanta, Georgia: "Evidence for an antirepressor function in phage P1."

R.H. Chesney, L. Mendelson and J.R. Scott, Department of Microbiology, Emory University, Atlanta, Georgia: "Superinfection immunity in *φamp*."

B.M. Steinberg and M. Gough, Department of Microbiology, State University of New York, Stony Brook: "Altered DNA synthesis in a mutant of *Salmonella typhimurium* that channels P22 toward lysogeny."

J.M. Pipas, C.E. Flick and R.H. Reeves, Institute of Molecular Biophysics, Florida State University, Tallahassee: "RNA polymerase mutants affecting development of phage P22."

TUESDAY EVENING, August 26

P.K. Tomich and D.I. Friedman, Department of Microbiology, University of Michigan, Ann Arbor: "λ *alt:* A phage exhibiting alternating immunities."

J.S. Salstrom, McArdle Laboratory, University of Wisconsin, Madison: "Inhibition of lambda $N^{-}nin^{5}$ growth by heteroimmune *N* function."

N.C. Franklin, Department of Biological Sciences, Stanford University, California: "Mutations of *N* and p_L limiting *N* operon function in lambda."

B. Jones and W.S. Reznikoff, Department of Biochemistry, University of Wisconsin, Madison: "Studies on λ*φ*80p*trp* transducing phages."

J.S. Salstrom and W. Szybalski, McArdle Laboratory, University of Wisconsin, Madison: Lambda nut_L mutants defective in *N* utilization for leftward transcription."

S. Adhya, M. Gottesman, D. Court, B. de Crombrugghe and C. Merril,* Laboratory of Molecular Biology, National Cancer Institute, and *Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, NIH, Bethesda, Maryland: "Gene controls by λ."

Ahmad Bukhari and Martha Howe, meeting organizers, captured sharing an idyllic moment between sessions. (*Photo by Sallie Chait*)

WEDNESDAY MORNING, August 27

B.G. Rolfe and J.H. Campbell,* Genetics Department, Research School of Biological Science, The Australian National University, Canberra; *Department of Anatomy, School of Medicine, University of California, Los Angeles: "Involvement of the *rex*-gene in the life cycle of phage λ."

D.B. Wilson, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: "The mode of action of the λ S gene product on *E. coli*."

J.H. Campbell and B.G. Rolfe,* Department of Anatomy, School of Medicine, University of California, Los Angeles; *Genetics Department, Research School of Biological Science, The Australian National University, Canberra: "Lysogenic conversion of *E. coli* by λ phage."

M. Belfort, N. Katzir, A. Oppenheim and A. Oppenheim, The Hebrew University-Hadassah Medical School, Jerusalem, Israel: "Analysis of λcII amber mutants and of phage regulatory events by polyacrylamide gel electrophoresis."

C. Epp and M. Pearson, Department of Medical Genetics, University of Toronto, Ontario, Canada: "Lambda early proteins synthesized in vivo."

A. Honigman, N. Kass and A. Oppenheim, The Hebrew University-Hadassah Medical School, Jerusalem, Israel: "Regulation of λ repressor synthesis."

A. Folkmanis, P. Mellon, J. Simuth and H. Echols, Department of Molecular Biology, University of California, Berkeley: "Purification and characterization of the *cro* protein of phage λ."

WEDNESDAY AFTERNOON, August 27

E.N. Jackson, D.A. Jackson, M.L. Adams and R.J. Deans, Jr., Department of Microbiology, University of Michigan, Ann Arbor: "*Eco*R1 restriction enzyme analysis of P22 DNA packaging."

A.I. Bukhari and A.L. Taylor,* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; *University of Colorado Medical Center, Denver: "Influence of insertions on the packaging of host sequences covalently linked to bacteriophage Mu DNA."

N. Sternberg and R. Weisberg, Laboratory of Molecular Genetics, National Institutes of Health, Bethesda, Maryland: "The role of head gene *D* in DNA packaging by λ."

A. Zachary and L.D. Simon, The Institute for Cancer Research, Philadelphia, Pennsylvania: "λ Head morphogenesis as seen in the electron microscope."

P. Youderian and J. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Length determination of the lambdoid tail."

J. Shaw, M. Pearson and C. Fuerst, Department of Medical Genetics, University of Toronto, Ontario, Canada: "Host range mutants of bacteriophage lambda synthesize an altered *J* protein."

A.R. Poteete and J. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Two new genes in P22 morphogenesis."

R.G. Shea and J. King, Massachusetts Institute of Technology, Cambridge: "Immunological analysis of phage P22."

THURSDAY MORNING, August 28

G.S. Roeder, Department of Medical Genetics, University of Toronto, Ontario, Canada: "Head assembly in bacteriophage T7."

J.R. Paulson, U.K. Laemmli and S. Lazaroff,* Department of Biochemical Sciences, Princeton University, New Jersey; *Department of Genetics, University of Washington, Seattle: "Head length determination in bacteriophage T4."

C.J. Castillo, P. Coon and L.W. Black, Department of Biochemistry, University of Maryland School of Medicine, Baltimore: "Studies of T4 head genes."

C.L. Hsiao and L.W. Black, Department of Biochemistry, University of Maryland School of Medicine, Baltimore: "Studies of gene 40 bacteriophage T4."

E.B. Goldberg, B.L. Hempstead, R.A. Levine and J. Silverstein, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: "A role for T4 gene 2 in phage morphogenesis."

J.E. McCullough and S.P. Champe, Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey: "Origin of the T4 internal peptides."

E. Kutter, The Evergreen State College, Olympia, Washington: "Control of T4 late-protein synthesis."

W.N. Choy and S.P. Champe, Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey: "Absence of a new trypsin-like enzyme in T4-infected cells."

THURSDAY EVENING, August 28

M. Salas, F. Jiménez, A. Camacho, R.P. Mellado, J.L. Carrascosa and E. Viñuela, Departamento de Biologia Molecular, Instituto G. Marañón, CSIC, Madrid, Spain: "Assembly of *Bacillus subtilis* bacteriophage ϕ29."

J.A. Mayo, S. Githens and G.C. Allen, Departments of Biological Sciences and Earth Science, University of New Orleans, Louisiana: "Structural characterization of bacteriophage XP12."

W. Maltzman, H. Echols, A. Folkmanis and A. Skalka, Department of Molecular Biology, University of California, Berkeley, and Roche Institute of Molecular Biology, Nutley, New Jersey: "Phage λ mutants that may affect regulation of DNA replication."

J.S. Glassberg, R. Slomiany and C. Stewart, Department of Biology, Rice University, Houston, Texas: "A screen for replication-deficient mutants."

J.M. Cregg and C.R. Stewart, Department of Biology, Rice University, Houston, Texas: "Timing of initiation of DNA replication in SPO1 infection of *Bacillus subtilis*."

P.L. Derstine and L.B. Dumas, Departments of Biological Sciences and Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois: "Replication of ϕX174 DNA in a temperature-sensitive dnaH mutant of *Escherichia coli* C."

J.R. Christensen and J. Halpern, University of Rochester, New York: "More about how lambda excludes T1 and complements the growth of T1 gene 4 mutants."

K. Dharmalingam and E.B. Goldberg, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: "Cellular localization of *E. coli* restriction activities of $r_{2.4}$ and r_6."

FRIDAY MORNING, August 29

V. Makin, F.R. Blattner and W. Szybalski, McArdle Laboratory and Department of Genetics, University of Wisconsin, Madison: "Effect of deletions and substitutions on recombination in coliphage lambda: Chiasma migration and high negative interference."

A.M. Breschkin and G. Mosig, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "Separate roles of phage T4 gene 32 protein in different steps of DNA replication and recombination."

R.J. Dannenberg and G. Mosig, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "EM visualization of replicating T4 DNA."

P.D. Sadowski, Department of Medical Genetics, University of Toronto, Ontario, Canada: "In vitro genetic recombination of bacteriophage T7 DNA."

H. Berger and D. Pardoll, Department of Biology, Johns Hopkins University, Baltimore, Maryland: "Mismatch recognition in T4 heteroduplex DNA."

L.S. Ripley, Department of Microbiology, University of Illinois, Urbana-Champaign: "Alkylation mutagenesis of bacteriophage T4."

R.E. Koch, Department of Microbiology, University of Illinois, Urbana: "Mutator activity of bacteriophage T4 gene-32 mutants."

J. Karam and J. Chao, Department of Biochemistry, Medical University of South Carolina, Charleston: "On the possible interaction between DNA polymerase and d-CMP hydroxymethylase of T4."

K. Horiuchi, G.F. Vovis, V. Enea, P. Model and N.D. Zinder, The Rockefeller University, New York: "Cleavage mapping of bacteriophage f1."

FRIDAY AFTERNOON, August 29

K. Van der Laan, L.B. Rothman-Denes and S.C. Falco, Department of Biophysics and Theoretical Biology, University of Chicago, Illinois: "Transcription program in N4-infected cells."

R. Konigs, C. Van den Hondel, L. Edens and J. Schoenmakers, Laboratory of Molecular Biology, University of Nijmegen, The Netherlands: "Regulation of gene activity in bacteriophage M13 DNA."

G.S. Gill and L.A. MacHattie, Department of Medical Genetics, University of Toronto, Ontario, Canada: "Studies on transcription from T1 DNA after infection of *E. coli* 159."

T. Young, Department of Biochemistry, University of Washington, Seattle: "Analysis of bacteriophage T4 mRNA synthesized in the presence of chloramphenicol by in vitro protein synthesis and by hybridization."

R.J. Frederick and L.R. Snyder, Department of Microbiology and Public Health, Michigan State University, East Lansing: "T4 anti-messenger RNA production with phage and host mutants."

C. Pachl, F. Hagen and T. Young, Department of Biochemistry, University of Washington, Seattle: "Overlapping polycistronic mRNA coded by bacteriophage T7."

D. Nakada, B.A. Hesselbach, Y. Yamada and P.A. Whitaker, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: "Early to late switch in T7 phage development."

P.S. Kohler and D.M. Green, Department of Biochemistry, University of New Hampshire, Durham: "Association of infecting SP82G DNA with host cell membrane."

M.R. McConnell and A. Wright, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: "Multiple stages in the adsorption of bacteriophage E^{15} to its host cell, *Salmonella anatum*."

J.T. Crawford, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: "Adsorption of T4 to *E. coli* B absolutely requires glucose in the LPS."

C.C. Pao and J.F. Speyer, Section of Genetics and Cell Biology, University of Connecticut, Storrs: "A mutant of bacteriophage T7 that fails to grow on lambda lysogens."

K. Kennedy, N. Thomas and H. Wiesmeyer, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "Complementation among ts mutants of PM2."

K.H. Korsten and R. Hausmann, Institut für Biologie III, der Universität, Freiburg, W. Germany: "Comparative studies with bacteriophages morphologically similar to T3 and T7."

A.B. Radue and R.H. Baltz, Eli Lilly and Company, Indianapolis, Indiana: "Isolation and characterization of phages for *Streptomyces fradiae*."

THE MOLECULAR BIOLOGY OF YEAST

Arranged by
DAVID BOTSTEIN, *Massachusetts Institute of Technology*
GERALD FINK, *Cornell University*

167 Participants

MONDAY EVENING, September 1

Introduction: G. Fink, Cornell University, Ithaca, New York

V.L. MacKay, R. Betz and W.D. Duntze, Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey, and Institut für Physiologische Chemie, Ruhr Universität Bochum, Germany: "An inducible sex pheromone synthesized by mating type *a* cells of *S. cerevisiae*."

R.K. Chan, Department of Genetics, University of Washington, Seattle: "The recovery of mating type *a* cells from cell-cycle arrest by alpha factor."

J. Hicks and I. Herskowitz, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: "Mating pheromones: Evidence for a new diffusible factor."

J. Hicks and I. Herskowitz, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: "Interconversion of yeast mating types."

J. Blamire, Department of Biology, Brooklyn College, City University of New York: "A new mutation involved in the determination of mating-type."

J.N. Strathern, J. Hicks and I. Herskowitz, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: "Analysis of mutations controlling mating and sporulation."

E. Cabib, A. Duran, R. Ulane and B. Bowers, National Institute of Arthritis, Metabolism and Digestive Diseases, National Heart and Lung Institute, NIH, Bethesda, Maryland: "Timing, function and localization of chitin synthesis in yeast."

Meeting organizers Gerald Fink (*left*) and David Botstein (*right*) — we can only guess at the point being debated. (*Photo by Sallie Chait*)

B. Byers, L. Goetsch and K. Shriver, Department of Genetics, University of Washington, Seattle: "A cortical ring of microfilaments at the base of the bud in *Saccharomyces cerevisiae*."

TUESDAY MORNING, September 2

M.W. Unger and L.H. Hartwell, Department of Genetics, University of Washington, Seattle: "A role for aminoacyl-tRNA in the control of cell division?"

C.E. Sripati and J.R. Warner, Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: "Messenger RNA metabolism in yeast."

N. Hynes and S. Phillips, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: "Studies of messenger-RNA in *Saccharomyces cerevisiae*."

J.F. Harper and P.T. Magee, Department of Human Genetics, Yale University School of Medicine, New Haven: "Evidence for a blocked 5′ terminus in poly A-associated spore mRNA."

F. Perrin, B. Winsor, F. Exinger and F. Lacroute, Laboratoire de Génétique Physiologique, IBMC, Strasbourg, France: "Central role of a methionine derivative in the regulation of RNA biosynthesis."

J.R. Warner, and S. Zinker, Department of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: "The ribosomal proteins of yeast."

C. Gorenstein and J.R. Warner, Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: "Control of the synthesis of ribosomal proteins."

D. Becker and J. Davies, Department of Biochemistry, University of Wisconsin, Madison: "Study of the structure of the yeast *Saccharomyces cerevisiae* ribosomes by way of phosphorylation."

S.G. Elliott, C.S. McLaughlin and E.A. Mash, Department of Molecular Biology and Biochemistry, University of California, Irvine: "Structure-function relationships of fungal products."

J.E. Haber, N. Pearson and D. Kimbrell, Department of Biology and Rosenstiel Basic Medical Science Research Center, Brandeis University, Waltham, Massachusetts: "Changes in expression of *rna* genes during sporulation."

J.M. Gentile and P.T. Magee, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: "Characteristics of the sporulation-dependent change in RNA polymerase in *Saccharomyces cerevisiae*."

TUESDAY EVENING, September 2

L. Skogerson, K. Bucher, U. Somasundaran and E. Wakatama, Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York: "Purification and properties of yeast protein synthetic factors."

R. Chandra and D.B. Mowshowitz, Department of Biological Sciences, Columbia University, New York: "Transcriptional and translational control of maltase during the cell cycle of *S. cerevisiae*."

J. Bossinger and T. Cooper, Department of Biochemistry, University of Pittsburg, Pennsylvania: "Sequence of molecular events involved in induction of allophanate hydrolase."

S.G. Oliver and C.S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California, Irvine: "Stringent control of RNA synthesis in yeast."

R. Rothstein, R. Esposito and M. Esposito, Committee on Genetics and Department of Biology, University of Chicago, Illinois: "A genetic fine structure analysis of *SUP*3 in *Saccharomyces*."

B. Ono and F. Sherman, Department of Radiation Biology and Biophysics, University of Rochester Medical School, New York: "Isolation and characterization of UAA suppressors in ψ^+ strains of yeast."

M.C. Brandriss, L. Soll, D. Botstein, J.W. Stewart* and F. Sherman,* Department of Biology, Massachusetts Institute of Technology, Cambridge; *Department of Radiation Biology and Biophysics, University of Rochester, New York: "Recessive lethal amber suppressors in yeast."

R.F. Gesteland, M. Wolfner, P. Grisafi, G. Fink, D. Botstein and J.R. Roth, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Yeast nonsense suppressors work efficiently in vitro."

R.S. Zitomer, R.L. Gibson and B.D. Hall, Department of Genetics, University of Washington, Seattle: "In vitro translation of cytochrome *c* mRNA."

J.R. Broach and J.E. Hopper, Division of Medical Physics, University of California, Berkeley, and Department of Biochemistry, University of Washington, Seattle: "Purification and in vitro translation of the enzymes of galactose metabolism from *Saccharomyces cerevisiae*."

WEDNESDAY MORNING, September 3

L. Prakash, Department of Radiation Biology and Biophysics, University of Rochester, New York: "Repair of pyrimidine dimers in nuclear and mitochondrial DNA of *Saccharomyces cerevisiae* following irradiation with low doses of UV light."

E. Silva-Lopez and R. Roth, Biology Department, Illinois Institute of Technology, Chicago: "Role of postreplicative DNA synthesis in meiotic gene conversion and reciprocal recombination."

C.W. Lawrence and R. Christensen, Department of Radiation Biology and Biophysics, University of Rochester Medical Center, New York: "Nucleotide sequence-specific UV mutagenesis."

C.W. Moore and F. Sherman, University of Rochester, New York: "Disproportionalities in recombination of *cyc1* alleles."

W.R. Boram, Department of Genetics, University of Washington, Seattle: "A mutation of *S. cerevisiae* which confers hypersensitivity to the alkylating agent methyl methanesulfonate, and is associated with increased mitotic recombination."

A. Nasim, Division of Biological Sciences, National Research Council of Canada, Ottawa: "Mutagenesis in radiation sensitive mutants of *Schizo-Saccharomyces pombe*."

S.W. Liebman, A. Singh and F. Sherman, Department of Radiation Biology and Biophysics, University of Rochester Medical School, New York: "A mutator that causes deletions of the region of the iso-1-cytochrome *c* gene in yeast."

A. Singh and F. Sherman, Department of Radiation Biology and Biophysics, University of Rochester Medical School, New York: "Genetic control of osmotic sensitivity of yeast."

H.L. Klein and B.E. Byers, Department of Genetics, University of Washington, Seattle: "Strand separation in yeast chromosomal DNA."

D.B. Finkelstein and R.A. Butow, Department of Biochemistry, University of Texas Health Science Center, Dallas: "Yeast DNA-binding proteins."

WEDNESDAY EVENING, September 3

J. Thorner and P. Senter, Department of Bacteriology and Immunology, University of California, Berkeley: "Isolation and preliminary characterization of yeast mutants conditionally defective in DNA synthesis."

C.P. Hollenberg, Max-Planck-Institute für Biologie, Abt. Beermann, Tübingen, W. Germany: "Agarose gel electrophoresis of intact yeast episomal DNA and its *Eco* R1 restriction endonuclease fragments."

M.C. Kielland-Brandt and W.L. Fangman, Department of Genetics, University of Washington, Seattle: "Analysis of chromosome-sized yeast DNA partially separated by centrifugation in a zonal rotor."

M. Olson, G. Page, A.K. Hopper, and B.D. Hall, Department of Genetics, University of Washington, Seattle: "Purification of yeast genes."

D. Botstein, G.R. Fink and P. Wensink,* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; *Rosenstiel Center, Biochemistry Department, Brandeis University, Waltham, Massachusetts: "Isolation of random segments of yeast DNA by joining with a bacterial plasmid."

P.A. Whitney and B.D. Hall, Department of Genetics, University of Washington, Seattle: "Complexity of the yeast genome."

S.K. Welch and B.D. Hall, Department of Biochemistry, University of Washington, Seattle: "Fraction of yeast nuclear DNA expressed as RNA transcripts."

J.H. Cramer, F. Farrelly and R.H. Rownd, Laboratory of Molecular Biology, University of Wisconsin, Madison: "Size and homogeneity of ribosomal RNA cistrons in *Saccharomyces cerevisiae*."

K. Nath and A.P. Bollon, Department of Biochemistry, University of Texas Health Science Center, Dallas: "Characterization of yeast DNA fragments generated by endonuclease R1."

THURSDAY MORNING, September 4

S. Henry, M. Culbertson, K. Atkinson, A. Brotsky and T. Donahue, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: "Inositol synthetase mutants in yeast."

J.G. Little and J.C. Game, Department of Biology, York University, Toronto, Canada, and Division of Microbiology, National Institute for Medical Research, London, England: "Analysis of thymidine monophosphate auxotrophs in yeast."

J. Delforge, F. Messenguy and J.M. Wiame, Institut de Recherches du CERIA and Laboratoire de Microbiologie de l'Universite de Bruxelles, Belgium: "The regulation of arginine biosynthesis in *Saccharomyces cerevisiae*: The specificity of *arg*R$^-$ mutations and the 'general control' of amino acid biosynthesis."

J. Bossinger and T. Cooper, Department of Biochemistry, University of Pittsburgh, Pennsylvania: "Failure of NADP-GDH to directly participate in nitrogen repression of the allantoin degradative system."

K.-B. Lam and J. Marmur, Departments of Biochemistry and Genetics, Albert Einstein College of Medicine, Bronx, New York: "Glycolytic pathway mutants of *Saccharomyces cerevisiae*."

D.B. Mowshowitz, Department of Biological Sciences, Columbia University, New York: "Induction of α-glucosidase."

H. Greer and G.R. Fink,* Department of Genetics, Development and Physiology, Cornell University, Ithaca, New York; *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Isolation of operator mutations in the *his 4* region of *S. cerevisiae*."

O. Yamada and B.G. Adams, Department of Microbiology, University of Hawaii, Honolulu: "Pleiotropic nature of the *gal 3* locus gene product in yeast."

N.A. Khan, Department of Biology, Brooklyn College, City University of New York: "Isolation and characterization of mutants affecting maltase synthesis in yeast."

J.A. Downie and F. Sherman, Department of Radiation Biology and Biophysics, University of Rochester Medical School, New York: "Mutations of the structural gene for iso-2-cytochrome *c*."

J.R. Pringle and A. Fiechter, Microbiology Institute, Swiss Federal Institute of Technology, Zurich, Switzerland: "Genetic analysis of reserve carbohydrate metabolism in yeast."

A.P. Bollon; Department of Biochemistry, University of Texas Health Science Center, Dallas: "Analysis of the *ilv 1* multifunctional gene."

THURSDAY AFTERNOON, September 4

C.W. Birky, Jr., Department of Genetics, Ohio State University, Columbus: "Possible consequences of gene conversion in a randomly mating population of yeast mitochondrial genophores."

P.S. Perlman and C.A. Demko, Department of Genetics, Ohio State University, Columbus: "Gene dosage and bias in mitochondrial genetics."

R.A. Butow and W.F. Bennett, Department of Biochemistry, University of Texas Health Science Center at Dallas: "Translational segregation of mitochrondrial ATPase and glyceraldehyde 3-phosphate dehydrogenase."

A.J.S. Ball, Biological Sciences, Brock University, St. Catharines, Ontario, Canada: "Glucosamine resistant mutants in yeast."

C.A. Biron, A. Louis, P. Boerner and T.L. Mason, Department of Biochemistry, University of Massachusetts, Amherst: "Mitochondrial assembly in temperature-sensitive mutants of *S. cerevisiae*."

E.M. Storm, B. Goldfinger, W. Perry and J. Marmur, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Cytoplasmically inherited temperature-sensitive respiratory deficient mutants of *Saccharomyces cerevisiae*."

A. Lewin, R. Morimoto, H.J. Hsu, H. Fukuhara and M. Rabinowitz, Departments of Medicine, Biochemistry and Biology, and the Franklin McLean Memorial Research Institute, University of Chicago, Illinois, and Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: "Restriction endonuclease analysis of mitochondrial DNA from genetically characterized cytoplasmic petite clones of *Saccharomyces cerevisiae*."

N. Hinckley, H. Fukuhara, H.J. Hsu and M. Rabinowitz, Departments of Medicine, Biochemistry and Biology, and the Franklin McLean Memorial Research Institute, University of Chicago, Illinois, and Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: "Mapping of mitochondrial transfer RNA genes and the identification of isoaccepting glutamyl tRNAs transcribed from the mitochondrial genome of *Saccharomyces cerevisiae*."

F. Hendler, G. Padmanaban, J. Patzer, R. Ryan and M. Rabinowitz, Departments of Medicine and Biochemistry, and the Franklin McLean Memorial Research Institute, University of Chicago, Illinois: "Translation of poly(A)-containing RNA from yeast mitochondria in an *E. coli* cell-free system."

C.S. Newlon, Department of Zoology, University of Iowa, Iowa City: "Mitochondrial DNA replication in cell division cycle mutants of yeast."

FRIDAY MORNING, September 5

R. Sumrada and T. Cooper, Department of Biochemistry, University of Pittsburgh, Pennsylvania: "Biochemical and genetic characteristics of urea uptake in *Saccharomyces cerevisiae*."

J.M. Becker and F. Naider, Department of Microbiology, University of Tennessee, Knoxville, and Chemistry Department, Richmond College, City University of New York, Staten Island: "Peptide transport in yeast."

H. Betz, Biochemisches Institut der Universität Freiburg, W. Germany: "Protein degradation and proteinases during yeast sporulation."

E.W. Jones, H.A. Lund and G.S. Zubenko, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: "Proteinase and peptidase mutants of *S. cerevisiae*."

E.G. Gollub, K.P. Liu, J. Dayan and D.B. Sprinson, Department of Biochemistry, Columbia University, College of Physicians and Surgeons, New York: "Heme mutations in *Saccharomyces cerevisiae*."

R.B. Wickner and M.J. Leibowitz, Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, Maryland: "Genetic studies of the killer character of *Saccharomyces cerevisiae*."

S.G. Oliver, C. Holm, P.A. Sutherland and C.S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California, Irvine: "Biochemical and physiological studies of the yeast virus-like particle."

H. Bussey, K. Al-Aidroos, R.G.E. Palfree and N.A. Skipper, Department of Biology, McGill University, Montreal, Canada: "Killer factor-like glycoproteins showing membrane mediated killing of *Saccharomyces cerevisiae*."

J. Conde, Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York: "Mating without caryogamy in *Saccharomyces cerevisiae*."

J. Bruenn and R.J. Roberts,* Department of Cell and Molecular Biology, State University of New York, Buffalo; *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Partial sequences of yeast killer-factor RNAs."

This meeting was partially funded by a grant from the National Cancer Institute, National Institutes of Health.

CELL MOTILITY

Arranged by
ROBERT GOLDMAN, *Carnegie-Mellon University*
THOMAS POLLARD, *Harvard University*
JOEL ROSENBAUM, *Yale University*

242 Participants

TUESDAY EVENING, September 9

Welcoming Remarks: J.D. Watson, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

A job well done, meeting organizers (*from left to right*) Thomas Pollard, Robert Goldman and Joel Rosenbaum seem to be enjoying the cocktail hour preceding the closing banquet. (*Photo by Ross Meurer*)

Opening Address: K. Porter, University of Colorado, Boulder, Colorado

Movie Session

WEDNESDAY MORNING, September 10

Session 1A

Chairperson: J. Adler, University of Wisconsin, Madison, Wisconsin

H. Hilmen and M. Simon, Department of Biology, University of California at San Diego, La Jolla: "Motility and the structure of bacterial flagella."

H.C. Berg, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: "Bacterial flagellar rotation."

D.E. Koshland, Jr., H. Warrick and B. Taylor, Department of Biochemistry, University of California, Berkeley: "The control of bacterial motility."

J. Adler, Departments of Biochemistry and Genetics, University of Wisconsin, Madison: "Structure and function of bacterial flagella and the effect of environmental stimuli on flagellar motion."

Y. Kikuchi and J. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: "The baseplate of bacteriophage T4."

L.M. Routledge, W.B. Amos, F.F. Yew and T. Weis-Fogh, Zoology Department, University of Cambridge, England: "New calcium-binding contractile proteins."

Session 1B

Chairperson: H.E. Huxley, MRC Laboratory of Molecular Biology, Cambridge, England

H.E. Huxley, MRC Laboratory of Molecular Biology, Cambridge, England: "Structural studies on muscular contraction."

E.W. Taylor, Department of Biophysics and Theoretical Biology, University of Chicago, Illinois: "Kinetics of the contraction cycle."

WEDNESDAY EVENING, September 10

Session 1B (continued)

J. Gergely, Department of Muscle Research, Boston Biomedical Research Institute; Department of Neurology, Massachusetts General Hospital; and Department of

Biological Chemistry, Harvard Medical School, Boston, Massachusetts: "Tropomyosin-troponin dependent control by Ca^{2+} of muscle contraction."

D.H. MacLennan, P.S. Stewart and P.C. Holland, Banting and Best Department of Medical Research, University of Toronto, Canada: "Composition, structure and biosynthesis of sarcoplasmic reticulum."

A.P. Somlyo and A.V. Somlyo, Department of Pathology, Presbyterian-University of Pennsylvania Medical Center and Departments of Physiology and Pathology, School of Medicine, University of Pennsylvania, Philadelphia: "Smooth muscle: Ultrastructure and function."

Session 2

Chairperson: O. Behnke, University of Copenhagen, Copenhagen, Denmark

E. Lazarides, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Actin, α-actinin and tropomyosin interaction in the structural organization of actin filaments in non-muscle cells."

K. Weber, Max Planck Institute of Biophysical Chemistry, Goettingen, W. Germany: "Visualization of tubulin containing structures by immunofluorescence microscopy: Cytoplasmic microtubules, mitotic figures and vinblastine induced paracrystals."

R. Pollack and D. Rifkin,* Department of Microbiology, State University of New York, Stony Brook; *The Rockefeller University, New York: "Plasmin causes the dissolution of actin-containing cables."

THURSDAY MORNING, September 11

Session 3A

Chairperson: O. Behnke, University of Copenhagen, Copenhagen, Denmark

R.D. Goldman, J. Schloss and J. Starger, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: "Structure and possible physiological functions of actin-like microfilaments."

G. Albrecht-Buehler, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Sensory function of microspikes during early spreading of 3T3 cells."

T.E. Schroeder, Friday Harbor Laboratories, University of Washington, Friday Harbor: "Role and properties of the contractile ring in cleavage contraction."

J.W. Sanger, Department of Anatomy, University of Pennsylvania, School of Medicine, Philadelphia: "Actin localization during cytoplasmic division."

G.M. Edelman and I. Yahara, The Rockefeller University, New York, New York: "Dynamics of cell surface modulation and growth control in mammalian cells."

R.R. Weihing, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: "Membrane association and polymerization of actin."

Session 3B Workshop: Membranes and Their Association with Contractile Proteins.

Chairperson: E. Korn, Laboratory of Cell Biology, National Heart and Lung Institute, NIH, Bethesda, Maryland

Participants: E. Korn, National Heart and Lung Institute, NIH, Bethesda, Maryland
M. Willingham, Department of Health, Education and Welfare, NIH, Bethesda, Maryland
M. Sheetz, Department of Physiology, University of Connecticut Health Center, Farmington
M. Karnovsky, Department of Pathology, Harvard Medical School, Boston, Massachusetts
G.M. Edelman, The Rockefeller University, New York, New York

Discussion

THURSDAY AFTERNOON, September 11

Session 4 Workshop: Use of Antibodies for Intracellular Localization of Proteins Involved in Cell Motility

Chairperson: G. Goldstein, Department of Medicine, University of Virginia Medical School, Charlottesville, Virginia

Participants: F.A. Pepe, Department of Anatomy, Medical School, University of Pennsylvania, Philadelphia: "Antibody labeling: Detectability in fluorescence and electron microscopy."
G. Fuller, University of Texas Medical School, Galveston
C. Fulton, Department of Biology, Brandeis University, Waltham, Massachusetts
E. Lazarides, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
J. Schollmeyer, University of Minnesota, Minneapolis
K. Weber, Max Planck Institute of Biophysical Chemistry, Goettingen, W. Germany
V. Kalnins, Department of Anatomy, University of Toronto, Canada

Discussion

THURSDAY EVENING, September 11

Session 5

Chairperson: R.D. Allen, Department of Biology, Dartmouth College, Hanover, New Hampshire

D. Bray and C. Thomas, Cell Biophysics Unit, Kings College, London, England: "Unpolymerized actin in non-muscle cells."

S. Hatano and K. Owaribe, Institute of Molecular Biology, Faculty of Science, Nagoya University, Japan: "Actin and actinin from myxomycete plasmodium."

L.G. Tilney and M.S. Mooseker, Department of Biology, University of Pennsylvania, Philadelphia: "I. Non-filamentous aggregates of actin in sperm. II. Actin filament-membrane attachment in the brush border of intestinal epithelial cells."

T.P. Stossel and J.H. Hartwig, Children's Hospital Medical Center, Boston, Massachusetts: "Interaction of contractile proteins of pulmonary macrophages: Effect of phagocytosis."

R. Cooke and J.A. Spudich, Department of Biochemistry and Biophysics, University of California, San Francisco: "Supramolecular forms of actin from amoebae of *Dictyostelium discoideum*."

FRIDAY MORNING, September 12

Session 6

Chairperson: H. Holtzer, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

R.S. Adelstein and M.A. Conti, Cardiology Branch, National Heart and Lung Institute, NIH, Bethesda, Maryland: "Phosphorylation of human platelet myosin."

T.D. Pollard, K. Fujiwara and P.M. Szamier, Department of Anatomy, Harvard Medical School, Boston, Massachusetts: "Assembly, fixation and intracellular localization of cytoplasmic contractile systems."

D.N. Jacobson, R.M. Johnke and M.R. Adelman, Duke University Medical Center, Durham, North Carolina: "Studies on motility in *Physarum polycephalum*."

V. Nachmias and A. Asch, Department of Anatomy, University of Pennsylvania, Philadelphia: "Polarity of actin in human platelet microspikes and synergistic regulation in *Physarum*."

R.E. Fine and A.L. Blitz, Department of Physiology, Boston University School of Medicine, Massachusetts: "Chemical and function studies of tropomyosin and troponin C from brain and other tissues."

D.L. Taylor, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: "Calcium sensitive models of amoeboid movement."

FRIDAY EVENING, September 12

Session 7

Chairperson: P. Satir, Department of Anatomy and Physiology, University of California, Berkeley, California

L.A. Amos, R.W. Linck and A. Klug, Medical Research Council, Cambridge, England, and Department of Anatomy, Harvard Medical School, Boston, Massachusetts: "Molecular structure of flagellar microtubules."

R.W. Linck, Department of Anatomy, Harvard Medical School, Boston, Massachusetts: "Fractionation of minor component proteins and α-tubulin from specific regions of flagellar doublet microtubules."

F.D. Warner, Department of Biology, Biological Research Laboratories, Syracuse University, New York: "Crossbridge mechanisms in ciliary motility."

I.R. Gibbons, E. Fronk, B.H. Gibbons and K. Ogawa, Pacific Biomedical Research Center, University of Hawaii, Honolulu: "Multiple forms of dynein in flagellar axonemes of sea urchin sperm."

C. Kung, Laboratory of Molecular Biology and Department of Genetics, University of Wisconsin, Madison: "Control of ciliary movement: Bioelectric and genetic studies."

G.B. Witman, Department of Biology, Princeton University, New Jersey: "Studies on *Chlamydomonas* mutants lacking the radial spokes and the central sheath and tubules."

S.L. Tamm, Zoology Department, Indiana University, Bloomington: "Properties of a rotary motor in eucaryotic cells."

B. Huang and D. Luck, The Rockefeller University, New York, New York: "Temperature-sensitive mutants of *Chlamydomonas reinhardi* defective in flagellar function."

C. Fulton and P.A. Simpson, Department of Biology, Brandeis University, Waltham, Massachusetts: "Selective synthesis and utilization of flagellar tubulin, or, how many different tubulin dimers are present in an organism?"

L. Wilson, K. Anderson and D. Chin, Department of Pharmacology, Stanford University School of Medicine, California: "Interaction of drugs with tubulin: Biochemical mechanisms of action."

R.J. Lasek and P.N. Hoffman, Department of Anatomy, Case Western Reserve, Cleveland, Ohio: "Axonal transport of cytoskeletal elements in neurons."

M.L. Shelanski, S. Yen and V. Lee, Department of Neuropathology, Harvard Medical School, Boston, Massachusetts: "Neurofilaments, glial filaments and glial fibrillary acid proteins from brain."

SATURDAY MORNING, September 13

Session 8

Chairperson: E. Taylor, University of Chicago, Chicago, Illinois

H.P. Erickson, Department of Anatomy, Duke University, Durham, North Carolina: "Facilitation of microtubule assembly of polycations."

J.B. Olmstead and G.G. Borisy, Department of Biology, University of Rochester, New York, and Laboratory of Molecular Biology, University of Wisonsin, Madison: "Viscometric characterization of microtubule polymerization in vitro."

F. Gaskin and J.S. Gethner, Departments of Pathology and Biophysics, Albert Einstein College of Medicine, Bronx, New York, and Department of Chemistry, Columbia University, New York: "Characterization of the in vitro assembly of microtubules."

R.C. Weisenberg, Department of Biology, Temple University, Philadelphia, Pennsylvania: "Role of nucleotides in microtubule assembly."

G.G. Borisy, K. Johnson, D. Murphy, and M. Marcum, Laboratory of Molecular Biology, University of Wisconsin, Madison: "Mechanism of microtubule assembly and characterization of tubule-binding proteins."

M. Kirschner, Department of Biochemical Sciences, Princeton University, New Jersey: "Studies on the mechanism of microtubule assembly."

SATURDAY AFTERNOON, September 13

Session 9A Workshop: Accessory Proteins in Microtubule Assembly

Chairperson: R. McIntosh, University of Colorado, Boulder, Colorado

Participants: R. Bloodgood and R. Sloboda, Department of Biology, Yale University, New Haven, Connecticut: "Initiation of microtubule assembly in vitro."

M. Kirschner, Department of Biochemical Sciences, Princeton University, New Jersey

D. Murphy, Laboratory of Molecular Biology, University of Wisconsin, Madison

B. Nagle, Department of Biology, University of Pennsylvania, Philadelphia: "Inhibition of spontaneous tubulin assembly by RNA and other polyanions: Evidence for a required protein."

E. Taylor, University of Chicago, Illinois

H. Erickson, Department of Anatomy, Duke University, Durham, North Carolina

Session 9B

Chairperson: B. Nicklas, Department of Zoology, Duke University, Durham, North Carolina

A. Forer, Department of Biology, York University, Downsview, Ontario, Canada: "Chromosomal movements: Actin filaments and birefringence."

J.R. McIntosh, W.Z. Cande, J.A. Snyder, E. Lazarides and K. McDonald, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: "Studies on the formation and function of the mammalian mitotic spindle."

B.R. Brinkley, D.P. Highfield and G.M. Fuller, Department of Human Biological Chemistry and Genetics, Graduate School of Biomedical Sciences, University of Texas Medical Branch, Galveston: "Tubulin antibodies as probes for mitotic mechanisms."

S. Inoue, Department of Biology, University of Pennsylvania, Philadelphia: "Chromosome movement by growth and melting of microtubules."

L.I. Rebhun and J. Nath, Department of Biology, University of Virginia, Charlottesville: "Involvement of sulfhydryls in cell division: Studies in vitro and in vivo."

Summary: E. Taylor, University of Chicago, Chicago, Illinois

This meeting was supported in part by grants from the National Institute of General Medical Sciences, the National Cancer Institute, and the National Science Foundation.

IN-HOUSE SEMINARS

Cold Spring Harbor in-house seminars were initiated to provide a semi-formal avenue for communication between the various research groups at the laboratory. They are particularly useful for research personnel who have joined the laboratory during the summer. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in defending, organizing and presenting their research. In addition to those listed below, seminars were given by many others involved in research at this laboratory.

September

16th. Dr. Gary Gunther, Rockefeller University, New York: "Surface alterations and mitogenesis in lymphocytes."

20th. Dr. Nelson Goldberg, University of Minnesota Medical School, Minneapolis: "Biological significance of cyclic-GMP."

October

9th. Dr. Bob Millette, University of Chicago, Illinois: "Synthesis and adenylation of mRNA in cells infected with herpes simplex virus."

25th. Dr. Mark Bretscher, Medical Research Council Laboratory, Cambridge, England: "Studies on the chemical structure of membranes."

November

4th. Dr. Paul Geshelin, Johns Hopkins University, Baltimore, Maryland: "Crosslinks in vaccinia DNA."

5th. Dr. Keith Roberts, John Innes Institute, Norfolk, England: "The crystalline glycoprotein envelope of a green alga."

6th. Dr. Dino Dina, University of Geneva, Switzerland: "Sequence arrangement in mRNA."

8th. Dr. Hardy Chan, University of Wisconsin, Madison: "The structural uniqueness of the lactose regulatory region."

15th. Dr. Tom Pollard, Harvard Medical School. Boston. Massachusetts: "Cytoplasmic actin and myosin as the basis of cellular movement."

20th. Dr. John Hopfield, Princeton University, New Jersey: "Kinetic proofreading: A new mechanism for reducing errors in biosynthetic processes requiring high specificity."

26th. Dr. Bill Sugden, Karolinska Institute, Stockholm, Sweden: "EBV."

December

4th. Dr. S.-I. Shin, Albert Einstein College of Medicine, New York: "Cellular tumorigenicity in the nude mouse."

5th. Dr. Douglas N. Foster, University of Utah, Salt Lake City: "Eukaryotic DNA polymerase activity in isolated nuclei."

9th. Dr. Terry Platt, Stanford University, California: "Ribosome-protected regions in tryptophan operon messenger RNA of *E. coli*."

11th. Dr. Joseph H. Coggin, Jr., University of Tennessee, Knoxville: "Retrogression in neoplasia."

16th. Dr. Gary Wilson, University of Rochester, New York: "Heterospecific transformation of *B. subtilis*."

18th. Dr. Dennis Gospodarowicz, The Salk Institute, San Diego, California: "Control of mammalian cell division in vitro and in vivo."

January

10th. Dr. Harold Weintraub, Princeton University, New Jersey: "Dissection of chromatin structure with proteases and nucleases."

14th. Dr. Gerard Roizes, MRC Mammalian Genome Unit, University of Edinburg,

Dietmar Kamp (*at board*) seems to have captured the attention of his audience during the lunchtime seminar. (*Photo by Robert Yaffe*)

Scotland: "The use of restriction endonucleases in the study of repeated DNA sequences in the eukaryote genome."

17th. Dr. Peter Wensink, Brandeis University, Waltham, Massachusetts: "Isolation and characterization of fragments of *Drosophila* DNA."

22nd. Dr. Aaron Shatkin, Roche Institute, Nutley, New Jersey: "The blocked methylated 5′ end of mRNA."

29th. Dr. John Chase, Harvard Medical School, Boston, Massachusetts: "Exonuclease VII of *E. coli*."

February

5th. Dr. Frank A. Pepe, University of Pennsylvania School of Medicine, Philadelphia: "The structure of the myosin filament of striated muscle from electron microscopy and antibody labeling."

12th. Dr. Vic Ling, Ontario Cancer Institute, Toronto, Canada: "Membrane mutants in cultured cells."

17th. Dr. Jack Griffith, Stanford University Medical School, Palo Alto, California: "SV40 mini-chromosomes."

19th. Dr. D. V. M. Reddy, University of Illinois, Chicago: "Wound tumor virus."

19th. Dr. C. Thomas Caskey, Baylor College of Medicine, Houston, Texas: "Biochemical characterization of 8-azoguanine resistance in Chinese hamster mutants."

28th. Dr. Michael Rosbach, Brandeis University, Waltham, Massachusetts: "Messenger RNA sequences in single copy DNA."

March

4th. Dr. Pat O'Farrell, University of California Medical Center, San Francisco: "Two-dimensional gel electrophoresis and the control of protein synthesis."

10th. Dr. William Haseltine, Massachusetts Institute of Technology, Cambridge: "The mechanism of initiation of reverse transcriptase."

11th. Dr. Michael Fried, Imperial Cancer Research Fund Laboratories, London, England: "Structure of polyoma virus DNA."

11th. Dr. Marc Van Montagu, Rijksuniversiteit, Ghent, Belgium: "Studies of large plasmids in crown-gall inducing strains of *Agrobacterium*."

12th. Dr. Mario R. Capecchi, University of Utah, Salt Lake City: "Mammalian cell genetics: A molecular approach."

14th. Dr. Bob Metzenberg, University of Wisconsin Medical Center, Madison: "Regulation of phosphorous metabolism in neurospora."

17th. Dr. Joan Brugge, Baylor College of Medicine, Houston, Texas: "Role of the SV40 gene A function in transformation."

19th. Dr. Bob Martin, National Institutes of Health, Bethesda, Maryland: "Characterization of temperature-sensitive mutants of SV40."

24th. Dr. Jonathan Jarvik, Massachusetts Institute of Technology, Cambridge: "Genetic methods of analysis of biochemical pathways."

April

2nd. Dr. John Cairns, Imperial Cancer Research Fund Laboratories, London, England: "Mutation, selection and the natural history of cancer."

3rd. Dr. Zac Cande, University of Colorado, Boulder: "Mitosis in lysed cell preparations."

4th. Dr. Ian Molineux, Medical Research Council, London, England: "The *E. coli* DNA binding protein: Interactions with DNA and enzymes."

29th. Dr. David Schlessinger, Washington University School of Medicine, Seattle: "RNA processing in relation to ribosome formation in *E. coli*."

May

9th. Dr. Scott Emmons, Carnegie Institution of Washington, Washington, D. C.: "Genetic duplications in phage lambda."

12th. Dr. Duane Eichler, Stanford Medical School, California: "Genetic and enzymological studies on the terminal stages of DNA replication in *E. coli*."

14th. Dr. Winston Salzer, University of California, Los Angeles: "Nucleotide sequence studies of satellite DNAs and hemoglobin messenger RNAs."

16th. Dr. George Poste, Roswell Park Memorial Institute, Buffalo, New York: "Studies on membrane fusion."

23rd. Dr. Margaret Clarke, University of California Medical Center, San Francisco: "Studies of cell movement: Contractile proteins of *Dictyostelium discoideum*."

26th. Dr. Lois Miller, Imperial Cancer Research Fund Laboratories, London, England: "The genetic map of polyoma virus."

UNDERGRADUATE RESEARCH PARTICIPATION PROGRAM

Summer 1975

Another 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, and since that year, 165 students have completed the course. It is a pleasure for us to recollect that David Baltimore, a joint recipient of the Nobel Prize for Physiology or Medicine in 1975, was a member of the first group of undergraduate research participants in 1959.

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) an increased awareness 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.

Ten college undergraduates, selected from a large number of applicants, take part in the program which is now mainly supported by laboratory funds. This year's students are listed below, with their laboratory sponsors and topics of research. We gratefully acknowledge the generosity of the Central General Hospital, Plainview, New York, which provided fellowships to support the work of Roslyn Feder, Vann Parker, Howard Rutman and Gary Struhl.

John Kent Chin, Yale University *Supervisor:* T. R. Broker	Characterization of ultraviolet radiation-sensitive mutants of bacteriophage T4
Paul Arthur Epstein, Princeton University *Supervisor:* R. F. Gesteland	The relationship between polarity suppression and internal reinitiation polypeptides in *Escherichia coli*
Roslyn Feder, Brooklyn College *Supervisor:* D. Botstein	Fractionation of suppressing tRNA from yeast cells
David Alan Goldberg, Yale University *Supervisor:* T. Maniatis	Direct DNA sequence analysis of bovine satellite DNA
Martin Jacobs, Duke University *Supervisor:* B. N. Apte	Polypeptide splicing in vivo and in vitro
Wilson H. Miller, Jr., Princeton University *Supervisor:* R. F. Gesteland	Cell-free protein synthesis in extracts from yeast
Julie Olson, Massachusetts Institute of Technology *Supervisor:* R. J. Roberts	Screening bacterial strains for new restriction endonucleases
Vann Parker, Duke University *Supervisor:* A. I. Bukhari	Genetic analysis of circular DNA molecules formed after prophage Mu induction
Howard I. Rutman, Harvard College *Supervisor:* M. Botchan	Phosphorylation of SV40 virion proteins
Gary Struhl, Massachusetts Institute of Technology *Supervisor:* G. Albrecht-Bühler	Two phases of locomotion in 3T3 mouse fibroblasts as revealed by haptotaxis phenomena

NATURE STUDY PROGRAM

The Nature Study Program is designed for elementary and high school students who wish to achieve a greater understanding of their environment. In summer and fall of 1975, 374 students participated in this program. Weather permitting, most of the courses were held outdoors, using both laboratory grounds and Uplands Farm Nature Preserve of the Nature Conservancy as resources. The Laboratory has built and equipped classroom-laboratories at Uplands Farm for the study of field specimens collected by students.

As the first step in the initiation of a new program in Marine Biology and Natural History, in October 1975 the Laboratory presented two courses to highly motivated high school students. These courses were taught at Uplands Farm and on the Laboratory's Cold Spring Harbor Marina site.

The continuing support of Heritage Federal Savings and Loan Association, Huntington, which provided scholarships for 20 students, and the Three Harbors Garden Club, which donated one scholarship, is greatly appreciated.

STAFF

Sanford Kaufman, M.S., M.P.A., Biology and Environmental Science Instructor, Hewlett High School. Program Director
Suzanne Ackley, M.A. candidate, Biology and General Science Teacher, Lynbrook High School
Linda Bovich, M.S., Science Teacher, Rocky Point High School
Ralph Chioffe, M.A., Teacher, Elwood Junior High School
Donn Dunn, M.S., Art and Photography Teacher, Hewlett High School
Syril Farber, Ph.D., Rutgers University
Steve Ferarro, Ph.D. candidate, SUNY, Stony Brook
George Hechtel, Ph.D., Department of Biology, SUNY, Stony Brook
Barbarajean Kaufman, M.S. candidate, Instructor, New York Institute of Technology
Fred Maasch, M. Ed., Biology Teacher, Islip High School
Harold O'Connors, Ph.D., Marine Sciences Research Center, SUNY, Stony Brook
Carlton Phillips, Ph.D., Department of Biology, Hofstra University
C. Donald Powers, Ph.D., Postdoctoral Research Associate, Marine Sciences Research Center, SUNY, Stony Brook
Jim Romansky, M.S., Biology Teacher, Bay Shore High School
Anthony Taormina, New York State Director of Environmental Conservation
Edward Tronlone, M.S., Science Teacher, Lynbrook High School

COURSES

General Nature Study
Advanced Nature Study
Elementary Geology
Advanced Geology
Bird Study
Seashore Life
Vertebrate Biology
Reptiles and Amphibians
Marine Biology
Nature Photography I, II
Introduction to Ecology
Fresh Water Life I, II
Conservation

Photos on facing page were taken, developed and printed by students in the Nature Photography course.

LABORATORY STAFF

November 1975

Director
J. D. Watson

Administrative Director
William R. Udry

Research Scientists
Guenter Albrecht-Buehler
Bal Apte
Michael Botchan
Thomas Broker
Ahmad Bukhari
Raymond Gesteland
Terri Grodzicker
Walter Keller
James Lewis
Thomas Maniatis
Michael Mathews
Richard Roberts
Joseph Sambrook
David Zipser

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Ramunas Bigelis
Alfred Bothwell
Keith Burridge
Louise Chow
Ellen Daniell
Sharyn Endow
George Fey
Denise Galloway
Richard Gelinas
John Hassell
Regine Kahmann
Dietmar Kamp
Sara Lavi
Eugene Lukanidin
William Topp
Sayeeda Zain

Graduate Students
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William E. Gordon, III
Daniel Kiessig
James Manley
Gek-Kee Sim

Research Assistants
Gregory Ballantyne
Julian Banerji
Joseph Bonventre
Cathleen Casserly
Sallie Chait
Barbara Doretsky
Susan Froshauer
Ronni Greene
Alan Johnston
Charles Jungreis
Robert Lancaster
Sheila Levings
Anita Lewis
Jayne Maritato
Robert McGuirk, Jr.
Norma Mendenhall
Phyllis Myers
Rosemary Oliveros
Diana O'Loane
John Scott
Randi Siemers
Luis Solari
Robin Thek
Louis Waldorf
Ingrid Wendel
Leslie Wheeler

Supporting Staff
Robert Borruso

Annual staff tug-of-war.

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This year the rope won!

FINANCIAL STATEMENT

Balance Sheet
Year ended October 31, 1975
With comparative figures for 1974

ASSETS

	1975	1974
Current Funds:		
Unrestricted:		
Cash	$ 126,878	$ 204,029
Accounts receivable	70,059	68,916
Inventory of books	167,085	84,781
Prepaid expenses	8,796	12,102
Due from restricted funds	36,595	—
Total unrestricted	409,413	369,828
Restricted:		
Cash	—	291,615
Grants receivable	605,651	387,237
Total restricted	605,651	678,852
Total current funds	1,015,064	1,048,680
Plant funds:		
Investment	39,197	39,685
Due from current funds	176,156	233,697
Land and improvements	581,496	579,197
Buildings	1,980,099	1,723,521
Furniture, fixtures and equipment	476,789	423,766
Books and periodicals	365,630	365,630
Construction in progress	276,993	21,497
	3,896,360	3,386,993
Less allowance for depreciation and amortization	686,331	516,077
Total plant funds	3,210,029	2,870,916

LIABILITIES AND FUND BALANCES

Current funds:		
Unrestricted:		
Accounts payable	58,347	98,854
Due to plant funds	176,156	233,697
Fund balance	174,910	37,277
Total unrestricted	409,413	369,828
Restricted:		
Due to unrestricted funds	36,595	—
Fund balance	569,056	678,852
Total restricted	605,651	678,852
Total current funds	1,015,064	1,048,680
Plant funds:		
Fund balance	3,210,029	2,870,916

Statement of Current Revenues, Expenditures and Transfers
Year ended October 31, 1975
with comparative figures for 1974

	1975	1974
Revenues:		
Grants	$1,661,421	$1,505,426
Indirect cost allowance on grants	856,344	663,050
Contributions	62,072	95,272
Robertson Research Fund contribution	275,000	320,012
Summer programs	151,235	129,821
Laboratory rental	19,633	18,765
Marina rental	31,300	30,000
Investment income	19,990	36,851
Book sales	382,221	297,247
Dining hall	160,021	105,971
Rooms and apartments	133,576	102,922
Other sources	24,747	2,967
Total revenues	3,777,560	3,308,304
Expenditures:		
Research*	1,593,093	1,515,896
Summer programs*	179,861	178,962
Library	56,361	80,637
Operation and maintenance of physical plant	502,136	421,311
General and administrative	387,595	344,173
Book sales*	224,145	227,452
Dining hall*	188,786	120,849
	3,131,977	2,889,280
Transfers:		
To unexpended plant funds	452,753	33,180
To plant funds	55,197	602,382
	507,950	635,562
Total expenditures and transfers	3,639,927	3,524,842
Excess (deficit) of revenues over expenditures and transfers	137,633	(216,538)

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

NOTE: The complete financial statements, as certified by our independent auditors, Peat, Marwick, Mitchell, Co., are available on request from the Comptroller, Cold Spring Harbor Laboratory.

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 grants which would result in 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 and the marine sciences, can only be undertaken 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

There are several ways to contribute securities:

(1) Your broker or bank may sell the securities and remit the proceeds directly to the Laboratory.

(2) If you wish to send the stock directly to the Laboratory, either a) endorse the certificate(s) in blank (no name transferee) by signing your name on the back of the certificate(s). Have your signature guaranteed on the certificate(s) by your bank or broker. Send the certificate(s) by *registered mail* to the Laboratory; or b) send *unsigned* certificate(s) along with a covering letter. Under separate cover, send a stock power executed in blank (one for each certificate and again with a signature guarantee) along with a copy of the covering letter. Both should be sent by first class mail to the Laboratory. Depreciated securities should be sold for your own account to establish a tax loss, then make your contribution to the Laboratory by check.

Bequests

Probably most wills need to be updated. Designating the Cold Spring Harbor Laboratory as a beneficiary insures that a bequest will be utilized as specified for continuing good.

Appreciated Real Estate or Personal Property

Sizeable tax benefits can result from such donations, some of which the Laboratory can use in its program, others can be sold after donation.

Life Insurance & Charitable Remainder Trusts

Can be structured to suit the donor's specific desires as to extent, timing and tax needs and, at the same time, increase the resources available for the work of the Laboratory.

Conversion of Private Foundation to "Public" Status or 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 the Cold Spring Harbor Laboratory."

For additional information, please contact the Administrative Director, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, N.Y. 11724, or call area code 516-692-6660.

GRANTS

November 1, 1974 — October 31, 1975

Grantor	*Principal Investigator–Program*	*Total Award*	*Duration of Grant*
NEW GRANTS			
National Science Foundation	Dr. Gesteland - Research	$ 135,000	1/1/75 - 12/31/77
	Dr. Albrecht-Buehler - Research	76,300	7/1/75 - 6/30/77
	Dr. Watson - Conference on Cell Motility	2,500	7/1/75 - 9/30/75
National Institutes of Health	Dr. Watson - General Research Support	145,638	1/1/75 - 12/31/75
	Dr. Bukhari - Career Development	110,000	5/1/75 - 4/30/80
	Dr. Hassell - Fellowship	13,000	10/1/75 - 9/30/76
	Dr. Watson - Conference on Cell Motility	24,500	6/30/75 - 5/31/76
American Cancer Society	Dr. Endow - Fellowship	8,500	1/1/75 - 12/31/75
	Dr. Lewis - Research	74,100	7/1/75 - 6/30/77
Eppley Foundation	Dr. Watson - Neurobiology	10,000	1/1/75 - 12/31/75
Anna Fuller Fund	Dr. Burridge - Fellowship	9,100	6/1/75 - 5/31/76
Cystic Fibrosis Foundation	Dr. Bukhari - Research	45,000	1/1/75 - 12/31/77
Alfred P. Sloan Foundation	Dr. Watson - Neurobiology Research	165,000	4/1/75 - 12/31/77
Jane Coffin Childs Memorial Fund for Cancer Research	Dr. Bothwell - Fellowship	10,500	7/1/75 - 6/30/76
CONTINUING GRANTS			
National Science Foundation	Dr. Bukhari - Research	60,000	8/1/74 - 7/31/76
	Dr. Roberts - Research	50,000	6/15/74 - 5/30/76
	Dr. Gesteland - Research	41,400	9/1/74 - 2/29/76
	Dr. Zipser - Research	80,000	1/1/74 - 12/31/75
	W. Udry - Symposium Support	5,000	4/1/75 - 9/30/75
	Dr. Pollack/Dr. Goldman - Research	115,600	5/1/73 - 4/30/75
National Institutes of Health	Dr. Pollack - Summer Training	354,459	1/1/70 - 5/30/75
	Dr. Zipser - Career Development	108,000	5/1/70 - 4/30/75
	Dr. Watson - Symposium Support	96,000	4/1/74 - 3/31/79
	Dr. Watson - Cancer Research Center	7,789,000	1/1/72 - 12/31/76
	Dr. Watson - General Research Support	148,850	1/1/74 - 12/31/74
	Dr. Zipser - Research	350,000	5/1/74 - 4/30/79
	Dr. Gesteland - Summer Workshops	194,000	4/1/74 - 3/31/77
	Dr. Watson - Neurobiology Training	160,812	5/1/74 - 4/30/77
	Dr. Gelinas - Fellowship	24,500	7/3/74 - 12/3/75
American Cancer Society	Dr. Sambrook - Fellowship	100,000	12/1/70 - 11/30/75
Helen Hay Whitney Foundation	Dr. Daniell - Fellowship	17,583	3/1/74 - 12/31/75
Alfred P. Sloan Foundation	Dr. Watson - Neurobiology Training	450,000	10/1/70 - 9/30/75
Volkswagen Foundation	Dr. Watson - Training Support	73,085	1/1/71 - 12/31/75
Energy Research & Development Administration	Dr. Watson - Symposium Support	8,000	6/3/75 - 6/10/75
Grass Foundation	Dr. Watson - Neurobiology Training	5,000	6/1/75 - 9/30/75

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Olney House (*Photo by Jack Richards*)

The Laboratory was founded in 1890 by several local philanthropists and the Brooklyn Institute of Arts and Sciences. The first chairman of the Board of Managers of the Laboratory was Eugene G. Blackford, who served from 1890 until his death in 1904. William J. Matheson succeeded him, serving until 1923.

In that year, when the Brooklyn Institute of Arts and Sciences withdrew from Cold Spring Harbor, the local supporters of the research formalized their efforts by incorporating as the Long Island Biological Association. Colonel T.S. Williams became the first Chairman of the new group. Jointly with the Carnegie Institution of Washington, LIBA continued to support and direct the research at Cold Spring Harbor Laboratory.

In 1962 the Laboratory was reorganized as an operating organization and LIBA relinquished its management responsibilities. During the past 13 years, LIBA's chief function has been to widen the interest of the community in the Laboratory and to help support it financially.

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Mrs. F. Carrington Weems
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Mrs. Alex M. White
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Mrs. John C. Wilmerding
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Mrs. Willis D. Wood
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Mrs. Ford Wright
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