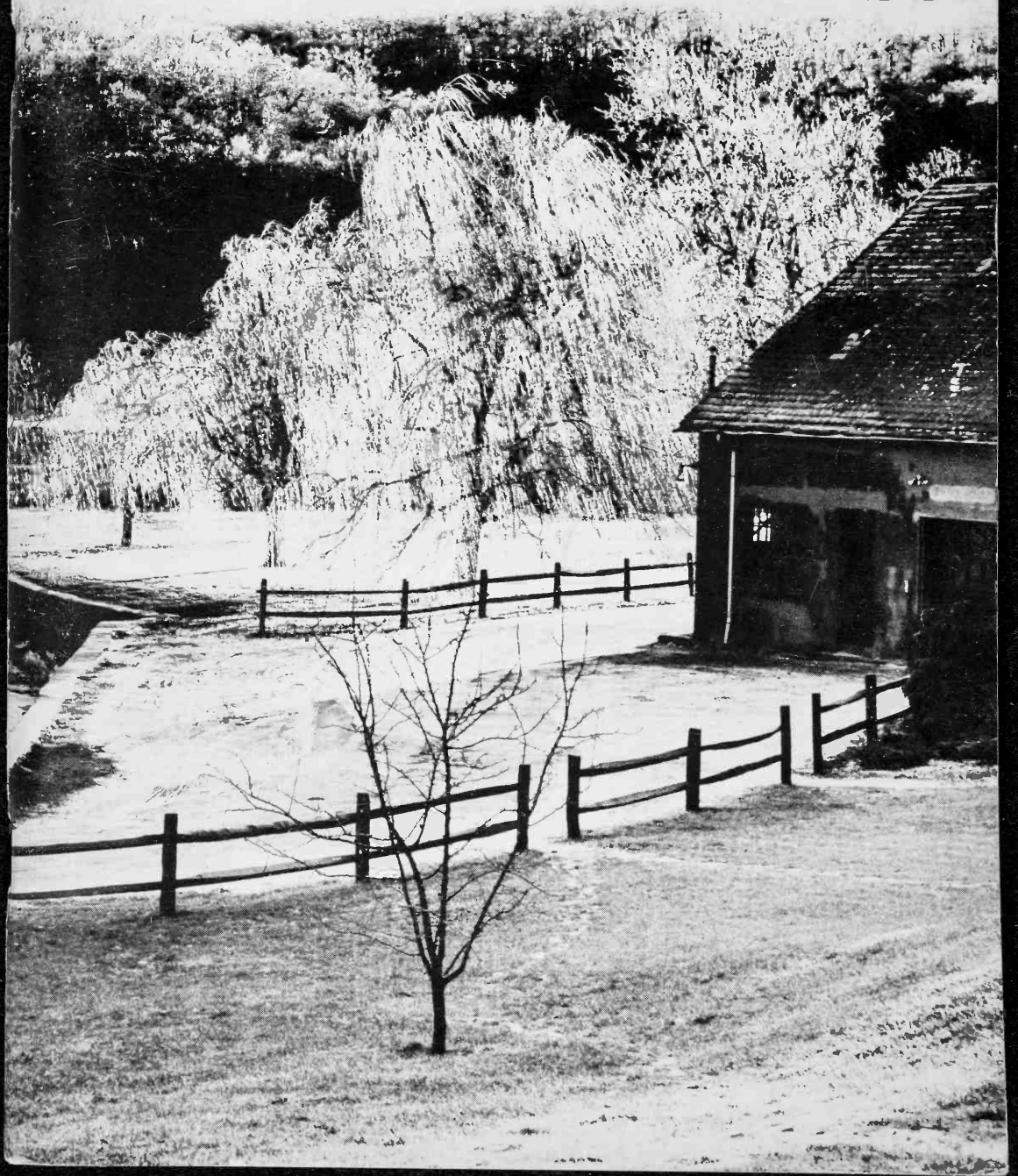
COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1973



ANNUAL REPORT 1973

COLD SPRING HARBOR LABORATORY

COLD SPRING HARBOR, NEW YORK

COLD SPRING HARBOR LABORATORY

Cold Spring Harbor, Long Island, New York

OFFICERS OF THE CORPORATION

Chairman: Dr. H. Bentley Glass

1st Vice Chairman: Edward Pulling

2nd Vice Chairman: Arthur D. Trottenberg

Secretary: Dr. Bayard Clarkson

Treasurer: Robert H. P. Olney

Assistant Secretary-Treasurer
William R. Udry

Laboratory Director: Dr. James D. Watson

Administrative Director: William R. Udry

BOARD OF TRUSTEES

INSTITUTIONAL TRUSTEES

Albert Einstein College of Medicine
Dr. J. T. August

The City University of New York
Dr. Norman R. Eaton

Columbia University
Dr. James E. Darnell, Jr.

Duke University Medical Center
Dr. Robert E. Webster

Harvard Medical School
Dr. Charles A. Thomas, Jr.

Long Island Biological Association
Dr. Edward Pulling

Massachusetts Institute of Technology
Dr. Maurice S. Fox

New York University Medical Center
Dr. Milton R. J. Salton

Princeton University
Dr. Bruce Alberts

The Rockefeller University
Dr. Norton D. Zinder

Sloan-Kettering Institute for Cancer Research
Dr. Bayard Clarkson

State University of New York, Stony Brook
Dr. H. Bentley Glass

University of Chicago
Dr. Robert Haselkorn

University of Wisconsin
Dr. Julian Davies

Wawepex Society of Cold Spring Harbor
Mr. Townsend Knight

INDIVIDUAL TRUSTEES

Dr. Reese F. Alsop

Dr. David P. Jacobus

Mrs. George N. Lindsay

Angus P. McIntyre

Robert H. P. Olney

Dr. Alexander Hollaender, Honorary Trustee

Walter H. Page

A. D. Trottenberg

Colton P. Wagner

Mrs. Alex M. White

William A. Woodcock

DIRECTOR'S REPORT

To the biochemist cancer now stands out like Mount Everest did to the alpinists of the late 1940's. Most certainly not the first very tall mountain to surmount, but something you always know is there and if you are strong and calm and make all the right advance preparations and then are more than a little lucky, you might with the help of many trained companions get to the top. You know all too well that you should wait for the right moment and not set off prematurely without knowing well the terrain of the lower crevasses. Too many of your friends, fresh from the successes of late youth, have started out without maps only to vanish from real science, leaving merely the remains of faded house organs containing smiling faces that have never grasped the essence of the enemy.

We wonder, of course, whether we will land in the same icy graveyard. Those who have disappeared before were not always silly and short-sighted but often scientists of the first rank, with no tolerance for the shoddy optimism of the second-rate. When last seen, however, they usually had become preoccupied with minor facts that had the smell of the irrelevant but which somehow had become that instant's great white hope in the fight against cancer. So today many respectable scientists still consider cancer research synonymous with lousy science. Whenever a well-known scientist announces that he has become interested in cancer, the stock reflex is to regard him as another cop-out, no longer good enough to compete for the increasingly limited money that goes for pure science, and so of necessity he is forced into the bountiful jaws of an overfunded National Cancer Institute.

Our problem, of course, is not whether uninformed outsiders regard us as has-beens, but whether our great effort of the past five years in reorganizing much of this Lab toward tumor virus research will pay off. Could we have jumped in too early, with a massive push toward the molecular nature of cancer only making sense when some totally unanticipated discovery at last gives cancer research an intellectual basis? Or if there already exist some real ideas to test, were there already enough good laboratories committed to their exploration? Did we enter an already overpopulated area of research or was there a vacuum waiting to be filled with intelligent minds?

My prejudiced guess is that in 1969 we chose the right time to go all out. The development in the early 1960's of cell culture techniques for the study of tumor viruses had at long last made technically feasible the study of cancer at the molecular level. The situation was in many ways similar to that of the phage world of 1945, then open for outside invasion because of the pioneering efforts of Delbruck, Hershey and Luria. Thanks to much patient work in a small number of far-sighted research groups (those of Dulbecco Eagle, Green, Rubin, Stoker and Puck quickly come to mind) cancer research was becoming a proper target for the molecular biologist. Yet for a variety of reasons, many connected to the widespread fatalism about the future American commitment to science engendered by the Vietnamese war, almost no new research groups were rising to the challenge. So we did not see how we could fail to leave our mark, that is, if we could get going on a large enough scale.

Key to our initial optimism were hints, now solidly confirmed, that an essential aspect of viral carcinogenesis was the insertion of one or more of the genes of the various tumor viruses into the chromosome(s) of the respective host cells. Here they must code for one or more specific proteins whose presence upsets the normal cellular metabolism, somehow changing it into a cancer cell. Given this way of thinking, the obvious question becomes how to find out the exact biochemical function(s) carried out by these cancer genes. Is always the same task involved or do different cancer viruses bring about cancer in fundamentally different ways? Answering this question, however, is not at all straightforward, and at pessimistic moments can seem like looking for a needle in a haystack. Most of the time, however, we think we have a fighting chance for we focus on the fact that the chromosomes of most known tumor viruses are

remarkably small. In the most favorable examples they have the potentiality of coding for only 6-10 small proteins, while with many other tumor viruses there are at most only 30 or so genes whose functions we must someday work out.

Thus at the heart of our approach in James Lab, as well as that in many other labs elsewhere, is the objective of working out all the details, no matter how seemingly obscure, in the multiplication of tumor viruses. Hopefully one or more of such facts will suddenly make sense of some essential aspect of the cancer cell. For example, for several decades the notion has existed that the biochemistry of the cancer cell strongly resembles that of embryonic cells, conceivably by a reversion of the pattern of gene transcription to that present in early embryological development. Thus a key element in any study of tumor viruses must be a study of their effects on the enzymes involved in transcription. Unfortunately the study of the transcription of the DNA of normal cells is still in a morass and not converging toward the truth nearly as fast as we would like. So what at first might seem a straightforward approach is not all that easy and may be only for those who are willing to wait many years for real answers.

Another way to proceed is to try to indentify proteins specific both for cells infected by tumor viruses and the cells they make cancerous. Hopefully, having found one such key viral gene product we might then be able to isolate it and see whether it possesses the enzymatic attributes that might predispose cells toward the cancerous appearance. But knowing where within the cell to look is not obvious, and for the time being the best approach may be to follow up the immunological findings that first came out of the National Institutes of Health. Work first initiated there by Huebner and Habel revealed that most tumor viruses induce within their respective tumors the presence of specific antigens, the so-called T(tumor) antigens. Given enough skill and time, some persistent scientist(s) should be able to isolate these tumor antigens in pure enough form to reveal their biological function(s). Unfortunately, this approach has so far yielded very limited dividends, conceivably both because the tumor antigens thus far examined are not very stable and because very few protein chemists have yet to see the unique challenge they present. Here at the Lab Klaus and Mary Weber are pushing this approach hard and hopefully will succeed. Most importantly, even if they don't soon pull off something very pretty, we must continue this approach. It is very hard not to believe that these tumor-specific substances are a vital clue to the essence of cancers.

Most clearly we must always be careful not to rush matters that are not obvious. On the other hand, if we felt that another generation of scientists would have to come along before we hit any jackpots, it would be very hard now to maintain research momentum. So much depends on when we think some true successes will emerge from the experiments currently being done in the better cancer oriented laboratories throughout the world. Now I would guess that within the next 5-10 years we will see the light, meaning we shall unambiguously know one or more of the biochemical reactions which make a cell cancerous. I realize, however, that this is the same time interval that I would have predicted five years ago and so I must further explain myself. It is not that nothing of consequence has happened over the past five years. I would guess that no serious scientist could have predicted that tumor virus research would move so fast within this interval. Our meeting here this past August on the small DNA tumor viruses (SV40, polyoma and adenoviruses) displayed an elegance of experimentation that formerly was thought would always remain the unique trademark of the phage world. Likewise the discovery in 1970 of the RNA-directed DNA polymerase (reverse transcriptase) by Temin and Baltimore has broken open the previously very murky world of the RNA tumor viruses and work with them now can be done with the sophistication that marks the best of science.

Most real answers about cancer nonetheless still look 5-10 years away since not unexpectedly we now realize our objectives to be much more complex than we might have guessed. For example, our thoughts about the physical organization of the chromosomes of RNA tumor virus are still very messy, and some really new ideas must emerge to set us off in the right direction. And the whole problem of the duplication of DNA, that once we hoped might be only moderately hard to unravel, has now emerged as a formidable obstacle even for the best molecular biologists working under optimal conditions. The main take-home lesson, however, is that important new facts are emerging at an ever increasing tempo, and it will be most surprising if something dramatic does not break soon.

In fact, there is a good chance that one such major event has already happened. This is the observation of Edward Reich and his collaborators at the Rockefeller University that virtually all highly malignant cells continuously release from their surfaces a highly specific proteolytic

enzyme that they call the "cell factor". This protein splitting enzyme then activates another proteolytic enzyme (plasminogen→plasmin) in the serum that surrounds the cancer cell. The resulting massive proteolytic activity created by this cascade-like process then acts back on the surface of the cancer cell degrading many of its surface proteins, thereby giving it a very different surface topology, and hence metabolism, from that of the equivalent normal cell.

Within a given animal, say the hamster, no matter what tumor virus brings about the cancerous transformation, each resulting hamster tumor cell releases the same "cell factor." This suggests that the cell factor is not coded by the transforming viral genome but represents a normal cellular enzyme, perhaps one that usually functions during embryonic life. Conceivably the "cancerous genes" of the tumor virus upset the normal controls over gene function in ways that turn on the functioning of large groups of embryonic genes. If this hypothesis holds water, then we find that the various classes of tumor viruses disrupt normal gene function in quite different ways. Yet the final result, the appearance of Reich's "cell factor," may always be the same. Working out the very many consequences of this discovery will create much excitement, and there are a number of areas where work here at Cold Spring Harbor can complement that of the Rockefeller team. Already Bob Pollack's group is interacting most effectively with them in studying the initial events that occur as tumor-virus-treated normal cells become cancerous.

Thus we no longer have any serious doubts whether the state of biological research has reached the point where a serious assault on the fundamental nature of cancer can be warranted. There is no point to wait 10-20 years to accumulate more basic knowledge before attacking cancer as such. On the other hand, we should have no delusion that we already know enough of the basic facts about how normal cells operate. We are woefully short of working maps for many vital biochemical pathways, and until they are put together we shall stumble more than we want. So hand in hand with direct assaults on the cancer cell must go parallel efforts on normal human cells. Failure to appreciate the magnitude of the remaining gap can only seriously set back the entire effort. So we must be very wary of prematurely placing too much emphasis on highly targeted research aimed at specific cancers like those of the lung and breast, when no comparable large-scale efforts are being directed toward the behavior of the corresponding normal cells. The fact that high quality cancer research can now be done should not let us forget that until recently most cancer research was getting nowhere, and unless we are vigilant, we will witness an absolute increase in the resources that generate uninterpretable data.

Now greatly hindering a wise distribution of the vast piles of new cancer money is the limited number of first-rate institutions that can rapidly respond with the creation of new staff positions and laboratories. Until recently very little cancer research was carried out in essentially academic institutions, with most such research being done in medically related environments, where the desire for the immediate payoff may be in the next room. Unfortunately, few of these hospital based laboratories are noted for their intellectual vigor and despite periodic surges after reorganization they have never developed into sites where our best young students learn to be scientists. But since they are so easily identifiable with the aims of targeted research and are very knowledgeable in knowing how to ask for cancer money, they have all markedly expanded since the "Conquest of Cancer" legislation was passed. In contrast, academic science has been getting relatively poorer and so not inclined to take on the cancer problem. Thus the number of younger scientists being well trained for cancer research may in fact be falling off rather than increasing. To be sure the total number of scientists engaged in cancer research is going up, but I fear the quality is going down.

Much of this blame must fall on witless directives from political hacks still packing power in the Office of Management and Budget and within the HEW's secretary's office. Their efforts to block the input of new scientific blood into the National Cancer Program have brought forth the image of the political commissar for the first time in American history. Equal discredit, however, lies in the leadership of the National Cancer Institute whose compulsion to please Congress with marginally effective new clinical facilities and cancer control programs always seems to take precedence over solid efforts that would effectively bring cancer research and the best brains of our country together. Their lack of foresight might be temporarily excusable if such developments would in any real way impede bringing more effective clinical treatment to the cancer patient. Compared, however, to clinical programs, pure research still costs very little, and a great opportunity is being lost by the failure of the NCI to seriously seek the collaboration of the best of our academic community.

The question, of course, should be asked—aren't the university scientists also to blame. Being good at science need not lead to ostrich-like behavior and prevent intelligent men from realizing that effective cancer research may cost piles of money and so individual university scientists must band together for joint efforts that will give them opportunities equivalent to the scientists who work in one of the major clinically sited cancer centers. Unless this happens, the hacks who still dominate so much of cancer research will never have the challenge of real competition for the big money. To date, however, there have been only hints of motion toward high powered cancer research from most major universities. Only MIT has taken a real plunge by its creation of a new center for cancer research that will give them facilities for work with cancer cells equal to those anywhere in the world. Unless other such bodies come forward, our country will be working far below its inherent potential for human betterment.

I, of course, realize that there is something unpleasant in the concept that the individual scientist working by himself may not be our best hope. The thought that cooperation between large groups may not only be helpful but also necessary if we are to understand cancer appears to require still another retreat from the Thoreau-like freedom we like to dream about. But we should remember that until they were almost to the summit, Hillary and Tensing were part of a large, well-organized group effort. And the mountain we aim to conquer is probably much higher.

Highlights of the Year

Creation of the Robertson Research Fund

Early this past June there came into existence a new body, the Robertson Research Fund, specifically set up to provide for research and teaching at this Laboratory. Distribution of monies from this fund will be managed by five trustees representing the Laboratory—myself, Walter Page, Robert Olney, Edward Pulling, William Udry—and four members of the Robertson family of Lloyd Harbor—Mr. Charles S. Robertson, the Fund's president, Mr. William S. Robertson, Dr. Walter Meier, and Mr. Eugene W. Goodwillie. Only income generated by investments will be available for use, the capital to remain inviolate to serve as a permanent endowment fund. A yearly income of some $250,000 is projected, a sum which should go far not only in allowing us to initiate innovative new research but in providing travel expenses, stipends, and tuition support for many of the young scientists who come here each year. For example, availability of these funds enabled us to continue this past summer our highly successful program of research by college undergraduates. Another most welcome use of the funds was support for the two month-long Phycomyces workshops organized by Max Delbruck in Davenport Laboratory. Robertson funds furthermore were a key item in equipping the west annex to James Laboratory, as well as in supporting the research in Demerec on SV40 proteins by Klaus and Mary Weber.

In a very real way, the creation of this Fund ranks as one of the most important events in our Lab's history since it gives us for the first time a partial independence from unpredictable fluctuations in Federal monies for science, a trend which has become increasingly pronounced over the past five years. For example, last spring when the National Science Foundation suddenly slashed its program to provide for summer research by college students, we never considered stopping our summer programs, knowing we might have Robertson Funds to back us up. Of course we realize that these funds are not sufficient to bail us out if all Federal money were to vanish, but this is very unlikely to happen as long as we remain one of the better institutions doing serious biology. With these new funds we thus have a potential, almost unmatched by any comparable research institution, for the support of the innovative and the young. Their future wise utilization represents a unique challenge which we must meet.

Cancer Center Grant Finishes Its Second Year

Our good fortune in possessing a large "center grant" from the NCI was made most apparent through the fast, if not at times frenetic, pace at which our staff has been able to carry out difficult experiments. While there has been much general criticism of this form of grant, which goes to an institution instead of to an individual, I believe we have demonstrated (as shown by the accompanying research summaries) that when you have a young and first-rate staff a center grant is a very powerful instrument for the rapid promotion of science. Most of our staff have been able to work more effectively than if they had to operate within the fiscal boundaries that now box in most scientists in receipt of their first grants. Most importantly it

has led to many very effective collaborations which have kept most of us keenly aware of what our bench mates are up to. Of course, like every institution, we have our loners, with some of them doing well—in most cases, however, I believe they produce well below their real potential when they try to do everything by themselves.

Greatly helping tumor virus research at James was the completion of the new west addition which gave us for the first time labs especially designed for the growth and isolation of animal viruses. Particularly important is that its existence brought to an end the demoralizing slow downs that had to occur during June and July when the cell culture and animal virus courses are taught on the second floor of James.

A Very Full Summer Program

Again this summer we offered nine advanced summer courses; three in the area of cancer cell biology, three in microbial genetics, and the remaining three related to neurobiology. As before, there were large numbers of applicants for the limited number of places in the cell culture and animal virus courses, and most interestingly the demand for places in the yeast genetics courses is steadily rising. In contrast, our bacterial genetics course, that aimed at an audience without prior knowledge, had diminished applications, most likely because it is now well taught in many university environments. So this year we will present a more advanced course in bacterial genetics directed toward persons already at ease with the beginning facts.

We again feel that our neurobiology courses went well and were especially pleased that our new effort, a course in the neurobiology of Drosophila led by Bill Pak of Purdue University, generated much enthusiasm among its students. So it will be repeated again this summer. Lack of space in the Animal House limits our neurobiology scope, but even so, we hope this summer to squeeze in an advanced workshop on the neurobiology of the leech to be organized by John Nicholls.

All our meetings continued to attract many of the best minds in their respective fields, and we were particularly encouraged by the increasing sophistication of the science presented during the SV40-polyoma-adenovirus and herpes meetings. Ending the summer was a very high powered gathering on Ribosomes organized by P. Lengyel, M. Nomura and A. Tissieres. Happily this meeting will lead to the next volume of our monograph series, hopefully coming out no later than next September.

Return of the Cairns Family to England

After dwelling in Cold Spring Harbor for almost ten years, John and Elfie Cairns returned in March to England where he will be the Director of the Mill Hill Laboratory at the Imperial Cancer Research Fund. First as our Director between 1963 and 1968, when he took over a Lab near death and brought it back to life, and later as an American Cancer Society Professor of Molecular Biology, John gave long and intelligent service to this institute as well as to the outside world of science. At the same time he remained with the top of the DNA world, giving with his famous PolA Mutant a shock to conventional thought that led quickly to a complete rethinking of the ways enzymes are used to replicate DNA.

Their departure leaves a great gap in our community, not only because of the loss of John's great scientific talents but also because their home in Airslie always radiated gracious and good natured concern for our staff and many visitors. I especially remember their hospitality before I married and often needed the encouragement provided by home cooked meals. Also to be greatly missed are their two sons William and Hugh, now both medical students in London and their daughter Vicki currently at the London School of Economics. Their ability to join in whenever needed was a major factor in the success of our various summer programs. They will be long missed for their straightforward assistance to everyone. We wish them all much future success in their native land.

Changes in Our Scientific Family

This year the size of our staff remained effectively the same as last, with James and Demerec Laboratories again tightly occupied. Valuable losses among our postdoctoral contingents have been Ernesto Bade who moved to a position in Gottingen, Germany, Peter Jeppesen who returned to the MRC lab in Cambridge, Peggy Anderson who moved on to the Mill Hill Lab of ICRF, and Jan Oey who moved to Konstanz, Germany. Ulf Pettersson, unfortunately, was tempted from our scientific staff back to Uppsala but not before he had converted much of James Lab to work on adenoviruses. Also a great loss was the departure of

Bill Sugden after four years in James Lab to George Klein's laboratory in Stockholm. One of the first occupants of the newly renovated facilities in James, he contributed immeasureably toward its establishment as a real force in tumor virus research. Happily with the cooperation of Columbia University his excellent work on RNA polymerase from human cells formed the basis of a Ph.D. thesis. Bill's work represents the first research done at the Lab that has led to the Ph.D., an event we hope will happen many more times in the future.

Additions to our scientific staff this past year have been Bernard Allet, who first came here to postdoc with Ray Gesteland, Bal Apte, originally a postdoc with Dave Zipser, and Terri Grodzicker, who after doing several postdoctoral years in bacterial genetics, came here last year to learn tumor virus research in James.

The LIBA Fund Drive Reaches Its $250,000 Goal

A source of great satisfaction occurred early in the spring when the Long Island Biological Association (LIBA) Fund drive reached its goal of $250,000, thereby enabling us to carry through its three objectives, the construction of the west addition to James Lab, the winterization of Blackford Hall, and the purchase of the Takami residence. We are indeed most grateful to Mr. Robert Olney and his committee, Mrs. Ward C. Campbell, Dr. Bayard Clarkson, Mr. Edward W. Kozlik, Mr. Angus P. McIntyre, Mr. Edward Pulling, and Mrs. Alex White for their splendid job in overseeing this much needed effort. Again we have a most pleasing demonstration of how fortunate we are to have such far-sighted and generous neighbors.

During early June a number of LIBA members again gave dinner parties for the speakers of our annual Symposium. Some 80 guests had the pleasure of most relaxing interludes away from the fast pace of Symposium papers. We wish to thank Mr. and Mrs. Arthur Crocker, Mr. and Mrs. Norris Darrell, Mr. and Mrs. Angus McIntyre, Mr. and Mrs. Robert Olney, Mr. and Mrs. Walter Page, Mr. and Mrs. Edward Pulling, Mr. and Mrs. Franz Schneider, Mr. and Mrs. Richard Storrs, Mrs. Alex White, and Mr. and Mrs. William Woodcock for helping continue this most pleasant tradition which dates back to the early years of the Symposium.

Mr. and Mrs. Robert Olney also came to our help with a lovely garden party for the participants of the Cell Proliferation Meeting. Many of their neighbors joined the Olneys in welcoming our guests and all present felt it was a most spirited success.

Blackford Hall Becomes Year Round

Blackford Hall, first built in 1906 to function as a summer dining hall and women's dormitory, finally became transformed this past year into a heated year-round building. Among the first reinforced concrete buildings in the United States, Blackford long symbolized Cold Spring Harbor to our summer visitors, for all the early Symposia were held there until the construction of the Vannevar Bush Lecture Hall in 1952. We had worried that modernization might destroy its links with our past, so we are greatly indebted to the architect Harold Buttrick who drew up the plans for the renovation as well as to Jack Richards and his staff for a remarkably effective job that has earned only praise from our many most surprised returning friends.

Initiation of an Annual Conference Related to Cell Proliferation

Our first occasion to use the new Blackford was a happy one — inauguration of a new series of conferences on cell proliferation. Our first such meeting, "The Control of Proliferation in Animal Cells," ably organized by Bayard Clarkson and Renato Baserga was held May 20-27. It brought some 100 cell biologists, biochemists, radiation biologists, immunologists and oncologists to what we believe was a singly important occasion. For the first time hints could be seen of an intellectual basis for judging the respective relevance of the various approaches to the question of how a cell decides to divide or not to divide. Interestingly many of the people present not only had never met before, but never previously had found an occasion to appreciate the obvious relations which were emerging between their various approaches. The papers presented at this meeting, like those of all subsequent cell proliferation conferences, will be published by the Laboratory. The first of these volumes, a massive book of some 1000 pages, will be out in early February.

Dedication of McClintock Laboratory

Our annual Symposium, which this year focused on chromosomes, provided the perfect

occasion for a brief ceremony in which the former "Animal House" was renamed McClintock Laboratory. Constructed in 1912 to further research in animal genetics and originally filled with pigeons and mice, the name "Animal House" was initially fitting. But now that it no longer has any animal quarters, its name has been increasingly confusing to visitors, especially following its transformation into a laboratory for neurobiology. In choosing a more suitable name we had no difficulty, as many of Barbara McClintock's most elegant cytological experiments were done there before she moved in 1952 into the then newly constructed Demerec Laboratory. Highlighting the occasion were Barbara's remarks which displayed her direct yet modest approach to science.

Our Tumor Virus Role Finally Appears

After a gestation period of almost four years, our much rewritten and updated book on "The Molecular Biology of Tumor Viruses" came off the presses early in August. It represents a collective effort in which a large number of leaders in tumor virology attempted to integrate all we know about tumor viruses into a text that would have wide readership throughout the world of experimental biology. Unlike most collections of reviews which are usually written for fellow experts, our purpose was a product that would also appeal to medical and graduate students as well as to practicing scientists in other disciplines. In charge of pulling together the final product was John Tooze, then of the Imperial Cancer Research Fund Laboratories, who spent several summers here during the tumor virus workshops. The final product we believe to be a great sucess, at least as judged by the quick pace of early sales. Most pleasing are indications that it is being widely adopted as a text.

Another recent laboratory publication is a small paperback book on "Biohazards in Biological Research" containing the proceedings of a meeting held last January at Asilomar in California to probe the question of the potential hazards to humans of the various viruses now used as research tools for understanding fundamental biological processes. Edited by Alfred Hellman of the National Cancer Institute, Michael N. Oxman of the Children's Hospital, Boston, and Robert Pollack of our Laboratory, this book represents a first look at a problem not very easy to define.

Apparently also a winner is a collection of reprints on animal cell culture, "Readings in Mammalian Cell Culture," assembled and commented upon by Bob Pollack. Despite the growing importance of this field, no one previously had brought together the leading articles on this subject, and so Bob's book is the first of its kind. Again we have been pleasantly surprised by the volume of our sales, and so despite our pricing it much lower than would any commercial publisher, we may come out in the black. Steady sales also are occurring in Jeffrey Miller's super lab manual "Experiments in Molecular Genetics". Not only is the book being widely read as a text in itself, we have already sold over 145 sets of the strain kit needed for laboratory adoption. It looks as though Jeffrey's efforts will remain very useful for many years to come.

Total Renovation of Davenport Lab

Davenport Lab, the site of our original phage and bacterial genetics courses, has for all too many years looked increasingly unequal to its role as a site for the teaching of high level molecular biology. Its floors were not strong enough to support high speed centrifuges and even walking upon them created vibrations that made light microscopic observations an unsettling affair. So last winter we began a complete rebuilding job, by which Jack Richards and his staff transformed a very shaky summer lab, lacking both heat and a dry basement, into a modern year-round building. Now it has a large chemical lab and photographic quarters on the ground floor, a kitchen, preparation rooms, and an EM facility on the first floor, and a sturdy teaching lab on the second floor. It is our intention to keep this building oriented toward genetics, with courses in the summer, and hopefully used by scientists on sabbatical during the remaining nine months. During this past summer the newly created basement space was most effectively used by Max Delbruck's phycomyces workshop.

Purchase of the Takami Residence

For over 50 years a stately late Victorian home and barn located on Bungtown Road on the way to the sandspit was the residence of the Takami family. With time it became almost completely surrounded by Laboratory controlled property and an obvious addition to our much needed housing, if it were ever to come on the market. This happened last spring when Alice Takami moved to Honolulu following the graduation of her children from local schools.

Fortunately, thanks to the success of the LIBA Fund drive, we were able to purchase this property in time for our summer session. Currently the ground floor forms an apartment for a staff member family, and the upper two floors provide rooms for unmarried staff and summer visitors.

Also now very effectively used by the Laboratory as a residence is a lovely little yellow frame house, also on Bungtown Road a little beyond the Takami house. The dwelling, which dates from the early whaling days of the 19th century, has been owned for many years by Mrs. James D. deTomasi, a daughter of Charles Davenport, our Laboratory Director during the first thirty years of this century. Negotiations have been concluded for its eventual sale to us which will complete our ownership of all the property siding on Bungtown Road.

Acquisition of Whaler's Cove Marina

Some ten years ago the eastern shore of the inner harbor of Cold Spring Harbor began to lose its essentially non-commercial character with the establishment of a small private marina under the name Whaler's Cove Yacht Club. Creation of this facility led to fears that it would expand in size sometime in the not too distant future. Were this to happen, we would lose the tranquility so very essential to our existence and become part of the commercial-urban spread which now dominates so much of the north shore of Long Island. For the past several years we thus have been most active to contain the Marina to its current size, always fearing that someday we might lose the battle. So when an occasion arose for the Laboratory to buy the Marina from the current owner, we seized it as an opportunity that were it to come in later years might have become prohibitively expensive. Now for the first time we possess inner harbor waterfront where boats can dock independent of the tides that leave us on the western shore dry much of the time. With time we hope to establish facilities on the Marina site for the study of marine biology. Given, however, the very expensive nature of marine science, some years may be required before the program can be effectively started.

Continued Uncertainties about Federal Funding for Science

Never within my memory has the Federal policy toward science seemed so ambiguous as during the past year. It opened with rumors that the next presidential budget would drastically slash if not eliminate funds for the training of new scientists. This news became official by late January to the horror of the bio-medical community who saw the floor drop out of many of their most effective programs. So I, like many others, traveled to Washington, D.C. to testify before Congress against the administration's mistaken policies. In talks before Senator Kennedy's Subcommittee on Health and Representative Roger's Public Health Committee, I found strong bipartisan congressional support for our objectives of continuing the flow of bright young people into experimental biology. But pessimism reigned everywhere since Congress was being told if it didn't like the new budget it should start impeachment proceedings or shut up. So it was hard not to feel very ineffectual, a mood I also felt as a member of the National Cancer Advisory Board where the official White House word was more money for targeted research even if it meant that many talented young scientists were no longer free to choose where the real excitement lies.

Immediately the only direct cutback that hit us was the loss of $50,000 that had been approved from the Institute for Mental Health for a training grant to support our neurobiology courses. This was money we hoped to use as a match for our Sloan gift, but in its absence we were forced to expend almost all of our remaining Sloan funds. We were also told by NIH that this was to be the last year for our training grant that had supported for some many years our summer courses in genetics and cell culture. Though we began steps that might generate other funds to cover part of their costs, we suspected that we would have to dig deeply into Laboratory funds to keep our training programs going.

Now, however, the outlook is much brighter. As a result of the Watergate affair, the White House may have given up its attempt to dismantle Congressional authority over the direction of our health programs. We are optimistic that not only will the training grants soon be reinstated but that much more money will go toward pure science. So if this Laboratory continues to submit good grant applications, there will be money to fund them. Hopefully I will need pay much less attention to the funding of science in general and concentrate more on seeing that the Laboratory chooses wisely in deciding what science should be done here.

Bentley Glass' Term as Chairman of the Board Comes to an End

Our by-laws limit continuous membership on our Board of Trustees to six years and so most reluctantly we had to accept the fact that Bentley Glass' term as Chairman of our Board of Trustees would end at our annual October meeting. Bentley took on the Chairmanship just before the possibility arose that I might be the Director. The fact that I knew I would be able to call for help from such a uniquely versatile scientist was a great plus in my decision to come here. My expectations of warm and wise counsel have been more than confirmed, and now it is even more obvious to all here why Bentley has been asked to preside over the destinies of so many distinguished organizations. Fortunately his close association with this Lab, which dates back almost forty years, need not cease. He joins Alexander Hollaender as an Honorary Trustee, and given that he lives nearby in Stony Brook, we expect to benefit from his presence for many more years to come.

Fortunately his successor as Chairman, Bob Olney, has long experience with the Lab first as a member of LIBA and then on our Board as our Treasurer and Chairman of the Finance Committee. So I look forward to an equally profitable partnership for the good of the Lab.

Our Administration Becomes Even More Professional

A most satisfying aspect of this past year has been the increased efficiency in the operation of our daily affairs. As long as we were a very small operation the need for real competence could always be avoided under the assumption that we could not afford it. But with our current staff size of approximately 100 and an annual budget of over $3,000,000 we have the opportunity to do things well. Thus our good fortune in having Bill Udry as Administrative Director becomes more obvious each year. Equally important is Jack Richards' ability as a builder for without it we would never have had the guts to save our old buildings no matter their various states of seemingly irreparable decay. Special mention must also be made this year of the arrival of Helen Parker to manage our summer programs. Before her presence here our hotel business had a chaotic aspect left best to old movies, and so it is most satisfying to have our summer operations now running as smoothly as our winter programs.

We Still Have a Long Way to Go

The creation of the Robertson Research Fund, the massive outpouring of excellent research, and the saving of the inner harbor from commercialism are events that only a few years ago would have seemed like unattainable dreams. So in many ways as we look back, this has been a most rewarding year for the Laboratory. Yet no matter how satisfying it is to reflect back on happy days, this Laboratory is not a place for retrospective thoughts—the future is what counts, and we must keep our wits to do well with it. Of course in looking ahead we hope we can continue to count on future support from our local neighbors, our scientific alumni, the charitable foundations, our industrial sponsors, and our participating institutions. Without their assistance we would not now be at the center of biology and capable of so much future good. Hopefully they will continue to view us as worthy of their trust.

If so, we will not soon be out of ideas.

December 30, 1973

J.D. Watson

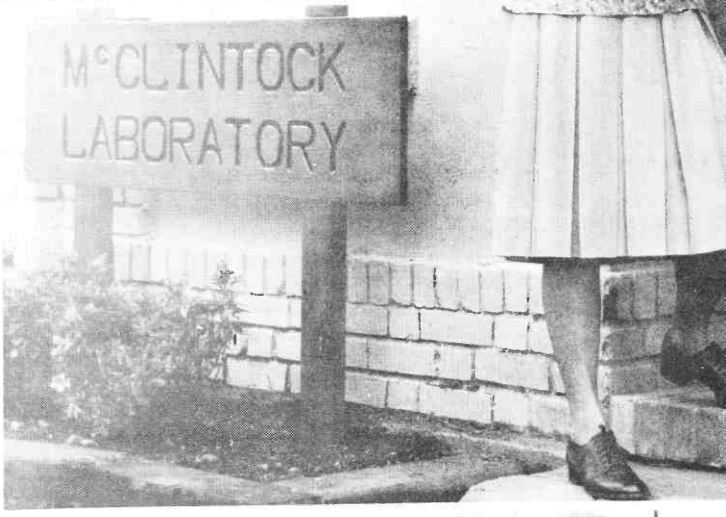


Photo Credits:
B. Glass, top left, by Robert F. Cohen
B. McClintock, top right, by Ross Meurer
J. Cairns, bottom, by Leonard Ramsler, Medical World News

YEAR-ROUND RESEARCH

MOLECULAR BIOLOGY OF TUMOR VIRUS

J. SAMBROOK
E. Allet
J. Arrand
D. Bachman
M. Botchan
R. Cohen
D. Day
S. Flint
R. Greene
T. Grodzicker
A. Jackson
W. Keller
F. Kelly
J. Kort
R. Lancaster
M. Lurye
G. McKenna
B. Miller
C. Mulder
B. Ozanne
U. Pettersson
P. Sharp
B. Shineberg
W. Sugden
N. Sullivan
I. Wendel

During the last year, work has continued in James Laboratory on five major topics — the transcription of adenovirus 2 and SV40 both *in vivo* and *in vitro*, the integration of viral nucleic acid sequences into the DNA of transformed cells, the isolation of variants from populations of transformed cells that carry specific genetic markers, the isolation of host-dependent mutants of viruses, and the purification and mechanism of action of RNase H and DNA-dependent RNA polymerase from mammalian cells.

Central to the progress we have made in attacking these problems has been the availability of a suite of restriction endonucleases, enzymes which introduce double-strand cuts into DNA at particular nucleotide sequences. During the first part of the year we spent a considerable effort developing an assay for these enzymes that was both rapid and specific. We adapted the technique of electrophoresis of DNA through agarose and/or polyacrylamide gels, and found that by incorporating a low concentration (0.5 μg/ml) of ethidium bromide into the gel and the running buffer we were able to stain DNA directly and could therefore eliminate the usual procedures involving staining and destaining, autoradiography or slicing of gels. The staining of DNA is sensitive enough to detect as little as 0.02 μg of DNA per band, and the assay is used routinely to monitor activity during purification of restriction enzymes as well as to analyze the patterns of restriction produced by the different enzymes on DNA templates. Using this technique, we have shown that the restriction system of *Hemophilus parainfluenzae* consists of at least two enzymatic activities, R·Hpa II, which cleaves SV40 DNA at a unique site, and R·Hpa I, which attacks at three different sites. The positions of all these cleavage sites on the SV40 genome have been mapped. By using Hpa I in conjunction with *E. coli* endonuclease R·RI (EcoRI), an enzyme which had been shown previously in our laboratory and elsewhere to cleave SV40 DNA once, we can divide the SV40 genome into four specific fragments which have proved valuable in studies of transcription of the viral genome.

We have also isolated a new restriction enzyme from a species of *Serratia* and we have made a major effort to map the sites of cleavage of this enzyme and EcoRI on various adenovirus DNAs. The *Serratia* enzyme (endonuclease R·Sa) appears to produce very few cleavages in most sorts of DNAs. Thus, the small DNAs of polyoma virus and SV40 are not cut at all, and neither T7 and mμ1 DNA nor the replicative forms of M13, f_1 or ØX174 DNAs are cleaved by the enzyme. Furthermore, high molecular weight mouse DNA is cut into large fragments (average molecular weight 18-20 x 10^6) and T5 and λDNAs are also cleaved infrequently. Consequently, it was surprising to find that the DNAs of most adenovirus serotypes generally appear to contain many recognition sites for R·Sa. We have examined the DNAs of seven different adenoviruses belonging to the three groups of these viruses: "highly" (Ad12); "weakly" (Ad3, Ad7), and "non-oncogenic" (Ad2, Ad5), for restriction by both R·Sa and R·EcoRI.

TABLE I

	Endonuclease EcoRI		*Endonuclease R·Sa*	
DNA	*no. of fragments*	*MW range*	*no. of fragments*	*MW range*
Ad2	6	13.5–1.1	11	4.5–0.15
Ad2$^+$ND$_1$	5	13.5–1.1	11	4.5–0.15
Ad5	3	19.0–1.8	12	4.2–0.15
Ad3	3	19.4–0.3	7	6.4–0.5
AD7 (E46$^-$)	3	19.4–0.3	7	6.4–0.5
Ad7 (cl 19)	2	20.0–3.0	7	6.4–0.5
Ad12	6	7.5–0.4	4	~ 8.0–1.4

Each of these DNAs was cut into a number of unique fragments. Table I gives a summary of the results obtained after analysis of the deproteinized DNA by electron microscopy and gel electrophoresis. Endonuclease EcoRI cuts each of these DNAs into a few small fragments and one large fragment that comprises 60-85% of the viral genome. It is this large fragment that contains most of the recognition sites for endonuclease R·Sa. Thus, a combination of these two enzymes cuts adenovirus DNAs into a limited number of fragments of a size useful for studies of transcription and integration.

The order of the fragments produced by endonuclease EcoRI has been established by one or more of the following methods for all the adenovirus genomes studied:

a) Partial denaturation mapping (with Hajo Delius);
b) heteroduplex mapping;
c) exhaustive digestion of partial digestion products.

Similar studies are in progress with adenovirus DNA cleaved by endonuclease R·Sa.

Work has also continued on the nature and localization of deletions and insertions that occur in SV40 DNA after passage of the virus at high multiplicities. We have found previously that such DNA has often become resistant to endonuclease EcoRI, and it now seems that the same DNA stocks contain many molecules that are resistant to endonuclease R·Hpa II. In addition, several such DNA preparations contain a species of DNA whose molecular weight is about 90-95% that of DNA extracted from plaque-purified virus. The nature of all these molecules is being investigated by heteroduplex mapping and restriction by the endonucleases isolated from *H. influenzae* (R·Hin).

Transcription of SV40

1) *During lytic infection:* Previous work, using the separated strands of SV40 DNA (Khoury *et al.*, Proc. Nat. Acad. Sci. USA *69*:1925 [1972]; Sambrook *et al.*, J. Mol. Biol. *70*:57 [1972]), has shown that the early and late species of SV40 RNA are transcribed from different strands of the viral DNA. In the past year, we have mapped the positions of these RNAs on the viral genome and we have determined the direction of transcription around the SV40 DNA molecule.

To map the location of the early and late regions of the viral genome, we used four specific fragments of SV40 DNA generated by sequential cleavage of closed circular viral DNA by the endonucleases EcoRI and R·Hpa I. The positions of these fragments are shown in Fig. 1. The ^{32}P—labeled fragments were separated by electrophoresis through polyacrylamide-agarose gels, and the two DNA strands of each fragment were isolated using asymmetric complementary RNA as described elsewhere (Sambrook *et al.*, J. Mol. Biol. *70*:57 [1972]).

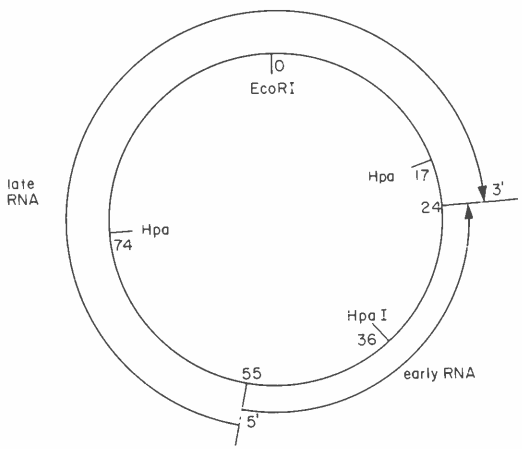


Figure 1

The separated strands of each of the fragments were sheared and hybridized to RNA extracted from polysomes or from whole cells at different times after infection. The results are shown in Table 2.

TABLE 2

		% of ^{32}P-labeled DNA entering hybrid at saturating levels of		
Fragment	*Strand*	*early RNA*	*late polysomal RNA*	*total cell RNA*
A	E	48	52	50
A	L	0	45	68
B	E	0	0	24
B	L	0	90	92
C	E	60	60	60
C	L	0	26	75
D	E	0	0	10
D	L	0	92	92
SV40	E	31	34	44
SV40	L	0	70	77

Early after infection, stable RNA complementary to part of the E-strand sequences of the contiguous fragments A and C was detected. True late polysomal RNA was complementary to part of the L-strand sequences of fragments A and C and to the total L-strand sequences of fragments B and D. For all fragments the sum of the *stable* transcripts of the E and L strands amounted to the equivalent of one strand of the viral DNA. By contrast, however, *total* RNA extracted from cells late in lytic infection hybridized to the equivalent of more than one strand of each of the four fragments. We believe that these symmetric transcripts correspond to those described by Aloni (Proc. Nat. Acad. Sci. USA *69*:2404 [1972]). Their function is unknown; they may represent the primary transcription product of SV40 DNA and they may be the precursors of the stable, asymmetric polysomal RNA species. Alternatively, they may be the product of some form of aberrant transcription, perhaps from integrated genomes, and they may play no part in productive infection.

To determine the orientation of transcription on the E and L strands of SV40 DNA, linear DNA molecules were prepared by cleavage of superhelical viral DNA by endonuclease EcoRI. This enzyme has been shown (Morrow and Berg, Proc. Nat. Acad. Sci. USA *69*:3365 [1972]; Mulder and Delius, Proc. Nat. Acad. Sci. USA *69*:3215 [1972]) to cleave SV40 DNA at a single site, by making single-strand scissions that are four bases apart (Hedgpeth *et al.*, Proc. Nat. Acad. Sci, USA *69*:3448 [1972]). Because the linear molecules contain a 3'-hydroxyl group and a protruding 5'-phosphoryl tail, they serve as primer·templates for DNA synthesis, and we were able to use RNA-dependent DNA polymerase isolated from avian myeloblastosis virus to incorporate ^{3}H-dTMP at each of the 3' ends of the linear DNA. The resulting molecules were then cleaved with endonuclease R·Hpa I, and the four DNA fragments were separated by electrophoresis, denatured and hybridized to asymmetric SV40 cRNA. From the pattern of hybridization of the fragments containing the labeled 3' ends, we conclude that transcription of SV40 proceeds in a clockwise direction on the L strand and in a counterclockwise direction on the E strand as drawn on the conventional map (see Fig. 1). If we assume that early RNA is transcribed from a contiguous section of the virus genome, then we can calculate from the saturation hybridization values shown in Table 2 that the 5' end of the stable species of early SV40 RNA maps at position 55, and the 3' end at position 24 on the SV40 map.

2) *In transformed cells:* We have used the separated strands of SV40 DNA to measure the percentage of the sequences of the virus genome that is present in RNA extracted from different lines of transformed mouse cells. We found that different lines of transformed cells display different hybridization patterns in that the proportion of the E-strand DNA sequences that is present in RNA varies from cell line to cell line, ranging from a low of 30% to a high of 75%. Because only 30-35% of E-strand DNA is represented in stable species of RNA during lytic infection, at least part of the viral RNA present in many transformants must be "anti-late."

The viral RNA sequences present in the highest concentrations are transcribed from a part of the SV40 genome contained in fragments A and C and are probably identical to the stable early sequences of viral RNA. Anti-late sequences, which are present in lower concentrations, hybridize only to fragments A and B and are therefore complementary to the block of E-strand sequences which stretches in a clockwise direction from map position 55. To explain how different amounts of the SV40 genome are transcribed in different transformed cell lines, we propose that the site on the viral genome at which integration occurs varies from one transformant to the next, and that transcription of the viral sequences starts in the anti-late region and finishes at the end of the early genes (position 24). Because the only set of RNA sequences present in every transformant is transcribed from the early region, we believe that the viral function responsible for maintenance of transformation must be an early gene product — a result which had already been predicted by others on the basis of genetic experiments.

Finally, we have reported previously (Sambrook *et al.*, J. Mol. Biol. *70*:57 [1972]) that occasional lines of SV40-transformed mouse cells contain very low concentrations of RNA which hybridize to L-strand DNA sequences. This RNA, which is found only in cell lines which contain large amounts (8-9 copies) of SV40 DNA per diploid amount of cell DNA, appears to be transcribed from all regions of the viral genome.

Transcription of adenovirus type 2

1) *During lytic infection:* In order to map the stable species of virus-specific RNA that are transcribed from adenovirus 2 DNA late during lytic infection, we have used the six fragments of the viral genome that are generated by cleavage of the intact DNA by endonuclease EcoRI (see Table 1). The order of these fragments along the genome has been determined by heteroduplex and denaturation mapping to be ABFDEC (Fig. 2).

^{32}P-labeled fragments were prepared and sheared to a length of approximately 300 nucleotides by boiling in alkali. After neutralization the fragments were incubated with a large excess of polysomal RNA extracted from KB cells 18 hr. after infection, and the percentage of DNA entering hybrid was measured by chromatography on hydroxylapatite. We found that at saturating levels of RNA, 50% of the radioactivity of complete adenovirus 2 DNA behaved as hybrid, and the same result was obtained with the DNA of each fragment. This result means that at late times during lytic infection, the equivalent of one strand of the viral DNA is transcribed into stable RNA.

Green *et al.* (Cold Spring Harbor Symp. Quant. Biol., vol. 35, pg. 803 [1970]) have shown that late adenovirus RNA is complementary to parts of the sequences of both strands of the viral DNA. In order to locate the positions of the strand switches, we repeated the experiment described above using intact DNA of each of the fragments. At saturating levels of RNA, we again found that 50% of the radioactivity of fragments D, E and F entered hybrid. However, greater than 70% of the radioactivity of fragments A, B and C behaved as hybrid. These results mean that there are at least three strand switches operative during synthesis of late adenovirus-specific, stable RNA — one located in fragment A, and one each in fragments B and C. There are no strand switches in D, E or F. The simplest model to account for these results is shown in Fig. 2.

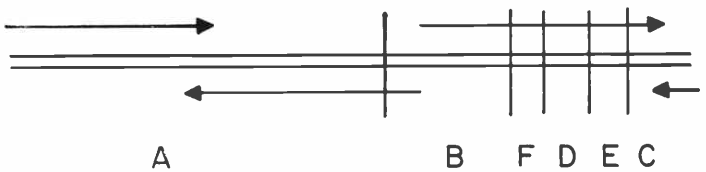


Figure 2

We have performed a large number of experiments to determine the orientation of transcription in different segments of the adenovirus genome. We have shown that the SV40 sequences contained in the adenovirus SV40 hybrid $Ad2^{+}ND_1$, are transcribed in a rightwards direction, and we know that these sequences are inserted into the adenovirus 2 genome at the border between fragments D and E. In addition, Patch *et al.* (Proc. Nat. Acad. Sci. USA *69*:3375-3379 [1972]) have shown that these SV40 sequences are transcribed from the L strand of $Ad2^{+}ND_1$ DNA — the same strand which is template for the adenovirus RNA synthesized from fragments F, D and E. Thus, we can be certain that the RNA transcribed from the segment of adenovirus DNA beginning somewhere in B and ending somewhere in C

is synthesized in a rightwards direction. Furthermore, hybridization experiments using adenovirus DNA which has been labeled specifically at the ends by DNA polymerase after treatment with exonuclease III indicate that RNA is synthesized from the end of fragment A in a rightwards direction and from the end of fragment C in a leftwards direction.

Clearly, late transcription of adenovirus 2 DNA is a complicated affair. However, given the availability of defined fragments of the genome, we hope to provide a fairly complete map of the stable RNA species.

2) *In vitro*: We have examined the transcription of adenovirus 2 DNA by *E. coli* RNA polymerase. In brief, the results we obtained are as follows:

cRNA is mainly asymmetric and has an average molecular weight of 1.5×10^6. It is complementary to 70% of the sequences of the L-strand and 30% of the sequences of the H-strand of the viral DNA, so that in total the equivalent of one complete strand of the viral genome is transcribed. The positions of the strand switches have been localized by hybridizing the RNA to the specific fragments of adenovirus 2 DNA obtained by cleavage of the genome by endonuclease EcoRI (Pettersson *et al.*, 1973). Competition hybridization experiments show that most of the viral sequences which are represented in stable RNA late during lytic adenovirus infection are transcribed by *E. coli* RNA polymerase *in vitro*. Initiation of RNA synthesis takes place at 5-7 locations on the adenovirus genome as determined by electron microscopy of transcription complexes after short periods of synthesis.

The use of restriction enzymes to investigate the structure of mammalian DNA

We have used restriction endonucleases to examine the organization of DNA within the mammalian genome and to investigate the integration of viral genomes in transformed cells.

1) *The organization of mammalian DNA*: After restriction by EcoRI, mouse DNA can be resolved into two components by electrophoresis through 1.4% agarose gels. The more slowly migrating component, which comprises about 10% of the total DNA, consists primarily of mouse satellite sequences. The size of the fragments of satellite DNA after cleavage by EcoRI ranges from 4×10^6 to 20×10^6 daltons, with the most common size being 8×10^6 daltons. These findings are consistent with the idea that the satellite has arisen as a repetition of a simple primeval sequence which has then diverged quite extensively.

After restriction by EcoRI, 90% of mouse DNA migrates in gels more rapidly than satellite DNA, and the size of the fragments of main band DNA ranges from 4×10^6 daltons to 2×10^5 daltons. The distribution of the sizes of the fragments is Poissonian, and the most probable fragment size is 1.5×10^6 daltons. This result — that no component of mouse DNA apart from the satellite DNA remains larger than 6×10^6 daltons after cleavage with EcoRI — is inconsistent with the hypothesis that eukaryotic DNA consists in large part of identical tandemly repeated sequences. If such sequences exist in main band mouse DNA, they cannot make up more than 0.5% of the total. Our data then do not support the "master-slave" hypothesis of Callan and his co-workers (Phil. Trans. Roy. Soc. [London] Series B, Biol. Sci. *243*:135 [1960]). However, we cannot preclude the idea that the mouse genome contains regions of tandemly arranged groups of related neucleotide sequences. Indeed, the fact that the model size of the fragments of main band DNA is slightly smaller than that predicted from its base composition tends to support this idea.

2) *Integration of SV40 sequences into transformed cell DNA:* High molecular weight SV3T3 DNA was digested to completion by endonuclease EcoRI, and the fragments were separated by electrophoresis through 1.4% agarose gels. The location of the viral sequences was determined by hybridization with radioactive SV40 cRNA. In the two cell lines so far examined, SV40 sequences were detected in two peaks. From the molecular weight of these peaks, we conclude first that the viral DNA is stably integrated, and second, that rearrangement of viral sequences does not take place continuously. Because the molecular weights of the fragments containing the SV40 DNA differ in the two cell lines, it seems that there is more than one site in the host DNA at which integration can occur. Furthermore, the amount of SV40 cRNA that hybridizes to each of the two peaks is different for each of the two cell lines. This result suggests that the site of integration within the viral genome can also vary. Before we can be sure of this, however, we have to be certain that the cells do not contain any partial copies of SV40 DNA. We are investigating this possibility by comparing the renaturation kinetics of the four specific fragments of SV40 DNA (Fig. 1) in the presence of DNA extracted from each of the two transformed cell lines.

3) *Adenovirus 2 sequences in the DNA of transformed rat cells*: We have examined the viral sequences integrated in one line (8617) of transformed rat cells using the method of Gelb *et al.* (J. Mol. Biol. *57*:129-145 [1971]) to determine the kinetics of reassociation of complete adenovirus 2 DNA and the DNA of each of the EcoRI viral fragments in the presence of transformed cell DNA. We find that each cell contains approximately 2 copies of about 40% of the sequences of the total viral DNA. The segments that are absent include all the sequences

of fragments B and F and 70-80% of those of fragment A. If this deletion occurred as a consequence of the act of integration, this result severely limits the number of possible mechanisms by which covalent attachment of viral sequences to host DNA can occur. We are currently testing other lines of adenovirus 2 transformed cells and we are attempting to determine the permutation of the viral sequences in cells of the 8617 line.

Isolation of variants from populations of transformed cells

1) *Concanavalin A-resistant cells*: During the past year we have continued to study lines of SV3T3 cells selected for resistance to concanavalin A. We have shown previously that these cells are similar in several respects to untransformed 3T3 cells. For example, they grow to low saturation densities and they are not agglutinated or killed by concanavalin A or wheat germ agglutinin. We now know that the concanavalin A-resistant cells also contain about the same level of cyclic AMP as 3T3 cells and that they do not form colonies in methocel. However, by contrast to untransformed cells, they will grow in low concentrations of serum, and they do not show a marked density-dependent inhibition of DNA synthesis. The cells of some concanavalin A-resistant lines contain an increased number of chromosomes. All of the revertants contain the same amounts of T-antigen, viral DNA and viral RNA as the parental SV3T3 cells, and the SV40 virus rescued from them by heterokaryon formation is indistinguishable in its growth and transforming properties from virus rescued from SV3T3 cells. The concanavalin A-resistant cells cannot be retransformed either by SV40 or MSV. Possibly, for SV40, this resistance may be a consequence of the failure of the virus to penetrate the cells. However, this explanation cannot apply to MSV for we have shown that, even in the absence of transformation, infectious MSV was produced by the cells and MLV-specific, RNA-dependent DNA polymerase could be detected in large amounts.

From these results we conclude that the concanavalin-resistant cells have reverted to an untransformed phenotype by a mechanism which involves a change in a cell-coded function and not because of a defect in an SV40-specific product.

We have isolated several clones of a new type of concanavalin A-resistant SV3T3 cells. Like the revertants described above, these cells are also resistant to the toxic effects of concanavalin A, they are not agglutinated by lectins unless they have been treated with proteases, and they do not form colonies in methocel. However, they grow to the same saturation density as SV3T3 cells in liquid medium. We feel that these results show that the surface change(s) that causes cells to become agglutinable is not necessary for growth to high saturation density, but may be involved in the decreased adhesive properties displayed by transformed cells.

2) *Revertants of MSV-transformed 3T3 cells:* In collaboration with Arthur Vogel, we have recently isolated several revertants of MSV-transformed 3T3 cells (line KA31) using as selective techniques resistance to 5-fluorodeoxyuridine, resistance to 5-bromodeoxyuridine and UV-irradiation, or resistance to concanavalin A. The revertant cells are indistinguishable from 3T3 cells in their saturation densities, serum requirements, growth in methocel, and density-dependent inhibition of DNA synthesis. All of them can be retransformed by MSV but not by SV40. Infectious MSV cannot be rescued from the cells selected with FdU or BrdU and UV-light after infection with MLV, but can be from those selected for resistance to concanavalin A. This latter class of cells can also be retransformed by super-infection with MLV.

3) *Cytochalasin-resistant cells:* We have shown previously that cytochalasin B acts differently on normal 3T3 mouse cells and on their SV40-transformed derivatives and, in particular, kills preferentially the transformed cells (Kelly and Sambrook, 1973). This differential killing effect was used to isolate cytochalasin B-resistant cells from populations of SV40-transformed 3T3 cells: After treatment with the drug (2 successive cycles of 6 days at 5 μ g/ml separated by a week's interval in drug-free medium and followed by two weeks in normal medium to allow growth of colonies), 1 in 10^5 of the original cells survived to give rise to colonies. Approximately one in ten of these colonies proved to be resistant to the drug, and the plating efficiency of these cells after treatment with cytochalasin B was now close to that of untransformed 3T3 cells. By contrast to the parent transformed cells which become multinucleated in the presence of the drug, these variants became only binucleated or at most, tetranucleated.

The resistant clones were further examined for their morphology, growth properties, presence and expression of the SV40 genome. One class of these variants resemble closely the "flat"revertants previously obtained by other methods, in that their growth properties are similar to those of normal cells; the viral genome is still present since T-antigen is detectable and virus can be rescued. In addition, this class of cytochalasin B-resistant cells has approximately twice as many chromosomes as the parental SV3T3 line, a property also shown by most other revertants of transformed cells.

A second class of resistant cells was obtained which, although they respond to cytochalasin B very much like the cells of the first class (i.e., they became bi- or tetranucleated in the presence of the drug and are resistant to its killing action), are very different in other properties. They are of the transformed phenotype and grow to high saturation densities; most of them are T-antigen negative. Two independently isolated T-antigen negative variants were examined further for the presence of the viral genome and found to contain only about half the amount of viral DNA present in the parent cell. The remaining viral genome was still transcribed in one case, but no virus-specific RNA could be detected in the second cell line.

Identification of the SV40 sequences still present in these cells is under way. We hope that studies of these and other cells which contain only fragments of the viral genome might help to localize specific functions (like expression of the T-antigen).

Karyotype analysis showed that one chromosome is missing from both cell lines, and we are using the banding technique in order to identify this chromosome. Such an identification and examination of other cytochalasin B-resistant cells derived from different SV40-transformed cells could provide another way of deciding whether the virus is integrated at random or in a specific region of the host genome.

Host-range mutants of viruses

Because we wanted to set up a system to obtain absolute as well as temperature-sensitive viral mutations, we have started to isolate host-range mutants of the non-defective $Ad2^{+}ND_1$ hybrid virus. Human adenovirus type 2, which grows well in human cells, replicates very poorly in monkey cells, although this defect can be overcome with co-infection with SV40. $Ad2^{+}ND_1$ grows equally efficiently in human cells and monkey cells presumably due to the insertion of SV40 sequences into the adenovirus 2 DNA (Levine *et al.*, J. Virology 7:343 [1971]). We used this property to select $Ad2^{+}ND_1$ host-range mutants which, while still growing and plaquing well on human cells, are now deficient in growth on monkey cells. Viruses with mutations in the integrated SV40 segment should comprise a class of mutants which have lost the enhancement function.

We have isolated several host-range mutants and have examined one (H39) in detail. Growth of H39 in monkey cells is increased by co-infection with adenovirus 2. Studies comparing the DNA of the mutant with $Ad2^{+}ND_1$, either by the cleavage patterns produced by EcoRI restriction endonuclease digestion or by heteroduplexing, reveal no differences. Furthermore, the same SV40 RNA sequences synthesized by $Ad2^{+}ND_1$ during lytic infection of monkey cells and human cells are also made by H39. However, the pattern of protein synthesis of $Ad2^{+}ND_1$ and H39 in monkey cells is quite different because the mutant is defective in the synthesis of several later proteins. In this respect it resembles adenovirus 2 which does not make its full complement of late proteins in infected monkey cells (Baum, Horwitz and Maizel, J. Virol. *10*:211 [1972]) and behaves like a mutant which has lost the enhancement function. In human cells, the proteins synthesized by H39 and the parental $Ad2^{+}ND_1$ are very similar with one exception. A 30,000 MW protein has been identified as present in ND_1 infected cells but not in Ad2 infected cells (Walter, Nature NB, Aug. [1973]; C. Anderson, unpublished results). This protein is also missing in H39 infected cells and may represent the mutant gene product.

Studies of complementation and recombination among the host-range mutants and isolation of revertants are currently in progress. We are also isolating mutants of $Ad2^{+}ND_1$ which are temperature sensitive in their growth on monkey cells.

Ribonuclease H

It has now been fairly firmly established that RNA-priming is a general mechanism for the initiation of new DNA chains in replicating DNA. This implies some mechanism of nucleolytic removal of RNA-primers prior to the ligation of newly synthesized DNA chains. As we suggested earlier, ribonuclease H-type enzymes may be responsible for this process. We, therefore, concentrated our efforts on the further characterization on RNase H activities both in *E. coli* and in tissue culture cells.

1) In collaboration with Dr. R. Crouch (NIH) we have isolated and purified two species of RNAse H from *E. coli*. The two activities can be separated chromatographically on phosphocellulose columns. Most work has so far been done with the activity eluting at the lowest ionic strength from phosphocellulose. This enzyme is a small (M.W. ca 20,000 daltons) protein with an isoelectric point of 9.5. The nuclease is highly specific for the degradation of RNA hybridized to complementary DNA. The degradation products are mono- and oligoribonucleotides terminated with 5'-phosphate groups. Like the 5S RNAse H from mammalian cells (see below), the *E. coli* enzyme is able to remove hybrid RNA covalently inserted into DNA of the colicinogenic factor E, of *E. coli*.

In the course of this work we have also investigated the three known DNA polymerases of *E. coli* for the presence of RNAse H-type activities. As reported earlier, the 5'-3' exonuclease

associated with DNA polymerase I is able to degrade RNA in DNA-RNA hybrids. However, this activity is not specific for the RNA strands but degrades the DNA strands as well. DNA polymerases II and III are completely free of nucleases attacking hybrid RNA.

2) *Tissue culture cells*: Human cells (KB) grown in suspension culture contain two RNAse H activities that can be separated by their different size. The larger species sediments at~5S in sucrose gradients and binds to DEAE cellulose. The smaller RNAse H sediments at 2.4S and has a high affinity to carbomethyl cellulose. It thus resembles the activities isolated from *E. coli*. Both nucleases are now being further purified and their intracellular localization is being investigated.

RNA-primed poly A-polymerases

We have detected and partially purified from human KB cells two enzymes which in the presence of an RNA-primer, ATP and Mn^{++} will polymerize poly A, most likely by covalently extending the priming RNA. The two activities can be separated by their differential affinity for ion-exchange columns. We intend to further purify and characterize these two enzymes and we hope to use them as tools for the general purpose of adding poly A to any RNA *in vitro*. Using oligo dT as primers and RNA-dependent DNA polymerase, it should then be possible to synthesize complementary DNA to any isolated RNA of interest.

PUBLICATIONS

Botchan, M., McKenna, G. and Sharp, P.A. An analysis of the organization of DNA in eukaryotic cells using restriction endonucleases. *Cold Spring Harbor Symp. Quant. Biol. 38* (in press).

Falcoz-Kelly, F. and Sambrook, J. (1973) The differential effect of cytochalasin B on normal and transformed cells. *Nature* 242:217—219.

Ozanne, B. and Lurye, M. Isolation and characterization of two classes of concanavalin A-resistant SV3T3 cells. *Control of Proliferation in Animal Cells.* Cold Spring Harbor Laboratory (in press)

Ozanne, B., Sharp, P.A. and Sambrook, J. (1973) Transcription of simian virus 40. II. Hybridization of RNA extracted from different lines of transformed cells to the separated strands of SV40 DNA. *J. Virol.* 12:90—98.

Ozanne, B., Vogel, A., Keller, W., Sharp, P.A. and Sambrook, J. Transcription of SV40 DNA. *4th Lepetit Symp. on Biol. and Medicine* (in press).

Pettersson, U. Replication of adenovirus 2 DNA. *J. Mol. Biol.* (in press).

Pettersson, U., Mulder, C., Delius, H. and Sharp, P.A. (1973) Cleavage of adenovirus type 2 DNA into six unique fragments by endonuclease R:R1. *Proc. Nat. Acad. Sci.* USA 70:200—204.

Pettersson, U. and Sambrook, J. (1973) The amount of adenovirus 2 DNA in the genome of transformed rat cells. *J. Mol. Biol.* 73:125—130.

Pettersson, U., Sambrook, J., Tibbetts, C. and Delius, H. Transcription of adenovirus 2 DNA by *E. coli* DNA-dependent RNA polymerase. *Virology.* (submitted).

Risser, R. and Mulder, C. Relative location of rearrangements in the DNA of defective simian virus 40. *Virology* (submitted).

Sambrook, J., Fenner, F., Mims, C.A., McAuslan, B.R. and White, D.A. *The Biology of Animal Viruses.* Academic Press (in press).

Sambrook, J., Jackson, A.H., Keller, W., Ozanne, B., Sharp, P.A. and Sugden, W. Transcription of simian virus 40. *Symp on Tumor Virus-Host Cell Interaction.* North Holland (in press).

Sambrook, J., Pettersson, U. and Sharp, P.A. An analysis of the viral sequences present in the DNA of cells transformed by adenovirus type 2. *J. Mol. Biol.* (submitted).

Sambrook, J. and Pollack, R.E. Transformation assays. *Methods in Enzymology, vol. 29.* Academic Press (in press)

Sambrook, J., Sharp, P.A. and Pettersson, U. Transcription of adenovirus 2 and SV40. *Oak Ridge Symposium on Control of Transcription in Eukaryotic Cells* (in press).

Sambrook, J., Sugden, B., Keller, W. and Sharp, P.A. Transcription of simian virus 40. III. Orientation of RNA synthesis and mapping of "early" and "late" species of viral RNA from lytically infected cells. *Proc. Nat. Acad. Sci.* USA (in press).

Sambrook, J., Sugden, B., Ozanne, B. and Sharp, P.A. Transcription of SV40. IV. Mapping of the viral RNA sequences in transformed cells. *Virology.* (submitted).

Sambrook, J. and Tooze, J. *Readings in Tumor Virology.* Cold Spring Harbor Laboratory (in press).

Sharp, P.A., Sugden, W. and Sambrook, J. (1973) Detection of two restriction endonuclease activities in *H. parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. *Biochemistry* 12:3055—3069.

Sugden, B. and Keller, W. (1973) Mammalian deoxyribonucleic acid-dependent ribonucleic acid polymerases. I. Purification and properties of an α-amanitin-sensitive ribonucleic acid polymerase and stimulatory factors from HeLa and KB cells. *J. Biol. Chem.* 248: 3777—3788.

MAMMALIAN CELL GENETICS

R. POLLACK
S. Arelt
C. Chang
J. Crisafulli
R. Goldman
N. Hopkins
P. Hough
P. Jeppesen
E. Maloy
J. Oey
R. Risser
J. Schwartz
C. Thomason
A. Vogel

The work of the cell culture laboratory has been directed in the most general sense toward understanding the differences between normal and transformed cells. Our attempts to do this have led us into a series of collaborations with other laboratories here and elsewhere. In particular, we have had the pleasure of working with Nancy Hopkins, Massachusetts Institute of Technology; Klaus and Mary Weber, on leave from Harvard University; Daniel Rifkin, Rockefeller University; Robert Krug, Sloan-Kettering Institute; Richard Goldsby, University of Maryland; Robert Goldman and Cheng Chang, Carnegie-Mellon Institute, Pittsburg; and Brad Ozanne, Peter Jeppesen, Phil Sharp and Joe Sambrook of the Tumor Virus Group here.

In September, Bob Goldman and Bob Pollack jointly purchased, with an Equipment Grant from the Human Cell Program of the National Science Foundation, a scanning electron microscope. Installed in the McClintock Building, this microscope will permit us to examine cell surface-specific phenomena, related to transformation, at very high resolution.

Transformation

A variety of selective assays have been used to monitor the transformation of normal cells into tumor cells. These assays take advantage of the ability of transformed cells to grow to high saturation densities, to grow in the absence of anchorage to glass or plastic, to form colonies or foci on monolayers of normal cells, to grow in reduced concentrations of serum components and to grow in disoriented patterns.

The transformation of mouse 3T3 cells by SV40 is usually monitored by the first assay and is a low frequency event. At very high virus doses (10^6 infectious units/cell) only 50% of the cells are transformed in this assay. We suspected that such selective transformation assays were not detecting the full range of cellular alterations which were induced by SV40. To isolate all possible types of SV40 transformants, a non-selective scan of clones of 3T3 cells arising after SV40 infection was carried out. Forty clones of SV40-infected 3T3 cells were picked without regard to morphology and analyzed in each transformation assay.

A density transformation assay carried out simultaneously on these infected cells showed a standard transformation frequency of 10%. However, the non-selective scan demonstrated that fully 90% of the clones differed from 3T3 cells in their ability to utilize low concentrations of serum.

The non-selective nature of this assay has allowed us to isolate many transformants which have not been previously described.

Clones of the first class, which are indistinguishable from 3T3 in these assays, may be unaffected by the virus or may be similar to the cryptic transformants of Smith *et al.*, (1971) if they carry SV40-specific sequences in unexpressed form (Smith *et al.*, 1972). They are presently being tested for SV40-specific DNA sequences.

Clones of the second class correspond to the standard transformants which have been described by many authors. The high proportion of these clones may in part be due to delayed transformation (Stoker, 1963; Todaro and Green, 1966). In fact, four of the clones looked normal by morphological criterion when they were picked. Subsequent to their cloning, they converted to transformants, perhaps because of the more rapid doubling time of transformed cells in 10% calf serum (16 hr vs. 23 hr). Reconstruction experiments on 3T3 and SV101 are currently in progress to test this hypothesis.

The third class of clones consists of cells which are altered in their ability to utilize low concentrations of serum. The saturation density of these clones is about twice that of 3T3. The mechanism by which this saturation density is maintained has not yet been investigated. We do not yet know if class III clones contain viral specific DNA sequences.

Serum transformants are expected from the work of others (Smith *et al.*, 1971; Scher and Nelson-Rees, 1972). What is surprising is the high proportion of clones which they represent, and the lack of viral T-antigen in these clones.

The last class of clones does not represent a uniform category of cells, but rather cells with a spectrum of growth properties which are intermediate between 3T3 cells and SV101 cells. The pattern of staining and level of complement-fixing T-antigen is significantly lowered from that of standard transformants. The saturation densities of such clones show a spectrum of values much as seen by Scher and Nelson-Rees (1972). Intermediate saturation densities seen in this class of clones may well be the result of an equilibrium between cell growth and cell death and detachment, as Scher and Nelson-Rees have demonstrated for their lines. The intermediate growth of these clones in methyl cellulose medium demonstrates that such clones are not comprised of a mixture of normal and standard transformed cells but rather comprised of cells each with a limited anchorage independence, similar to the abortive transformants of Stoker (1968). Such cells differ from abortive transformants in that they continue to grow slowly throughout the three week assay. It will be quite important to know if the few dense

colonies which such lines form on 3T3 monolayers in fact represent segregation of standard transformants.

We have shown in this work that the effect of SV40 on 3T3 cells is more varied and of a greater extent than previously thought to be the case. Whether the variety of cell growth patterns seen in this study is a result of an inherent instability of 3T3 cells due to their aneuploid chromosome complement is not yet clear.

This range could result from a mechanism of SV40 transformations that affected each cell somewhat differently, leading to the simultaneous appearance of every different type of transformant. Alternatively, the range may be the result of secondary alterations that become possible only after a primary virus-mediated alteration, such as serum utilization, has occurred.

In any event, the results reported here demonstrate that SV40 is capable of bringing about a variety of stable changes in growth properties of the majority of infected cells. Which of these changes, if any, is related to the ability of a cell to proliferate uncontrolledly in a host animal is not yet clear.

Reversion

1) Growth control of F1SV101

3T3 mouse fibroblasts in 10% calf serum cease to increase in number at confluence. SV40-transformed 3T3 cells lose their sensitivity to this growth control and so are able to reach multilayered cell densities. We previously described a polyploid revertant line F1SV101, isolated from a SV40-transformed cell, that has a low maximum cell density (Pollack *et al.*, 1968), despite the continued presence in this revertant of SV40-specific DNA, RNA and T-antigen. We have examined the mechanism by which the revertant maintains a low saturation density and find that at confluence, the revertant and 3T3 are both reduced in mitotic index, fraction of cells synthesizing DNA and rate of DNA synthesis. The transformed cell does not respond to confluence. None of the lines sheds intact cells into the medium at confluence.

2) Selection of serum-revertants

SV101, the SV40-transformed subline of the mouse fibroblast line 3T3, is both serum and density transformed, since it grows in both 1% and 10% calf serum, and grows beyond confluence in 10% calf serum. Negative selection at low cell density in 1% calf serum or in 10% agamma-depleted serum has permitted direct recovery of serum-revertant sublines of SV101. These sublines are unable to grow in 1% calf serum.

Although negative selection at high cell density in 10% calf serum is known to permit recovery of density-revertant sublines of SV101, density-revertants maintain a transformed, low serum requirement for growth. However, all serum-revertants isolated so far also grow to low saturation densities.

Although the serum revertants cannot grow in 1% calf serum, they do make DNA in the restrictive serum. It appears that the cells are slowly going through the cell cycle and detaching from the plate in 1% calf serum. The low saturation density of the serum revertants in 10% calf serum also results from cell shedding at confluence.

3) Selection of revertants with colchicine

Treatment of SV40-transformed mouse cell line SV101 with colchicine permits the isolation of polyploid revertant sublines which have lower saturation densities than the parent SV3T3 cells. These low saturation density lines have also reverted to a high serum requirement for growth and are unable to form colonies in methocel. Normal SV40 has been recovered from these revertants.

3T3 cells are more resistant to colchicine than SV3T3 cells at all cell densities. Colchicine-revertants do not display a 3T3-like resistance to colchicine at low density, but do survive colchicine at confluent cell densities, presumably due to their increased contact inhibition.

4) Gratuitous reversion

Once cells reverted in any one of the transformed properties (density, serum, anchorage, lectin susceptibility) have been isolated, one may ask if reversion in one property leads to the gratuitous reversion of the other transformed properties and if any other alterations in cellular physiology consistently accompany reversion. Density revertants of SV40-transformed 3T3 cells isolated with FdU or BrdU are reverted in their ability to grow in semi-solid medium, but maintain a transformed serum requirement. Colchicine selected density revertants also show a decreased ability to grow in methocel and have also reverted to a 3T3-like serum requirement. Thus selection of density revertants with different drugs leads to the isolation of revertants with different properties.

Selection of variants with a higher serum requirement for growth surprisingly also leads to reversion in saturation density. Thus selection with either restrictive serum yielded serum -sensitive lines with reduced saturation densities. However, selection in different restrictive sera lead to the isolation of revertants with different properties with regard to growth in methocel. Serum revertants isolated in 1% calf serum are unable to form colonies in methocel, while variants selected in agamma-depleted serum can grow in methocel. The reason for this difference is unknown, but the behavior in methocel is correlated with the cyclic AMP levels and agglutinability of these lines. The 1% serum revertants contain high cyclic AMP levels when grown in sparse culture in 10% calf serum and are also not agglutinable by wheat germ agglutinin in these growth conditions. The agamma serum revertant in sparse culture has a SV3T3-like cyclic AMP level and is agglutinable.

5) Retransformation of revertants

The serum and density revertants cannot be retransformed by SV40. However, murine sarcoma virus can morphologically retransform all revertant lines tested. The MSV-transformed revertants grow to high saturation density in 10% calf serum, and can form colonies in methocel. MSV transformation does not restore the ability to grow in 1% calf serum to the serum revertants.

A revertant of an MSV transformed non-producer Balb 3T3 line was isolated which can no longer grows in methocel. This revertant grows to low saturation density in 10% calf serum and grows poorly in 1% calf serum. It can be retransformed by a mixture of MSV and MLV but not by MLV alone.

6) Cyclic AMP

To investigate the role that cAMP may play in the regulation of cell growth, we have measured intracellular cyclic AMP levels in 3T3, SV40-transformed 3T3, and revertants under three growth conditions: sparse culture in excess serum, confluence in excess serum, and sparse culture in low concentrations of serum. The first condition compares the cyclic AMP levels among cultures that are all growing at similar rates. The latter two conditions are restrictive for some lines but not others. In growing cultures, the concentration of cyclic AMP was higher in 3T3 and most revertants than in SV40 transformed 3T3. However, growing cultures of one type of serum revertant maintained a low cAMP concentration.

No rise in cAMP occurred in any line subjected to density inhibition.

A steep rise in cAMP was seen in cultures of lines unable to grow in sparse culture and low serum. When serum was decreased these rises in cAMP occurred after a one day delay. However, when serum was increased, cAMP fell within minutes.

Preliminary studies suggest enucleation (see below) results in a permanent rise in cyclic AMP despite the presence of high serum.

Enucleation

1) Replating

Following recovery of enucleates from the effects of cytochalasin B we have been able to remove them from their substrates with 0.25% trypsin-EDTA solution. We carefully observe the cells during trypsinization, and as soon as they begin to round up and lose some of their contacts with the substrate, the trypsin-EDTA solution is removed and replaced with a few ml of normal medium. The medium is then rapidly pipetted in and out of the petri dish containing the coverslip, thereby removing the enucleates and suspending them in the normal medium.

The suspension of enucleates can be replated immediately into Falcon dishes or onto glass coverslips. In order to enhance re-attachment and spreading of enucleates, they are concentrated into small drops of normal medium on the substrate. Within a few hours, the plate is flooded with medium. The effect of this crowding is to enhance the attachment and spreading of the enucleates. The enucleates are also capable of attachment and spreading at low cell densities; however, the process is slower.

Utilizing this procedure we have demonstrated that rounded up enucleated cells are capable of normal attachment, spreading and shape formation. For example, enucleated 3T3 cells attach and spread out over the substrate in a fashion which is identical to whole 3T3 cells. The same is true for enucleated BSC-1 cells and BHK-21 cells. These observations demonstrate that cytoplasm contains the information necessary for cell attachment, spreading and shape formation.

Other physiological phenomena are also seen in enucleates. They are capable of normal pinocytosis and membrane ruffling. We have also demonstrated that contact inhibition of membrane ruffling occurs normally in enucleated BSC-1 cells and that cell locomotion may be seen in enucleated BHK-21 cells.

2) Continued macromolecular synthesis

Rates of DNA, RNA and protein synthesis in normal and enucleated BSC-1 monkey cells and Balb-C 3T3 mouse cells were compared by following the incorporation of ^{3H}-thymidine, ^{3H}-uridine or ^{3H}-leucine into material precipitable by acid from lysates of cells incubated with these precursors. In order to normalize the data, rates of incorporation were referred to the concentration of protein, which had been previously pre-labeled by incubating the cells for two generations before enucleation with ^{35}S-methionine containing medium. Cells were enucleated as described by Pollack and Goldman (1973) by centrifugation in the presence of 10 μg/ml of cytochalasin B. BSC-1 cells were spun on plastic coverslips inverted in cytochalasin B medium at 12,000 rpm for 10 min, when an enucleation efficiency of over 95% could be obtained. In the case of Balb-C 3T3 cells, the plastic coverslips were pre-coated with callagen to aid adhesion of the cell monolayer and spun at 15,000 rpm for 15 min, giving an efficiency of enucleation of over 99%, although some cells were lost.

Cells were pulsed for a 2 hr period with the tritiated precursors up to 16 hr after enucleation, then lysed with SDS and precipitated with TCA. The results showed that incorporation into both DNA and RNA drop immediately after enucleation to background levels which agree well with the efficiencies of enucleation, confirming that the nucleus is removed intact by treatment with cytochalasin B. On the other hand, incorporation of ^{3H}-leucine, after a drop of about 30% immediately after enucleation decays only slowly thereafter, with a half-life of some 12-16 hr. The initial drop must reflect either an unstable class of messenger RNAs with a very short half-life, or alternatively a disruption to a small extent of the protein synthesizing apparatus caused by enucleation procedure.

In different experiments performed with enucleated BSC-1 cells, SDS gel electrophoresis of polypeptides pulse labeled with ^{35}S-methionine at several time intervals after enucleation did not show any obvious alteration in band pattern as detected by autoradiography, when compared with non-enucleated controls. Thus any proteins which are specified by short-lived mRNAs do not appear to be present in sufficient quantities to be detected by the gel method.

In order to test for the necessity of protein synthesis in the attachment and spreading of enucleated BSC-1 and BHK-21 cells, we have suspended them in medium containing 20 g/ml cycloheximide and replated them as described above. Under these conditions, cell attachment, spreading and shape formation are normal for periods of observation to 7 hours following enucleation.

Since microtubule and microfilament assembly seem to be involved directly in cell attachment, spreading and shape formation in nucleated BHK-21 and BSC-1 cells, it seems likely, based on the above observations, that pools of subunits to these cytoplasmic fibers are present in enucleated cytoplasm. These types of observations emphasize that the cytoplasm contains storage forms of molecules or subunits which may be assembled or disassembled during various types of physiological activity in the absence of nuclear information.

3) Ultrastructure

A preliminary electron microscopic survey of enucleated BHK-21 cells indicates that the ultrastructure of spread enucleates is normal. A normal distribution of mitochondria, golgi, ribosomes, etc., is seen in enucleated BHK-21 cells. In addition there is a normal distribution of microfilaments, microtubules and 100 Å filaments.

4) Overlapping

We have initiated attempts to answer some basic questions about the behavior of normal vs. transformed cells in the absence of nuclear genetic information by utilizing the trypsinization and replating procedure. Our initial experiments have involved the use of enucleated 3T3 and Py3T3 cells. When these enucleates are replated into drops and cells are observed at the periphery of the drops, it becomes obvious that the property of piling up or overlapping of cells is maintained by Py3T3 enucleates while 3T3 enucleates at the edge of the drop show relatively minimal overlaps. Thus it appears that nuclei are not necessary for the maintenance of these properties of normal and transformed cells. These observations also indicate that the cytoplasm is capable of maintaining normal contact inhibition in enucleated 3T3 cells and a lack of contact inhibition in enucleated Py3T3 cells. From this type of experiment, it looks as if this aspect of the behavior of transformed cells (overlapping or lack of contact inhibition) does not require the presence of oncogenic virus genes which are located in the host cell nucleus, although other aspects of the transformed state probably do require a nucleus.

5) Longevity

Although the enucleates appear quite healthy for periods between 12-18 hr, they

begin to look rather unhealthy after longer time intervals. We are in the process of attempting to prolong the viability of enucleates by crowding and the use of conditioned medium. Crowding the enucleated cells into small areas seems to increase the length of time during which the cells appear healthy with regard to such parameters as membrane ruffling, pinocytosis and cell shape. For example, by crowding 3T3 enucleated cells some of them retain a fairly normal shape, even at 28 hr following enucleation and replating.

6) Virology

No newly synthesized virus could be recovered from enucleate fragments in earlier studies of viral replication because cells containing nuclei were always present in large numbers during the infection. However, we were able to infect relatively pure enucleated BSC-1 monolayers with poliovirus and were able to recover newly synthesized infective poliovirus. This showed that monkey cell nuclei are not necessary for uncoating, translation and replication of poliovirus RNA and for the assembly of infectious virus.

The replication of more complex RNA viruses such as SV5 and influenza may also proceed in enucleated cytoplasms of susceptible cells, and those viruses are under examination. By the same token, enucleates should, in principal, serve as excellent material for the synthesis of RNA or DNA directed by exogenous DNA or RNA, whether viral or cellular.

PUBLICATIONS

Goldman, R., Berg, G., Bushwell, A., Chang, C., Dickerman, L., Hopkins, N., Miller, M., Pollack, R. and Wang, E. (1973) Fibrillar systems in cell motility. *Ciba Foundation Symposium* 14:83-107.

Goldman, R. and Pollack, R. Uses of enucleated cells, in *Methods of Cell Physiology, vol. 9 (ed D. Prescott)* Academic Press (in press).

Goldman, R., Pollack, R. and Hopkins, N. (1973) Preservation of normal behavior by enucleated cells in culture. *Proc. Nat. Acad. Sci.* 70:750-754.

Hellman, A., Oxman, M. and Pollack, R., editors (1973) *Biohazards in Biological Research.* Cold Spring Harbor Laboratory.

Oey, J., Vogel, A. and Pollack, R. Intracellular cyclic AMP concentration responds specifically to growth regulation by serum. *Proc. Nat. Acad. Sci.USA* (in press).

Ozanne, B., Vogel, A., Sharp, P., Keller, W. and Sambrook, J. Transcription of SV40 DNA sequences in different transformed cell lines. *Lepetit Colloq.* (in press).

Pollack, R., editor (1973) *Readings in Mammalian Cell Culture.* Cold Spring Harbor Laboratory.

Pollack, R. (1973) The culture of mammalian cells, pp. 75-172, in *The Molecular Biology of Tumour Viruses (ed. J. Tooze)* Cold Spring Harbor Laboratory.

Pollack, R. and Goldman, R. (1973) Synthesis of infective poliovirus in BSC-1 monkey cells enucleated with cytochalasin B. *Science* 179:915-916.

Pollack, R. and Hough, P.V.C. The cell surface and malignant transformation. *Annual Reviews of Medicine* (in press).

Pollack, R. and Vogel, A. (1973) Isolation and characterization of revertant cell lines. II. Growth control of a polyploid revertant line derived from SV40-transformed 3T3 cells. *J. Cell. Physiol.* 82:93-100.

Risser, R. and Pollack, R. Biological analysis of clones of SV40-infected mouse 3T3 cells, in *Control of Proliferation in Animal Cells (ed. B. Clarkson and R. Baserga)* Cold Spring Harbor Laboratory.

Sambrook, J. and Pollack, R. Isolation and culture of cells — basic methodology for cell culture-cell transformation (X.A.3.), in *Methods in Enzymology.* Academic Press (in press).

Vogel, A., Oey, J. and Pollack, R. Two classes of revertants isolated from SV40-transformed 3T3 mouse cells, in *Control of Proliferation in Animal Cells, (ed. B. Clarkson and R. Baserga)*, Cold Spring Harbor Laboratory (in press).

Vogel, A., Ozanne, B. and Pollack, R. DNA content in normal transformed and revertant mouse cell lines. *First Annual Life Sciences Symposium — Mammalian Cells: Probes and Problems.* Los Alamos Scientific Laboratory (in press).

Vogel, A. and Pollack, R. (1973) Isolation and characterization of revertant cell lines. IV. Direct selection of serum-revertant sublines of SV40-transformed 3T3 mouse cells. *J. Cell. Physiol.* 82:189-198.

Vogel, A. and Pollack, R. Methods for obtaining revertants of transformed cells, in *Methods in Cell Physiology, vol 8 (ed. D. Prescott)* Academic Press (in press).

Vogel, A., Risser, R. and Pollack, R. Isolation and characterization of revertant cell lines. III. Isolation of density-revertants of SV40-transformed 3T3 cells using colchicine. *J. Cell Physiol.* 82:181-198.

R. GESTELAND
B. Allet
C. Anderson
M. Anderson
J. Atkins
P. Baum
J. Bruenn
R. Cloward
P. DeLucia
P. Grisafi
F. Jeppesen
P. Jeppesen
P. Maloy
J. Manley
R. Solem

Read-through protein of R-17

Close examination of proteins made *in vitro* from bacteriophage R-17 RNA reveals a minor product larger in molecular weight than the synthetase protein by about 40-50 amino acids (65,000 MW; 60,000 for the synthetase). The amount of this protein is only about 7% of the synthetase, but this fraction increases to about 15% if extracts from a UGA suppressing strain are used for the *in vitro* synthesis. This result suggests that the minor protein is the result of read-through beyond a UGA termination triplet due to suppression by a UGA level reading in the wild-type extracts. That this read-through product comes from the synthetase gene is suggested by the fact that addition of coat protein to the *in vitro* reaction mixtures represses synthesis of both the synthetase and read-through protein, while not affecting coat synthesis. The implication is that the termination signal at the end of synthetase cistron is UGA and that if suppression occurs, synthesis of the polypeptide chain continues on to another terminator or to the 3'-end of the RNA. Fingerprint studies and end group analyses are in progress to confirm these data.

The synthesis and processing of Adenovirus 2 proteins

HeLa or KB cells infected with adenovirus 2 yield progeny virus 18 to 48 hr after infection. The synthesis of virus-induced proteins in these cells was examined by labeling infected cultures with ^{35}S-methionine at various times after infection and analyzing the labeled proteins on SDS-polyacrylamide gels. The structural viral polypeptides were first detected approximately 15 hr after high-multiplicity infection; at about the same time there was a drastic decrease in host protein synthesis. Twenty-two virus-induced methionine-containing polypeptides were found; 14 of these appear in mature virions. Together these polypeptides account for about 70% of the theoretical coding capacity of adenovirus 2. Further analysis of these proteins indicated that at least 2 of the virion polypeptides, components VI (24,000D) and VII (18,500 D), were derived from precursor polypeptides of 27,000 D and 20,000 D, respectively. This was shown by pulse-chase experiments and, in the case of component VII (the major core protein), by tryptic fingerprinting. Conversion of the precursors to virion components occurs by the loss of 15-20 amino acids and appears to be a late step in virion assembly. Newly synthesized virus have small, but significant amounts of the precursor forms which are later cleaved to the mature product. Empty particles appear to be precursors to full particles and contain at least one polypeptide precursor (27K, precursor to VI) but no core proteins, core precursor, or viral DNA. Virus assembly can be inhibited by inhibiting protein synthesis with cycloheximide, raising the growth temperature to 42° C, or by the addition of proflavin. Each of these treatments also reduces the rate of conversion of both precursors to their respective products providing further evidence that cleavage is normally coupled to virus assembly. Attempts to determine if cleavage occurs near the carboxy or amino terminus of the precursors has thus far not been successful due to the difficulty in obtaining sufficient pure precursor protein.

When adenovirus 2 infects cultures of African green monkey cells (e.g., CV-1), little mature virus is produced even though all late viral-specific RNA appears to be synthesized. Virus production can be restored by co-infection with SV40 virus, or by infection of an adenovirus 2-SV40 hybrid virus which contains the SV40 region responsible for U-antigen induction. CV-1 cells infected at high multiplicity by adenovirus 2 were found to synthesize all but two of the previously identified adenovirus-induced polypeptides; these are the fiber protein (VI) and the precursor to component VI (27K). The synthesis of these two proteins is restored when the adenovirus-SV40 hybrid Ad ND is used to infect CV-1 cells. After Ad_2ND_1 infection of either HeLa or CV-1 cells, an additional virus-induced band with a molecular weight of 30,000 D is found. The relationship of this component to the SV40 U-antigen is currently being investigated. In addition, we are attempting to determine how SV40 enhances adenovirus synthesis in monkey cells. To do this we plan to examine the translation of *in vivo* and *in vitro* synthesized viral RNA in protein synthesizing systems derived from human and monkey tissue culture cells. If appropriate systems can be constructed, it will then be possible to ask whether SV40 U-antigen directly affects translational specificity.

Ribosome binding sites on SV40 mRNA

Experiments are in progress aimed at identifying ribosome initiation sites on SV40 mRNA so that we can determine their nucleotide sequences and map their location on the SV40 genome. Very high specific activity cRNA is made *in vitro* using α-^{32}P-triphosphates, SV40 form I DNA and *E. coli* RNA polymerase. This RNA, which is predominantly early strand RNA, binds to mouse liver ribosomes in the presence of rabbit reticulocyte initiation factors, GTP, etc., and met $tRNA_f$. The binding appears specific since uncharged tRNA or met $tRNA_m$ will not substitute for met $tRNA_f$. The amount of ^{32}P-RNA remaining bound after mild treatment of the complexes with RNase suggests that at most one or two binding

sites are found per genome equivalent of SV40 early RNA. There are difficulties with the experiment; these stem mainly from variable properties of this α-^{32}P-triphosphate and unusual aggregation problems of this *in vitro* synthesized RNA. However, these problems can be sorted out so that we can reproducibly detect binding to specific sites. We hope to separate these sites by conventional techniques, sequence them, and map them by hybridization back to restriction endonuclease fragments of SV40 DNA.

Promoter mutant of lac operon

A mutant of *Escherichia coli* selected to continue expression of the *lac* operon after a short pulse induction has been characterized as a new promoter mutant. The mutant is partially constitutive for β-galactosidase synthesis and on induction with IPTG, synthesizes this enzyme at a maximal differential rate 8-10 times that of the induced wild-type parent. This is accompanied by synthesis of a 25-fold excess of *lac* mRNA over the wild type, and this RNA has a near-normal half-life. This implies that in the induced mutant up to 20% of the mRNA should be from the *lac* operon.

The mutation maps with the I gene, so that the expression of the *lac* operon from this presumed new promoter must result from read-through for I through O and P and into Z.

The physiological properties of the mutant are being examined along with the properties of the new promoter.

Lambda DNA anatomy

Work has continued on transcription and translation of phage λ DNA. In particular, we have been exploiting specific DNA fragments produced by restriction endonucleases. The R·R1 endonucleases from *E. coli* cleaves linear λ DNA into six fragments which have been mapped by comparison of digestion products from various deletion and deletion substitution derivation of phage λ . One of these fragments (MW 4.5 x 10^6) contains the complete immunity region. One of the restriction enzymes from *Hemophilus parainfluenzae*, Hpa II, cleaves λ DNA into about 100 fragments, and those larger than 3 x 10^5 daltons have been mapped, and many of the cleavage sites in the immunity region and the b2 region have been identified. The early leftward promoter and operator ($O_L P_L$) are on a fragment 375 base pairs long which also contain the beginning of the N gene. The early rightward operator and promoter ($O_R P_R$) are in one of the largest fragments (~1.5 x 10^6 daltons) which has at least the complete CRO gene. A more thorough analysis of the immunity region has now been made using in addition restriction enzymes from *Hemophilus influenzae* (Hin) and *Hemophilus egypticus*. More than ten cleavage sites were identified and accurately mapped within the immunity region. Examination of the fragments digested by Hin enzyme revealed that DNA sequences for repressor binding overlap those for early promoters. This was shown by the finding that 2 mutations in P_L , sex 1 and sex 3, abolish the Hin cleavage site in O_L.

PUBLICATIONS

Allet, B. (1973) Fragments produced by cleavage of λ deoxyribonucleic acid with the *Hemophilus parainfluenzae* restriction enzyme Hpa II. *Biochem.* 12:3972—3977.

Allet, B., Jeppesen, P.G.N., Katagiri, K.J. and Delius, H. (1973) Mapping the DNA fragments produced by cleavage of lambda DNA with endonuclease R_1. *Nature* 241:120.

Allet, B., Katagiri, K.J. and Gesteland, R.F. (1973) Characterization of polypeptides made *in vitro* from bacteriophage lambda DNA. *J. Mol Biol.* 78:589—600.

Anderson, C.W., Baum, P.R. and Gesteland, R.F. (1973) Processing of adenovirus 2-induced proteins. *J. Virol.* 12:241—252.

Crawford, L.V. and Gesteland, R.F. (1973) Synthesis of polyoma proteins *in vitro*. *J. Mol. Biol.* 74:627—634.

Gesteland, R.F. and Kahn, C. (1972) Synthesis of bacteriophage λ proteins *in vitro*. *Nature New Biol.* 240:3—6.

Schreier, M.H., Staehelin, T., Gesteland, R.F., Spahr, P.F. (1973) Translation of bacteriophage R17 and QβRNA in a mammalian cell-free system. *J. Mol. Biol.* 75:575—578.

Boedtker, H. and Gesteland, R.F. The physical properties of RNA bacteriophage and RNA bacteriophage RNA. *Chapter for RNA. Bacteriophage Book.* (*ed. N. Zinder*) Cold Spring Harbor Laboratory (in press).

Bruenn, J. and Hollingsworth, H. A mutant of *E. coli* with a new, highly efficient promotor for the lactose operon. *Proc. Nat. Acad. Sci.* USA (in press).

Jeppessen, P. A method for separating DNA fragments by electrophoresis in polyacrylamide concentration gradient slab gels. *Analytical Biochemistry* (in press).

Allet, B. Separation of promoter sites in lambda DNA by "Specific Endonucleases". *J. Mol. Biol.* (in press).

MOLECULAR GENETICS

D. ZIPSER
B. Apte
A. Bukhari
M. Howe
H. Lund
D. Mattmuller
L. Rawluk
T. Razzaki
D. Ruthig
B. Shineberg

The molecular genetics section has been working on problems connected with operon expression, primarily in *Escherichia coli*. Originally our work was focused on the problem of polarity, a phenomenon characteristic of operons. However, by following a variety of different leads which have logically developed in this work, we are now involved in a considerably broader set of projects. Most of them are connected in one way or another with the use of the *lac* operon system in *E. coli*.

Our current work is conveniently divided into four categories. These are (1) translational punctuation, (2) specific polypeptide degradation, (3) integration of phage Mu, and (4) *in vivo* polypeptide splicing. This year there have been several new developments in our laboratory. Among those of most interest are: (a) The discovery of polypeptide splicing, that is, the synthesis *in vivo* of one protein from two genes; (b) the finding that the genetic locus which controls specific polypeptide degradation is the same as the well-known *lon* mutation, which is involved in the formation of capsid and also a variety of other functions of *coli*; (c) the isolation of mutations in phage Mu which allowed Mu-induced bacterial mutations to revert. All of these new results have appeared within approximately the past year. They have all been made possible, however, by the availability to us of an accurately characterized genetic and physiological system in *E. coli*. This system, of course, is the *lac* operon. It has been quite heartening to us to find that many new developments can come from an old and well-known system.

PUBLICATIONS

Abelson, J., Boram, W., Bukhari, A.I., Faelen, M., Howe, M., Metlay, M., Taylor, A.L., Toussaint, A., van de Putte, P., Westmaas, G.C. and Wijffelman, C.A. (1973) Summary of the genetic mapping of prophage Mu. *Virology* 54:90—92.

Apte, B.N. and Zipser, D. (1973) *In vivo* splicing of protein: one continuous polypeptide from two independently functioning operons. *Proc. Nat. Acad. Sci.* USA 70:2969—2973.

Bade, E.G. (1972) Asymmetric transcription of bacteriophage Mu-1. *J. Virol.* 10:1205—1207.

Bukhari, A.I. and Metlay, M. (1973) Genetic mapping of prophage Mu. *Virology* 54:109—116.

Bukhari, A.I. and Zipser, D. (1973) Mutants of *Escherichia coli* with a defect in the degradation of nonsense fragments. *Nature New Biol.* 243:238—241.

Howe, M. (1973) Prophage deletion mapping of bacteriophage Mu-1. *Virology* 54:93—101.

Howe, M. (1973) Transduction by bacteriophage Mu-1. *Virology* 55:103—117.

Zeldis, J., Bukhari, A.I. and Zipser, D. (1973) Orientation of prophage Mu. *Virology* 55:289—294.

ELECTRON MICROSCOPY

H. DELIUS
G. Bourguignon
M. Metlay
K. Sweeney
A. Wilde

Molecular weight determination by electron microscopy

The molecular weights of fragments produced by the action of endonuclease R:R_1 and endonuclease R·Sa on adenovirus DNAs of serotypes 2, 3, 5, 7 and 12 have been determined by length measurements on samples mixed with standards of known molecular weight.

In collaboration with Dr. D. Kolakofsky (Geneva), the molecular weight of Sendai virus RNA was determined as 5.5×10^6 daltons, using the method of attaching 32-protein to the RNA in order to stretch it out.

Partial denaturation mapping of adeno DNAs

Partial denaturation mapping was used to determine the position of adenovirus DNA fragments produced by restricting endonuclease R·R_1 in relation to the intact DNA. Mixtures of the fragments and of the intact DNA were partially denatured with alkaline in the presence of formaldehyde. A random sample of 150 to 250 molecules were measured. After sorting them according to their length, the denaturation maps were plotted. From the different length classes representative molecules were selected for the construction of denaturation histograms. By fitting the histograms of the fragments to the partial denaturation map of the intact DNA, in most cases an unambiguous position of the fragment on the original DNA map could be established. The fragment sequences (indicated by starting with the fragment at the terminus that is less denatured) were determined for adeno 2 DNA (A, B, F, D, E and C), adeno 3 DNA (A, C, and B), adeno 5 DNA (A, C, and B), and adeno 12 DNA(C, D, B, E, F and A).

Analysis of in vitro *transcription complexes*

The method of extending RNA chains by the attachment of 32-protein for length measurements of nascent RNA *in vitro* transcription complexes was applied to the analysis of SV40, lambda, and adenovirus 2 DNA transcription by *E. coli.* polymerase.

Circular SV40 DNA was converted to linear molecules by treatment with endonuclease $R \cdot R_1$. The efficiency of the linear DNA as a template for transcription by *E. coli* RNA polymerase is lower than that of the superhelical DNA, but in this case about half of the RNA chains found in the transcription complexes originated at a single promoter. This promoter is located 0.15 fractional length from one end of the DNA molecule and synthesis proceeds toward this end.

The analysis of *in vitro* transcription of lambda DNA yielded RNA patterns in good agreement with known promoter sites and directions of synthesis. In wild-type lambda DNA the main promoters were located at 0.79 and 0.74 fractional length, initiating rightward and leftward RNA synthesis, respectively, and around 0.5 fractional length initiating bi-directional synthesis within the b2 region. In lambda p*bio* (30-7 *nin*5) DNA the promoter at 0.74 fractional length is deleted, and the rightward promoter in the b2 region causes read-through into the *bio* region.

The most active promoters in adenovirus 2 DNA for *in vitro* transcription by *E. coli* polymerase seem to be located preferentially in one half of the DNA molecule. With the help of this orientation, a diagram of nascent RNA chains was constructed from the measurements on adeno 2 transcription complexes. Between 6 and 9 promoter positions can be recognized in this diagram. Comparison of this pattern with the RNA diagrams obtained from transcription of endonuclease $R \cdot R_1$ fragments of adeno 2 DNA by *E. coli* polymerase gave evidence that the less active half of the DNA represents the region occupied by the A-fragment, and that the most active promoter is located in the D-fragment.

Electron microscopy of T5 DNA

The positions of single-strand nicks in the T5 DNA relative to the partial denaturation map have been determined. In addition to the nicks already known, a nick was found very close to one end (on the side close to the high-GC region), probably corresponding to the nick located at a distance of 0.08 fractional length from the other end, with both nicks lying within the redundant region.

Analysis of the replicative DNA from T5-infected *E. coli* cells shows that, as in the case of T4 DNA, DNA synthesis can be initiated at several sites within the chromosome. At later stages (15 min after infection) circular molecules with a circumference of unit length T5 DNA minus the 8% of the redundant region were observed.

PUBLICATIONS

Mulder, C. and Delius, H. (1972) Specificity of the break produced by restricting endonuclease R_1 in simian virus 40 DNA, as revealed by partial denaturation mapping. *Proc. Nat. Acad. Sci.* USA 69:3215.

Sigal, N., Delius, H., Kornberg, T., Gefter, M. and Alberts, B. (1972) A DNA-unwinding protein isolated from *Escherichia coli*: Its interaction with DNA and with DNA polymerases. *Proc. Nat. Acad. Sci.* USA 69:3537.

Petterson, U., Mulder, C., Delius, H. and Sharp, P.A. (1973) Cleavage of adenovirus type 2 DNA into six unique fragments by endonuclease $R \cdot R_1$ *Proc. Nat. Acad. Sci.* USA. 70:200.

Westphal, H., Delius, H. and Mulder, C. (1973) Visualization of SV40 *in vitro* transcription complexes. *Lepetit Colloq. Biol. Med.* 4.

NUCLEIC ACID CHEMISTRY

R. ROBERTS
J. Arrand
J. Ashton
P. Myers
B. Smith

The main aim of this laboratory is to investigate the use of restriction endonucleases as tools in the field of nucleic acid sequence analysis. Three lines of inquiry have been pursued.

Sequencing

A new, fast method for examining directly short terminal sequences on double-stranded DNA has been explored. This technique utilizes silkworm nuclease, a nonspecific endonuclease which, under our conditions, generates predominantly trinucleotides as terminal digestion products. Because of the mode of cleavage, stretches of DNA lacking a 5'-terminal phosphate residue give rise to unique products upon complete digestion with this enzyme, and these products can easily be isolated by virtue of their electrophoretic properties. In this way the 5'-terminal trinucleotide sequences of restricted fragments can be deduced, and in many cases this leads directly to the complete sequence of the recognition site of the restriction endonuclease.

New endonucleases

In addition to building up a collection of the known restriction endonucleases, we have begun an active search for new ones. Examination of *Hemophilus aegyptius* has shown the presence of two new restriction endonucleases in addition to the one previously described. The purification and characterization of these new enzymes is under way. One of them fails to cleave SV40 DNA, although it cleaves most DNAs with considerable frequency.

Mapping

Work is in progress, in collaboration with J. Sambrook, to produce a physical map of the adenovirus-2 genome by the use of restriction endonucleases. Fragments produced by enzymes from *Hemophilus influenzae* and *Hemophilus parainfluenzae* have been partially ordered with respect to the known sites of cleavage by the restriction endonuclease Eco R_1. One of these fragments, produced by the enzyme Hpa I (from *H. parainfluenzae*) is approximately 600 nucleotides in length and contains one end of the linear adenovirus-2 DNA molecule. This in turn is cleaved into two fragments by endonuclease Z from *H. aegyptius*, of which one 325 nucleotides in length now contains the terminal sequence. An enzyme is presently being sought which will cleave this fragment further to produce a piece of DNA, containing the end, that is sufficiently small for sequence analysis to be undertaken.

PROTEIN CHEMISTRY

P. GREENAWAY
D. LeVine

This section is interested in structural aspects of the interactions between proteins and nucleic acids.

The endonuclease associated with purified SV40 particles.

Wild-type and mutant SV40 particles contain an associated endonuclease that is capable of degrading exogenous DNA. Digestion of bacteriophage λ DNA with this enzyme produced nucleotides which were relatively small (sedimenting in alkaline sucrose gradients at 7-10 S) and which had a heterogeneous length distribution. Efficient labeling of these nucleotides with polynucleotide kinase was obtained only after prior treatment with bacterial alkaline phosphatase, and thus the terminal nucleotides predominantly contained 5 -phosphoryl groups. The 5'-terminal mono- and oligonucleotides of the digestion products, isolated after polynucleotide kinase labeling, were shown to be heterogeneous. It was therefore concluded that the SV40-associated endonuclease is not highly specific with respect to the nucleotide sequence at which it cleaves. This study does not, however, rule out the possibility that purified SV40 particles contain more than one endonucleolytic activity.

DNA and protein methylases in chicken embryo cells

Restriction and modification enzymes (specific DNA endonucleases and DNA methylases) have been identified in many bacterial strains; these enzymes together form an efficient defense mechanism against the expression of foreign DNA obtained, for example, by bacteriophage infection. Restriction and modification enzymes may play a similar role in higher organisms but they have yet to be identified in eukaryotic cells. We are therefore investigating this possibility using soluble extracts from chicken embryo cells.

Nuclei from chicken embryo cells were isolated by velocity sedimentation through high concentrations of sucrose and then disrupted to release the soluble enzymes. These enzymes were fractionated by ammonium sulfate precipitation and each fraction was assayed for the presence of DNA endonucleases and DNA methylases. All fractions examined contained nucleases capable of degrading exogenous DNA, but enzymes giving specific fragmentation patterns were not detected. A potentially interesting ATP-dependent DNA endonuclease was observed. A DNA methylase was detected in the proteins precipitated by 20% (w/v) ammonium sulfate and was purified to approximately 70% homogeneity. The enzymic activity was dependent on the presence of Mg^{++} and DTT and was stimulated by ATP. S-adenosyl methionine was the methyl group donor and the enzyme methylated the DNA isolated from a number of different sources. The product of methylation was 5-methyl cytosine.

A protein methylase was also isolated during these studies. Partial purification by ion-exchange and exclusion chromatography gave preparations that were still highly heterogeneous. The enzyme, at this state of purity, carried out endogenous protein methylation, again using S-adenosyl methionine as the methyl group donor, but it was stimulated by the addition of exogenous proteins, particularly histones. This enzyme again required Mg^{++} and DTT for optimal activity but there were no other cofactor requirements. It was inhibited by certain heavy metals, e.g., Hg^{++}, Cu^{++}, Fe^{+++}, and was competitively inhibited by S-adenosyl methionine and S-adenosyl homocysteine. The product of the reaction was the methylated derivative of either lysine, arginine or histidine.

PUBLICATIONS

Greenaway, P.J. and LeVine, D. (1973) Binding of *N*-acetylneuraminic acid by wheat-germ agglutinin. *Nature New Biology* 241:191—192.

Greenaway, P.J. and LeVine, D. (1973) Amino acid compositions of simian virus 40 structural proteins. *Biochem. Biophys. Res. Commun.* 52:1221—1223.

Greenaway, P.J. and Murray, K. (1973) Polynucleotides associated with histone preparations. *Biochem. J.* 131:585—591.

Greenaway, P.J. (1973) A study on the cleavage site specificity of the endonuclease associated with purified SV40 particles. *FEBS Letters* 34:193—197.

DNA REPLICATION

E. BADE
L. Buzzo
W. Schröder

The most interesting property of temperate phage Mu-1 is, no doubt, its ability to integrate at many sites of the chromosome of *E. coli* K12, apparently without need for a large recognition sequence. The analysis of the structure and function of Mu DNA should give useful information for our understanding of the integration process.

Structure and replication of Mu DNA

Following our initial observation with Hajo Delius, that the DNA of temperate phage Mu has a "variable end," we further characterized this end of the phage DNA and found that it contains mainly *E. coli* DNA sequences. We have also partially succeeded in constructing a physical map of Mu DNA by using several restriction enzymes.

From our studies on the replication of Mu, showing that both bacterial and phage DNA appear in superhelical DNA during phage replication, we have proposed a model that suggests packaging from a hybrid replication intermediate containing both *E. coli* and phage DNA, as origin for the variable (*E. coli* DNA-containing) end. Different aspects of this model are being tested experimentally.

Transcription of Mu

Mu prophages, integrated such that the direction of Mu transcription is the same as the direction of host transcription in the *lac* Z gene, are efficiently transcribed upon induction of the *lac* operon. The transcripts hybridize to the heavy strand of the phage DNA, from which 95% of the Mu-specific RNA is transcribed. It is not yet clear if the transcripts are hybrid *lac*-Mu mRNA molecules or not.

Generalized transduction by Mu, excision of Mu

We have found that Mu transduces preferentially a small region of the *E. coli* chromosome, different from the origin of replication. At this time we do not know if this result indicates a transient integration of Mu in that region, prior to replication, or if it is simply the result of preferential packaging of DNA of a specific composition or a specific location within the cell. Mu prophages do not excise cleanly from the host chromosome after induction, as other temperate phages, notably lambda, are known to do. In specific cases a certain amplification of genes close to the site of integration of the prophage was found after induction.

A model for the integration of Mu has been developed. One specific prediction of our model, that Mu might generate deletions upon integration, has been largely confirmed by M. Howe. On our efforts at developing an *in vitro* system for the integration of Mu, we have no results to report yet.

PUBLICATIONS

Bade, E.G. (1972) Asymmetric transcription of bacteriophage MU 1. *J. Virol.* 10:1205.

Bade, E.G. The molecular biology of bacteriophage Mu 1. *XI. Latin American Congress of Physiological Sciences* (in press).

Schröder, W., Bade, E.G. and Delius, H. Participation of *E. coli* DNA in the replication of phage Mu 1. (Submitted for publication).

Bade, E.G. and Buzzo, L. Preferential transduction of some segments of the *E. coli* K12 chromosome by temperate phage Mu 1. (Manuscript in preparation).

Bade, E.G. and Delius, H. Characterization of the "variable end" of phage Mu 1 DNA. (Manuscript in preparation).

PROTEINS IN SV40 INFECTED AND TRANSFORMED CELLS

K. WEBER
M. Weber
E. Lazarides

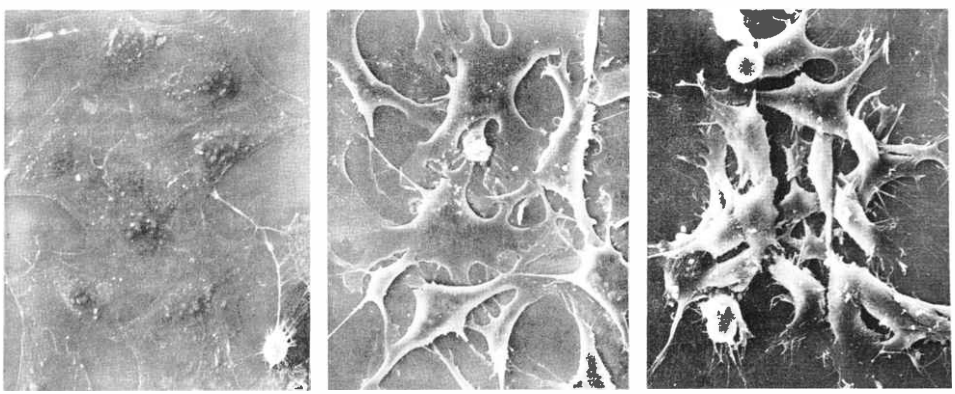
Sedimentation analysis of T-antigen in CV1 cells infected with SV40 and tsA-type mutants of SV40

Infection of CV1 cells with SV40 at 32° or 41°C produces a single T-antigen species as judged by sedimentation analysis of crude cell extracts. All the complement-fixing activity sediments at 15 S regardless of the temperature during the infection. Inhibition of DNA synthesis by ara C does not change the S value, although the amount of T-antigen is reduced.

*ts*A7 is an early SV40 mutant isolated by P. Tegtmeyer. This mutant is non-permissive at 41° and permissive at 32° and is thought to have a defect in the initiation step of viral DNA synthesis. We have studied the sedimentation properties of T-antigen in *ts*A7-infected CV1 cells at 32° and 41°. T-antigen shows the normal S value of 15 S if cells have been infected at the permissive temperature. Infection at the non-permissive temperature yields T-antigen sedimenting predominantly at 8 S and only a minor amount at 15 S. Similar results have been obtained with the *ts*A30 mutant.

Protein chemistry on SV40 capsid proteins

In order to characterize the major capsid proteins of SV40 we have started amino terminal analysis of these proteins. SDS gel electrophoresis is used as the isolation procedure of the individual polypeptide chains. The major capsid protein (VP1, molecular weight 48,000) shows the amino terminal sequence Ala-Pro-Thr-Lys . . . . Current work concentrates on the purification of the minor structural proteins, especially VP3 (molecular weight 27,000). Similar work is being done for polyoma virus.



Scanning Electron Micrographs of Normal 3T3 and Polyoma and SV40 Transformed 3T3 Cells
Photo on left shows normal 3T3 cells demonstrating normal contact inhibition.
Middle photo is of PY3T3 cells showing lack of contact inhibition which results in extensive overlapping of cells.
This loss of contact inhibition is also evident in the photo on the right of SV3T3 cells.

POST GRADUATE TRAINING PROGRAMS

SUMMER 1973

Since its inception the postgraduate course program at Cold Spring Harbor Laboratory has aimed to meet the rather special need of training in new inter-disciplinary 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 teaching 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 an up-to-date covering of current research work.

1) ANIMAL CELL CULTURE — June 11 - July 1

The program included the basic techniques required for the initiation, maintenance, and storage of mammalian cells in culture. Using cell lines and primary cultures, exercises focused on such essential parameters as growth, saturation density, contact inhibition, synchrony, cloning, karyotypes, differentiation and transformation by oncogenic viruses. In addition, other useful techniques which were covered were the production of viable cell hybrids, Sendai-mediated cell fusion, large scale enucleation with Cytochalasin B, radioautography, microscopy, differential agglutination of cells using plant lectins, and the induction and selection of auxotrophic variants. Experiments were carried out by the students individually and in groups, and there were demonstrations and lectures by invited guests.

INSTRUCTORS

Coon, Hayden, Ph.D., National Cancer Institute, Bethesda, Maryland
Goldman, Robert, Ph.D., Carnegie-Mellon University, Pittsburgh, Pennsylvania
Hsie, Abe, Ph.D., Oak Ridge National Laboratory, Oak Ridge, Tennessee

ASSISTANTS

Chang, Cheng-Ming, Ph.D., Carnegie-Mellon University, Pittsburgh, Pennsylvania
Rahe, Ann, B.S., National Cancer Institute, Bethesda, Maryland

PARTICIPANTS

Addison, Randolph R.A., B.S., Cornell University, Ithaca, New York
DeRobertis, Edward M., M.D., Instituto Investigaciones Bioquimicas, Buenos Aires, Argentina
Duguid, John R., B.S., California Institute of Technology, Pasadena, California
Feldberg, Ross S., Ph.D., Brandeis University, Waltham, Massachusetts.
Frischauf, Anna-Maria, Ph. D., Max-Planck Institut f. Biophys. Chemie, Austria
Gargus, John J., M.A., Case Western Reserve University, Cleveland, Ohio
Louie, Marjorie, B.S., University of Chicago, Illinois
Herrmann, Richard, Dr. Rer. Nat., California Institute of Technology, Pasadena, California
Hochstadt, Joy, Ph.D., Worcester Foundation for Experimental Biology, Worcester, Massachusetts
Hammarström, Sven R., M.D. & Ph.D., Karolinska Institutet, Stockholm, Sweden
Kowit, Joel D., Ph.D., Harvard Medical School, Boston, Massachusetts
Kuebbing, Daniel, A.B., University of Miami School of Medicine, Florida
Kuempel, Peter L., Ph.D., University of Colorado, Boulder, Colorado
Pfahl, Magnus, Ph.D., The Salk Institute, San Diego, California
Siu, Chi-Hung, B.A., University of Chicago, Illinois
Sugino, Yoshinobu, Ph.D., National Institutes of Health, Bethesda, Maryland
Voss, Ruth M., Ph.D., Sloan Kettering Institute, New York City, New York
Wang, John L., A.B., The Rockefeller University, New York City, New York
Willecke, Klaus, Ph.D., Institut fur Genetik der Universitat, Germany
Zipser, Birgit, Ph.D., Albert Einstein Medical School, Bronx, New York

SEMINARS

Harry Eagle, Albert Einstein School of Medicine, *Some aspects of cell regulation in culture.*
James Weston, University of Oregon, *Contact inhibition of movement: Behavioral manifestations of cell contact or cell surface interactions.*
Thomas Pollard, Harvard Medical School, *Cytoplasmic actin and myosin: The molecular basis of movement in cells.*
Abe Hsie, Oak Ridge National Laboratory, *Control of phenotypic expression by cAMP and hormones in cultured mammalian cells.*
Wesley McBride, National Cancer Institute, *Transfer of genetic information by isolated metaphase chromosomes.*
Richard Davidson, Harvard Medical School, *Studies on the genetics of mammalian cells in culture: BUdR dependence, cell hybridization, and Herpes virus.*
Elizabeth Neufeld , National Institutes of Health, *Hyposomal disease studied in cell culture.*
Jay Unkeless, Rockefeller University, *A proteolytic enzyme correlated with oncogenic transformation.*
Louis Siminovitch, University of Toronto, *Isolation and characterization of mutants of somatic cells.*
Stu Heywood, University of Connecticut, *Mechanisms of gene expression during myogenesis.*
Kenneth Kramer, National Institutes of Health, *Genetic heterogeneity in* xeroderma pigmentosum.
Gordon Sato, University of California, San Diego, *Lessons drawn from ten years of research in cell culture.*
Elliot Levine, Wistar Institute, *Methods for mycoplasma detection in cell cultures.*
Hayden Coon, National Cancer Institute, *Recombination in mammalian mitochondrial DNA.*
John Minna, National Institutes of Health, *Expression of phenotype in hybrid somatic cells derived from the nervous system.*
Joanna Olmsted, University of Wisconsin, *In vitro assembly of neurotubule protein.*
Matthew Scharff, Albert Einstein School of Medicine, *Mutations in immunoglobulin production in mouse myeloma cells.*
Hiroyuki Hirumi, Boyce Thompson Institute, *Methods in invertebrate cell culture.*

2) MOLECULAR BIOLOGY AND GENETICS OF YEAST — June 11 - July 1

This program emphasized the major laboratory techniques used in the genetic analysis of yeast: tetrad analysis, mitotic recombination, fine structure mapping. The isolation and characterization of both chromosomal and cytoplasmic mutants was undertaken. Biochemical studies were performed with chromosomal and mitochondrial mutants.

INSTRUCTORS

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

ASSISTANT

Fried, Howard M., Cornell University, Ithaca, New York

PARTICIPANTS

Blumenthal, Tom, Ph.D., Harvard Biological Laboratories, Cambridge, Massachusetts
Geisinger, Henry W., B.S., Harvard Medical School, Boston, Massachusetts
Goldthwaite, Claire D., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Gough, Simon P., Licentiatus Scientarium, Rockefeller University, New York City, New York
Greer, Helen A., B.A., Massachusetts Institute of Technology, Cambridge, Massachusetts
Groot, Gerardus S.P., Ph.D., University of Amsterdam, Holland
Jones, Irene M., Ph.D., Grinnell College, Grinnell, Iowa
Pahel, Gregory L., B.A., Massachusetts Institute of Technology, Cambridge, Massachusetts
Roberts, Jeffrey W., Ph.D., Harvard Biological Laboratories, Cambridge, Massachusetts
Roth, John R., Ph.D., University of California, Berkeley, California
Rothman-Denes, Lucia B., Ph.D., University of Chicago, Illinois
Sprague, Jr., George F., B.S., Yale University, New Haven, Connecticut
Sripakash, Kadaba S., Ph.D., Yale University, New Haven, Connecticut
Villadsen, Ingrid S., Ph.D., York University, Ontario, Canada
Warner, Jonathon R., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Wolf, Jr., Richard E., Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Leland H. Hartwell, University of Washington, *Genetic control of cell division in yeast.*
-- *Integration of the cell cycle with the life cycle.*
Calvin S. McLaughlin, University of California, Irvine, *Mutations and antibiotics that affect protein synthesis (I).*
-- *Mutations and antibiotics that affect protein synthesis (II).*
Jonathan R. Warner, Albert Einstein College of Medicine, *Ribosome biosynthesis in yeast.*
Julius Marmer, Albert Einstein College of Medicine, *DNA in yeast.*
Thomas Manney, Kansas State University, Manhattan, *Genetic control of mating in yeast.*
Herschel L. Roman, University of Washington, *Recombination studies in yeast.*
Gottfried Schatz, Cornell University, *Biogenesis of cytochrome oxidase in yeast.*
-- *Impaired mitochondrial assembly in yeast mutants.*
Robert K. Mortimer, University of California, Berkeley, *Genetic mapping in yeast.*
-- *Gene conversion.*
C.F. Robinow, University of Western Ontario, London, *Morphology of mitosis and meiosis in yeast.*
Gerald R. Fink, Cornell University, *The "Killer" phenomenon in yeast.*
-- *The regulation of histidine biosynthesis in yeast.*
Fred Sherman, University of Rochester, *Genetic control of cytochrome c in yeast.*
-- *Nonsense suppressors in yeast.*
Christopher Lawrence, University of Rochester, *Genetic control of UV mutagenesis in yeast.*
Rochelle S. Esposito, University of Chicago, *Physiological and genetic studies of sporulation-deficient mutants.*

3) THE BASIC PRINCIPLES OF NEUROBIOLOGY – June 11 - July 1

The aim of the program was to instill the basic principles of neurobiology in students previously ignorant of that field. During the first two weeks, lectures and discussions emphasized electrophysiology and neuroanatomy. The students were encouraged to develop a sound, intuitive understanding of electrophysiological principles, by a stress on the "equivalent circuit" approach; and of neuroanatomical principles by an emphasis on development. The visual system was used extensively as a model for the central nervous system in general. In the final week, guest lecturers presented seminars on research areas of great current interest. Concurrently, demonstrations were given which included synaptic transmission in rat neuromuscular junctions, the electrophysiology of the leech, the response of mud-puppy retinal ganglion cells to light, and the dissection of the human brain.

INSTRUCTORS

Kelly, James P., Ph.D., Harvard Medical School, Boston
Kelly, Regis B., Ph.D., University of California Medical Center, San Francisco, California
Sargent, Peter B., B.A., Harvard Medical School, Boston, Massachusetts
Van Essen, David, Ph.D., Harvard Medical School, Boston, Massachusetts

PARTICIPANTS

Akin, Ethan J., Ph.D., The City College of New York, New York, New York
Buas, Michael, M.S., University of Maryland, College Park, Maryland
Dewey, Michael J., B.S., University of Pennsylvania, Philadelphia, Pennsylvania
Dierker, Michael L., B.S., E.E., Washington University, St. Louis, Missouri
Fields, Kay L., Ph.D., University College, London, England
Gordon, Adrienne S., Ph.D., University of Konstanz, Germany
Howe, Herbert C., Ph.D., Cold Spring Harbor Laboratory, New York
Hucho, Ferdinand, Ph.D., University of Konstanz, Germany
Lin, Shin, Ph.D., University of California, San Francisco, California
Marty, Alain N., University of Paris, France
Pochobradsky, Jiri, M.Sc., The American University, Washington, D.C.
Proctor, William R., B.A., Oak Ridge National Laboratory, Oak Ridge, Tennessee
Ready, Donald F., B.A., California Institute of Technology, Pasadena, California
Seifert, Wilfried W., Ph.D., The Salk Institute for Biological Studies, San Diego, California
Siddiqi, Obaid, Ph.D., Tata Institute of Fundamental Research, Bombay, India
Simantov, Rabi, M.Sc., Weizmann Institute, Rehovot, Israel
Strickland, Sidney, Ph.D., University of Michigan, Ann Arbor, Michigan
Vitto, Anthony Jr., B.A., Oak Ridge National Laboratory, Oak Ridge, Tennessee
Webb, Watt W., Sc.D., Cornell University, Ithaca, New York
Weill, Cheryl L., B.S., University of California, San Francisco, California

SEMINARS

John Nicholls, Harvard Medical School, *Introductory lecture.*
-- *Ionic basis of resting and action potentials.*
-- *Experimental evidence for that basis.*
-- *Passive electrical properties of nerves.*
-- *Chemical and electrical synapses.*
-- *Synaptic permeability changes.*
-- *Quantal release of transmitter.*
-- *Current research on the leech.*
James Kelly, Harvard Medical School, *Synaptic anatomy.*
J.M. Ritchie, Yale University School of Medicine, *Ionic pumps.*
David Van Essen, Harvard Medical School, *Sensory transduction.*
Maxwell Cowan, Washington University Medical School, *Developmental neuroanatomy (I).*
-- *Developmental neuroanatomy (II).*
-- *Developmental neuroanatomy (III).*
-- *Developmental neuroanatomy (IV).*
-- *Developmental neuroanatomy (V).*
Motoy Kuno, University of North Carolina, Chapel Hill, *Motor system (I).*
-- *Motor system (II).*
Dennis Baylor, University of Colorado Medical School, *Retinal anatomy and physiology (I).*
-- *Retinal anatomy and physiology (II).*
David Hubel, Harvard Medical School, *Visual perception (I).*
-- *Visual perception (II).*
-- *Visual perception (III).*
-- *Visual perception (IV).*
Regis Kelly, University of California Medical Center, *Biochemistry of synaptic transmission (I).*
-- *Biochemistry of synaptic transmission (II).*
Cyrus Levinthal, Columbia University, *Wiring of simple nervous systems.*
Alan Finkelstein, Albert Einstein College of Medicine, *Artificial membranes.*
William Hagins, NIAMD, National Institutes of Health, *Visual receptors.*
Peter Schiller, Massachusetts Institute of Technology, *Oculomotor systems.*
Abe Flock, Woods Hole, Massachusetts, *Lateral line organ.*
Michael Dennis, University of California, San Francisco, *Development of neuromuscular junctions.*
Thomas Woolsey, Washington University School of Medicine, *Mouse somatosensory cortex.*
Jean Rosenthal, Yale University, *Regulation of ACh receptors.*
R. Rahaminoff, Harvard Medical School, *Facilitation at the neuromuscular junction.*

4) ANIMAL VIRUS — July 5 - July 25

This program consisted of lectures, discussions, and laboratory exercises including: preparation of primary cell cultures; growth of continuous tissue culture cell lines; growth of virus stocks and their titration by plaque assay and hemagglutination; purification and electron microscopic examination of virions; electrophoretic analysis of viral nucleic acids and proteins; determination of virus-specific macromolecules in infected cells by autoradiography and cell fractionation techniques; assay of virus-induced and virion-associated enzymes; effects of interferon and other inhibitors on virus replication; translation of viral RNA by mammalian cell-free extracts; and virus-mediated oncogenic transformation and cell fusion. In addition to the formal laboratory exercises, there were opportunities for carrying out individual research projects.

INSTRUCTORS

Kates, Joseph, Ph.D., State University of New York at Stony Brook, New York
Shatkin, Aaron J., Ph.D., Roche Institute for Molecular Biology, Nutley, New Jersey

ASSISTANTS

Lucas, Joseph, Ph.D., State University of New York at Stony Brook, New York
Radke, Kathryn L., B.S., University of Connecticut
Saborio, Jose, M.D., Roche Institute of Molecular Biology, Nutley, New Jersey

PARTICIPANTS

Benz, Wendy, M.D., The Johns Hopkins University, Baltimore, Maryland
Brega, Agnese, Ph.D., Laboratorio de Genetica Biochimica ed Evoluzionistica, Pavia, Italy
Celma, Maria L., Ph.D., Albert Einstein College of Medicine, Bronx, New York
d'Eustachio, Peter G., B.A., The Rockefeller University, New York City, New York
Dressler, David, Ph.D., Harvard University, Cambridge, Massachusetts
Gomez, Beatriz, Dr. Ver. Nat., Universidat Nacional Autonoma de Mexico, Mexico 20, D. F.
Jay, Gilbert W. H., B.Sc., Harvard University, Cambridge, Massachusetts
Karu, Alexander E., Ph.D., University of California, Berkeley, California
Kruppa, Joachim, Ph.D., New York University School of Medicine, New York City, New York
Linney, Elwood A., Ph.D., University of California at San Diego, La Jolla, California
Meiss, Harriet K., Ph.D., New York University School of Medicine, New York City, New York
Morrison, Trudy G., Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts
Nicholson, Audrey M., B.S., Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee
Parkhurst, James, Ph.D., University of Wisconsin, Madison, Wisconsin
Rizzino, Arnold A., M.S., State University of New York at Stony Brook, New York
Roeder, Robert G., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Scott, Walter A., Ph.D., University of California, San Francisco, California
Takano, Toshiya, M.D., National Institutes of Health, Bethesda, Maryland
Wiley, Don C., Ph.D., Harvard University, Cambridge, Massachusetts
Yang, Shen-Kwei, Ph.D., Yale University, New Haven, Connecticut

SEMINARS

E. Robbins, Albert Einstein College of Medicine, *Cell cycle.*
G. Fareed, Harvard University, *DNA synthesis.*
P. Lengyel, Yale University, *Interferon.*
A. Shatkin, Roche Institute of Molecular Biology, *Reovirus and other double-stranded RNA viruses.*
R. Compans, The Rockefeller University, *Lipid-containing RNA viruses.*
J. Sambrook, Cold Spring Harbor Laboratory, *SV40 transcription.*
B. Roizman, University of Chicago, *Herpes viruses.*
P. Sharp, Cold Spring Harbor Laboratory, *Ad-SV40 hybrid viruses.*
J. Maizel, Albert Einstein College of Medicine, *Enteroviruses.*
H. Lodish, Massachusetts Institute of Technology, *Protein synthesis.*
A. Huang, Harvard University, *Rhabdoviruses.*
D. Baltimore, Massachusetts Institute of Technology, *RNA tumor viruses.*
J. Kates, State University of New York at Stony Brook, *Pox viruses.*
T. Grodzicker, Cold Spring Harbor Laboratory, *Isolating animal virus mutants.*
J. Lucas, State University of New York at Stony Brook, *Adenoviruses.*
A. Granoff, St. Jude's Children's Research Hospital, Memphis, Tennessee, *Frogs, viruses and cancer.*
-- *Ts mutants of FV_3 virus defective in encapsulation.*
R. Risser, Cold Spring Harbor Laboratory, *T antigen in virus-transformed cells.*
R. Pollack, Cold Spring Harbor Laboratory, *Cell hybrids.*
R. Roeder, Washington University, *Mammalian RNA polymerases.*
T. Benjamin, Harvard University, *DNA tumor viruses.*
M. Botchan, Cold Spring Harbor Laboratory, *Integration of viral DNA in SV40-transformed cells.*
B. Ozanne, Cold Spring Harbor Laboratory, *Cell surfaces of normal and transformed cells.*
S. Penman, Massachusetts Institute of Technology, *RNA synthesis.*
A. Vogel, Cold Spring Harbor Laboratory, *Properties of revertants of transformed cells.*
F. Kelly, Cold Spring Harbor Laboratory, *Karyotype analysis.*

5) BACTERIAL GENETICS — July 5 - July 25

The program included lectures and experiments covering the elements of bacterial genetics. Mutants, revertants, suppressor mutations were analyzed genetically by conjugation, transduction, and growth tests. A specialized transducing phage (*Ø80dlac*) was used in diploid studies on the *lac* operon. In addition, students participated in a class project to estimate the number of essential genes coded by the *Escherichia coli* chromosome. Finally, each student undertook a research project applying the techniques learned in the introductory part of the course.

INSTRUCTORS

Gross, Julian D., Ph.D., University of Edinburgh, Scotland
Scaife, John G., Ph.D., University of Edinburgh, Scotland
Taylor, Austin L., Ph.D., University of Colorado Medical Center, Denver, Colorado

ASSISTANTS

Finnegan, David, Ph.D., Oxford University, England
Marble, Mary Gene, B.A., University of Colorado Medical Center, Denver, Colorado

PARTICIPANTS

Archer, Luis J., Ph.D., Gulbenkian Institute of Science, Oeiras, Portugal
Baulieu, Laurent, Maitrise de Physique, Ecole Normale Superieur de Saint-Cloud, France
Chambers, Robert W., Ph.D., New York University Medical Center, New York City, New York
Culbertson, Michael R., Ph.D., Albert Einstein College of Medicine, Bronx, New York
De Robertis, Edward M., M.D., Instituto Investigaciones Bioquimicas - Fundacion Campomar, Buenos Aires, Argentina
Flessel, C. Peter, Ph.D. , University of San Francisco, California
Hermann, Monique, D.Sc., University of Illinois, Urbana, Illinois
Johnson, Paul H., Ph.D., California Institute of Technology, Pasadena, California
Malathi, Vazhappilli, Ph.D., New York University Medical Center, New York City
Mangel, Walter F., Ph.D., University of California, Berkeley, California
Masker, Warren E., Ph.D., Stanford University, Palo Alto, California
Miller, Terry L., M.S., University of Illinois, Urbana, Illinois
Pühler, Alfred, Ph.D., Institut für Mikrobiologie, Universität Erlangen, Germany
Rakow, Brenda J., M.S., Upjohn Company, Kalamazoo, Michigan
Reuben, Roberta C., B.A., Columbia University, New York City, New York
Schwartz, Joseph A., Ph.D., Richmond College, City University of New York, Staten Island, New York
Setoguchi, Yoshiko, Ph.D., Hunter College, New York City, New York
Shih, Jason C. H., Ph.D., Cornell University, Ithaca, New York
Stern, Linda K., B.A., Albert Einstein College of Medicine, Bronx, New York
Tal, Moshe, Ph.D., Technicon-Israel Institute of Technology, Haifa, Israel

SEMINARS

E.P. Kennedy, Harvard Medical School, *Biosynthesis and function of membrane lipids.*
F.W. Studier, Brookhaven National Laboratory, *Genetics and physiology of bacteriophage T7.*
R. Losick, Harvard University, *RNA polymerase and sporulation.*
D. Kaiser, University of Minnesota, *Bacterial mutants unable to propagate phage lambda.*
Reed Wickner, National Institutes of Health, *In vitro DNA synthesis.*
R.C. Deonier, California Institute of Technology, *Mapping of ribosomal RNA genes on defined segments of the* E. coli *chromosome by electron microscope heteroduplex methods.*
Ellis Englesberg, University of California, Santa Barbara, *Further studies on the mechanism of positive control in the L-arabinose operon.*
Klaus Weber, Cold Spring Harbor Laboratory, *Reinitiation in the* lac i *gene.*
J.R. Beckworth, Harvard Medical School, *Interaction of cyclic nucleotides and RNA polymerase at the* lac *promoter site.*
N. Willetts, University of Edinburgh, *Studies on the F factor of* Escherichia coli *K12.*
Jeremy Thorner, Stanford University, *Recent studies on mutants of* E. coli *DNA polymerase I and T4 DNA polymerase.*
M. Levine, University of Michigan, *Lysogeny with phage P22: a dual control system.*

6) EXPERIMENTAL TECHNIQUES IN NEUROBIOLOGY — July 5 - July 25

The program focused on the marine snail *Aplysia californica,* whose nervous system consists of unusually large cells ranging from 50μm to almost 1 mm in diameter, although some experiments were also done on the land snail, *Helix aspersa.* As a result of the large size of its cells, technical problems involved in intracellular electrical recordings were considerably reduced. Equally facilitated were the problems involved in dissection of individual neurons for biochemical and morphological studies.

Emphasis was placed on the following experimental techniques: intracellular recording of controlled membrane potentials, intracellular injection of ions, ionophoretic application of drugs, physiological and electrical stimulation of selected activities. These techniques were used in explaining the following problems of cellular neurophysiology: resting potential, action potential, the sodium potassium pump, and ionic and pharmacological bases of synaptic transmission.

INSTRUCTORS

Ascher, Phillippe, D.Sc., Ecole Normale Superieure, Paris, France
Kehoe-Ascher, JacSue, Ph.D., Ecole Normale Superieure, Paris, France
Thomas, Roger C., Ph.D., Bristol University, Bristol, England

ASSISTANT

Leon, Vivienne, A. B., The Rockefeller University, New York City, New York

PARTICIPANTS

Baylor, Stephen M., M.D., Yale University School of Medicine, New Haven, Connecticut
Dewey, Michael J., B.S., University of Pennsylvania, Philadelphia, Pennsylvania
Honegger, Hans-Willi, Ph.D., Zoologisches Institut, Koln, Germany
Howe, Herbert, Ph.D., Cold Spring Harbor Laboratory, New York
Kebabian, John W., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Nipomnick, Elliot S., B.A., Stanford University, Palo Alto, California
Rao, Anjana, M.S., Harvard University, Cambridge, Massachusetts
Ready, Donald F., B.A., California Institute of Technology, Pasadena, California
Siddiqi, Obaid, Ph.D., Tata Institute of Fundamental Research, Bombay, India
Simpson, Paul, M.D., National Institutes of Health, Bethesda, Maryland
Stanley, Jeff, A.B., Harvard College, Cambridge, Massachusetts
Vogel, Zvi, Ph.D., National Institutes of Health, Bethesda, Maryland

SEMINARS

M. Fourtes, National Institutes of Health, *Photoreceptors.*
L. Cohen, Marine Biological Laboratory, Woods Hole, Massachusetts.
S. Kater, Iowa University, *Behavioral neurobiology of a pond snail.*

7) TUMOR VIRUS WORKSHOP — July 30 - August 12

The program was concerned with the various aspects of viruses known to produce tumors in animals or transformation in culture. Emphasis was placed upon 1) viral replication, 2) integration of the viral genome into the cellular DNA, 3) the genetic and biochemical evidence of functions needed for replication and/or cellular transformation, 4) the similarities and differences between RNA and DNA tumor viruses, and 5) structure of the viral genomes.

The workshop was divided into lectures, discussion groups, student presentations, a tour of the laboratory facilities at Cold Spring Harbor, and some of the participants worked in the laboratory.

INSTRUCTORS

Levine, Arnold, Ph.D., Princeton University, New Jersey
Todaro, George, Ph.D., National Institutes of Health, Bethesda, Maryland
Wyke, John, Ph.D., Imperial Cancer Research Fund, London, England

PARTICIPANTS

Allaudeen, H. S., Ph.D., Yale University, New Haven, Connecticut
Bilello, John A., M.A., Albert Einstein College of Medicine, Bronx, New York
Birg, Francoise, M.Sc., Unité de Recherche, Marseille, France
Bornkamm, Georg, M.D., Institut für Klinische Virologie, Erlangen, Germany
Carter, Timothy H., Ph.D., University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania
Eisinger, Magdalena G., D.V.M., Sloan-Kettering Institute for Cancer Research, New York City, New York
Flint, Sarah Jane, B.Sc., University College, London, England
Forchhammer, Jes, M.D., Ph.D., University Institute of Microbiology, Copenhagen, Denmark
Graessmann, Adolf, Dr. habil., University of Sao Paulo, Sao Paulo, Brazil
Hough, Paul V. C., Ph.D., Brookhaven National Laboratory, Upton, New York
James, Eric, Ph.D., University of South Carolina, Columbia, South Carolina
Kamely, Daphne, B.S., Harvard University, Cambridge, Massachusetts
Metz, David H., Ph.D., Children's Hospital Medical Center, Boston, Massachusetts
Moelling, Karin, Ph.D., Robert Koch-Institut, Berlin, West Germany
Moyer, Rex C., Ph.D., Trinity University, San Antonio, Texas
Rapp, Ulf R., M.D., University of Wisconsin, Madison, Wisconsin
Rozenblatt, Shmuel, M.Sc., Weizmann Institute of Science, Rehovot, Israel
Schafer, Thomas W., Ph.D., Schering Corporation, Bloomfield, New Jersey
Wasi, Safia, Ph.D., University of Toronto, Ontario, Canada
Wolf, Hans, M.A., Institut für Klinische Virologie, Erlangen, Germany

Auditor:
Daniell, Ellen, A.B., University of California at San Diego, La Jolla, California

SEMINARS

G. Todaro, National Institutes of Health, *Role of viruses in cell transformation.*
-- *Endogenous cat viruses.*
M. Baluda, University of California, Los Angeles Medical School, *Nature and replication of RNA tumor viruses.*
L. Gross, Bronx Veterans Hospital, *Mouse leukemia viruses.*
R. Gallo, University of Michigan Medical School, *Leukemia viruses and leukemia.*
J. Wyke, Imperial Cancer Research Fund, London, *RNA virus genetics.*
A.J. Levine, Princeton University, *Polyoma and SV40 DNA replication.*
P. Tegtmeyer, Case Western Reserve University, *SV40 and polyoma virus genetics.*
U. Pettersson, Cold Spring Harbor Laboratory, *Adenoviruses.*
A.S. Levine, National Cancer Institute, *An introduction to adeno/SV40 hybrids.*
C. Anderson and R. Gestland, Cold Spring Harbor Laboratory, *Protein synthesis in adeno and SV40 infected cells.*
P. Sharp, Cold Spring Harbor Laboratory, *The use of restriction enzymes in mapping DNA viruses.*
B. Roizman, University of Chicago, *Herpes viruses.*
M. Martin, National Institute of Allergy, Infectious Diseases, *Virus and cellular nucleic acid hybridization.*
W. Folk, University of Michigan Medical School, *Virus rescue.*
M. Fried, Imperial Cancer Research Fund, *Defective polyoma DNA.*
J. Sambrook, Cold Spring Harbor Laboratory, *Transcription of tumor viruses DNAs.*
E. Scolnick, National Cancer Institute, *Mammalian sarcoma viruses.*
J.M. Bishop, University of California, San Francisco, *Mammary tumor viruses: modes of replication.*
-- *Synthesis and integration of the provirus of RNA tumor viruses.*
W. Hardy, Sloan-Kettering Institute for Cancer Research, *Cat leukemia: infectious disease?*
D. Rifkin, Rockefeller University, *Role of surface proteases in transformation.*
B. Ozanne, Cold Spring Harbor Laboratory, *The cell surface and transformation.*
R. Pollack, Cold Spring Harbor Laboratory, *The phenotype of transformed cells.*
A. Hellman, National Cancer Institute, *Biohazards in tumor virus research.*

8) MOLECULAR CYTOGENETICS — July 30 - August 19

The cytogenetics program consisted of lectures, discussions and lab exercises on eukaryotic chromosomes. Experimental approaches combined biochemical and cytological analyses.

INSTRUCTORS

Pardue, Mary Lou, Massachusetts Institute of Technology, Cambridge, Massachusetts
Walker, Peter M. B., Edinburgh University, Scotland

ASSISTANT

Longer, Pamela, Massachusetts Institute of Technology, Cambridge, Massachusetts

PARTICIPANTS

Dippell, Ruth V., Ph.D., Indiana University, Bloomington, Indiana
Folk, William R., Ph.D., University of Michigan, Ann Arbor, Michigan
Freedman, Victoria H., B.A., Albert Einstein College of Medicine, Bronx, New York
Holland, Christie A., B.A., Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee
Hourcade, Dennis H., B.S., Harvard University, Cambridge, Massachusetts
Kelly, Francoise, M.D. & Ph.D., Cold Spring Harbor Laboratory, New York
Lehrach, Hans, Dipl. Chem., Max-Planck Institut f. Biophys. Chemie, Gottingen, Germany
Manduca de Ritis, Paola, Laurea, Albert Einstein College of Medicine, Bronx, New York
McKay, Ronald D. G., B.S., University of Edinburgh, Scotland
McKenna, Gillies, B.S., Cold Spring Harbor Laboratory, New York
Skinner, Dorothy M., Ph.D., Oak Ridge National Laboratory, Oak Ridge, Tennessee
Tomizawa, Jun-ichi, Ph.D., National Institutes of Health, Bethesda, Maryland
Wiche, Gerhard, Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey
Yao, Meng-Chao, B.S., University of Rochester, New York

SEMINARS

Burke Judd, University of Texas, *An examination of the one cistron:one chromomere concept.*
Aimee Bakken, Yale University, *Visualization of transcription in the eukaryotic genome.*
Peter M.B. Walker, Edinburgh University, Scotland, *Related sequences and multiple genes.*

Mary Lou Pardue, Massachusetts Institute of Technology, *Repeated sequences in eukaryotic chromosomes.*
Hewson Swift, University of Chicago, *Nucleic acids in agal chloroplasts and leucoplasts.*
Orlando J. Miller, Columbia College of Medicine, *Mechanisms and applications on mammalian chromosome banding.*

9) NEUROBIOLOGY OF DROSOPHILA MELANOGASTER — July 30 - August 19

The program was designed to introduce workers who had no previous experience in this area to the techniques involved in studying the neurobiology of *Drosophila melanogaster.*

Discussions centered around the genetics, anatomy, physiology, and behavior of the recently isolated neurological mutants. The laboratory work included the methods of handling the fruit fly, methods of mutation and selective screening for appropriate mutants, and introduction to the basic electrophysiological recording techniques to study some of the mutants.

INSTRUCTORS

Deland, Michael, Ph.D., Purdue University, Lafayette, Indiana
Heisenberg, Martin, Ph.D., Max-Planck Institute for Biology, Tubingen, Germany
Pak, W. L., Ph.D., Purdue University, Lafayette, Indiana

ASSISTANTS

Healy, Christine, Purdue University, Lafayette, Indiana
Wu, Chun-Fang, Purdue University, Lafayette, Indiana

PARTICIPANTS

Arsenault, Molly L., B.A., Colorado University, Boulder, Colorado
Chang, Mei-Ven M., B.S., Purdue University, Lafayette, Indiana
Cline, Thomas W., A.M., Harvard University, Cambridge, Massachusetts
Homyk, Theodore Jr., B.S., Vanderbilt University, Nashville, Tennessee
Koenig, Jane, M.S., University of California at Los Angeles, California
Kyrala, Judith W., M.Sc.Phys., Arizona State University, Tempe, Arizona
McMilin, Kenneth D., B.A., University of Oregon, Eugene, Oregon
Udin, Susan R., B.S., Massachusetts Institute of Technology, Cambridge, Massachusetts
Warner, Cynthia K., M.S., University of Tennessee, Oak Ridge, Tennessee

SEMINARS

William D. Kaplan, City of Hope National Medical Center, *Genetics and behavior of neurological mutants of* Drosophila.
Kazuo Ikeda, City of Hope National Medical Center, *Study of genetically coded neural activities.*
Thomas Hanson, California Institute of Technology, *Neurogenesis of the* Drosophila *eye.*
Douglas Kankel, California Institute of Technology, *Genetic mosaics and behavior mutants.*
Jeffrey Hall, California Institute of Technology, *Neurobiology of* Drosophila *courtship.*
David Suzuki, University of British Columbia, *Studies on temperature-sensitive locomotor mutants of* Drosophila melanogaster.
Chip Quinn, California Institute of Technology, *Learning in higher organisms.*
-- *Conditioned behavior in* Drosophila.

10) PHYCOMYCES WORKSHOP — July 6 - August 31

The *Phycomyces* Workshop was held during July and August in the new basement lab of Davenport. A large group participated in the daily meetings, practical lessons in the production of heterokaryons, and research projects. The highpoint of the summer was the first *Phycomyces* meeting which attracted 29 participants. During an intense week a large amount of new work on a wide variety of subjects was presented in the formal sessions amid much informal discussion on future work.

Pat Burke coordinated the whole group in a search for auxotrophic mutants. This work is very laborious since despite treatment with nitrosoguanadine the yield is low, one mutant in 10^3 survivors. Nonetheless, five new mutants were found and characterized as to their specific requirement. *Kostia Bergman* observed that germlings can be separated from ungerminated spores because they settle out of suspension at a much greater rate. He found

that the proportion of a nicotinic acid requiring mutant in a mixture with a white phototrophic strain could be increased four times by growing the spores overnight in minimal medium, allowing them to settle for one hour, and then testing the supernatant. Repeated cycles of this technique should increase the enrichment obtained. *Arturo Eslava* used nitrosoguanadine to find revertants of three auxotrophs requiring different supplements. All three were revertable by nitrosoguanadine at a frequency of 1 in $5X10^{-4}$ survivors. This work will be continued to find optimal conditions for forward and back mutation using nitrosoguanadine and other mutagenic agents. The analysis of revertants would be useful to prove that concomitant biochemical and phototropic defects are due to a single point mutation but their frequency must be increased by an order of magnitude before we can hope to find revertants to normal phototropism.

Tamotsu Ootaki and *Peter Fischer* continued complementation studies on mutants with abnormal phototropism. So far, four complementation groups have been identified and definite proof is now available that the 15 mutants studied are all recessive. The results are compatible with a previous classification scheme based on phenotypic studies and their striking simplicity suggests that only a small number of gene products are involved in phototropism.

Max Delbruck and *Enzo Russo* used Giemsa staining to study the proliferation of nuclei during germination of spores. The number of nuclei began to increase at 90-120 minutes and then proceeded to double every 2 to 2.5 hours. This doubling time had been predicted from chemical measurements of the amount of DNA present at 18 hours made by Ruth Dusenberry at Caltech. In the 90 minutes following a heat shock the nuclei swell and show at least 5 thread-like structures which may be chromosomes (similar structures were seen with Feulgen staining).

Since indoleacetic acid, a molecule clearly implicated in the growth and phototropism of higher plants, is present in *Phycomyces*, numerous workers have tried to demonstrate a physiological role. *Sielke Sievers*, who previously demonstrated specific indoleacetic acid binding by *Phycomyces* extracts, was unable to find such a function but is determined to keep trying.

Margarete Petzuch used the excellent electrophysiological equipment available in McClintock Lab to once again search for electrical signals involved in light detection by the sporangiophore. She proved that the resistance of the cell wall is too great to provide a route for the type of current flow seen in other photoreceptor cells but could not rule out the possibility that in small regions such a path exists between the membrane and the cell wall.

The complex regulatory interactions between β-carotene synthesis and sexual differentiation were the subject of much discussion and experimentation this summer. *Dick Sutter* presented clear evidence that trisporic acid (TA), which induces the start of sexual differentiation in both mating types, is present in appreciable quantity only when the two mating types can synthesize it in tandem. That is, each mating type makes, presumably from β-carotene, a precursor which it is unable to convert to TA, but which the other mating type can convert to TA. Thus, little or no TA is made and no sexual differentiation occurs unless both mating types are grown together. Many *car* mutants, mutants altered in the amount or type of carotenes synthesized, do not complete the normal process of sexual differentiation when mated with a wild type of the opposite sex. Since the two mating types are morphologically identical, it has been difficult to observe which of the two strains is not performing. *Delbruck* and *Sutter* attempted to resolve this problem by observing matings that took place across a dialysis membrane which the mycelia cannot penetrate. Technical problems concerning impermeability of the membrane to TA and its precursors were encountered but should be surmountable. *Joanne Whitaker* continued work on her observation that the amount of β-carotene synthesized per gram is increased by growing two mating types together. This stimulation may take place through the influence of TA or its precursors. During the past year *Eslava* made the surprising observation that all *carA* mutants can be induced to form β-carotene by the addition of vitamin A or β-ionone, molecules which share structural features with TA, to the medium. He has also shown that the inducer is not incorporated into β-carotene, that the synthesis occurs by the normal route, and that the effect occurs in wild type as well. This summer he showed that protein synthesis is involved since *carA* strains do not become yellow if the inducer is added after treatment with 50 μg/ml of cyclohexamide.

Since the Cold Spring Harbor *Phycomyces* Workshop of 1967 we have known that C5, an albino mutant, shows a dramatic increase in geotropic bending speed. *Burke* and *Fischer* tested strains now known by complementation tests to be altered in the function of the same *carB* gene as C5. Most of these strains showed a similar change in geotropism. This discovery should be further tested and confirmed by analysis of revertants to normal *carB* function since it may be the first clear clue to the mechanistic basis of geotropism.

COORDINATOR

Bergman, Kostia, Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts

ASSISTANTS

Alvarez, Maribel, M.S., California Institute of Technology, Pasadena, California
Mitchell, Bonnie, B.S., Washington University Medical School, St. Louis, Missouri
Parker, Vann, Duke University, Durham, North Carolina

WORKSHOP MEMBERS

Burke, Patricia, Ph.D., University of California, Santa Cruz, California
Delbruck, Max, Ph.D., California Institute of Technology, Pasadena, California
Eslava, Arturo P., Ph.D., Universidad de Sevilla and California Institute of Technology, Pasadena, California
Fischer, Peter, M.S., California Institute of Technology, Pasadena, California
Hubmann, Franz-Herbert, M.D., Ph.D., California Institute of Technology, Pasadena, California
Jacobs, Terry, Ph.D., Research Division, IBM, San Jose, California
Jan, Y. N., M.S., California Institute of Technology, Pasadena, California
Malhotra, Sudarshan, Ph.D., University of Alberta, Edmonton, Canada
Ootaki, Tamotsu, Ph.D., Yamagata University, Japan
Ortega, Ken, M.S., University of Colorado, Boulder, Colorado
Petzuch, Margarete, Ph.D., University of Konstanz, Germany
Russo, Vincenzo, Ph.D., Max-Planck-Institut, Berlin, Germany
Sievers, Sielke, M.S., Max-Planck-Institut, Berlin, Germany
Sutter, Richard, Ph.D., West Virginia University, Morgantown, West Virginia
Whitaker, Joanne, B.S., West Virginia University, Morgantown, West Virginia

SEMINARS

Klaus Thompson, Universitat Freiberg, Germany, *Auxin binding by membranes of corn coleoptiles.*
Gerhard Meissner, Vanderbilt University, *Reconstitution of the sarcoplasmic reticulum.*
Martin Heisenberg, Max-Plank Institut fur Biologische Kybernetic, Tubingen, *System analysis of* Drosophila *behavioral mutants.*
William Pak, Purdue University, *A* Drosophila *mutant with no receptor potential.*
Peter Ray, Stanford University, *The synthesis and transport of plant cell wall polysaccharides.*
Karl Kaissling, Max-Planck Institut fur Verhaltensphysiologis, *How* Bombyx mori *smells its mate.*
Walter Keller, Cold Spring Harbor Laboratory, *Eukaryotic RNA polymerases.*

PHYCOMYCES MEETING — August 6 - August 11

Tuesday, August 7

M. Delbruck, California Institute of Technology, Pasadena, *General Introduction.*
Y.N. Jan, California Institute of Technology, Pasadena, *Chitin synthetase: its properties, location and control.*
T.J. Leighton, University of Massachusetts Medical School, *Chitin synthetase: control by effectors.*
R.J. Cohen, University of Florida Medical School, Gainesville, *Cyclic AMP levels during a response to light.*
W.A. Scott, The Rockefeller University, New York, *Cyclic AMP and* Neurospora *morphology.*

Wednesday, August 8

E.D. Lipson, California Institute of Technology, Pasadena, *System analysis of the light growth response using automatic tracking and white noise programs.*
D.S. Dennison, Dartmouth College, Hanover, N.H., *The phototropic merry-go-round.*
K. Bergman, University of Massachusetts Medical School, Worcester, *Mycelial photo-responses and the phenotypic classification of* mad *mutants.*
T. Ootaki, Yamagata University, Japan, *How to make heterokaryons and complementation of* mad *mutants.*
W. Shropshire, Jr., Smithsonian Institution, Washington, D.C., *Phytochrome from rye seedlings.*



Thursday, August 9

Y.N. Jan, California Institute of Technology, Pasadena, *The avoidance response.*
R.J. Cohen, University of Florida Medical School, Gainsville, *Volatile substances produced by* Phycomyces.
E.K. Ortega, University of Colorado, Boulder, *The analysis of spiral growth using the fungus bowl.*
V.E.A. Russo, Max-Planck-Institut, Berlin, *Approaches to the chemical analysis of the growth response.*

Friday, August 10

L. Jan, California Institute of Technology, Pasadena, *Rhodopsin-like protein from* Halobacterium halobium.
S. Godfrey, University of Pennsylvania Medical School, Philadelphia, *Synchronization of sporangium formation in plucked sporangiophores.*
M. Delbruck for R. Dusenberry, California Institute of Technology, Pasadena, *Phyco DNA: GC ratio, complexity, repetitious fraction.*
S.K. Malhotra, University of Alberta, Edmonton, Canada, *Electron microscopy of* Phycomyces *spores.*
M. Delbruck, California Institute of Technology, Pasadena, *Sexual genetics.*
R.P. Sutter, West Virginia University, Morgantown, *Chemistry and physiology of sexuality in the mucorales.*

Saturday, August 11

A.P. Eslava, Universidad de Sevilla, Spain, *The control of β-carotene synthesis in* Phycomyces.
H.C. Rilling, University of Utah Medical Center, Salt Lake City, In vitro *studies of the early steps in carotene synthesis.*
R. Harding, Smithsonian Institution, Washington, D.C., *Temperature dependence of the photoinduction of carotene synthesis in* Neurospora.

COURSE ASSISTANTS

DAVENPORT LABORATORY
V. Parker
B. Root

JAMES LABORATORY
E. Jamieson
J. Kohm
D. Tullier
F. Walker

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

The chromosomes of higher cells have long held a central role in the imagination of our better biologists. We know them however only at a semi-molecular level that generates more frustration than satisfaction. Even the appearance of better and better electron microscopes has not dispelled much of the fog that still envelopes long familiar terms like the centromere or chromatin. The EM works best when highly regular structures are involved, a situation that unfortunately does not hold for highly convoluted chromosomes.

Now, however, there are very good reasons for believing that the essential structural features of chromosomes may be resolved over the next decade. Ingenious applications of the electron microscope, as well as the development of a new armada of powerful techniques for molecular biology, now create the sense of excitement that characterizes regions of science which have begun to move rapidly toward a complete solution of their key problems.

So the moment seemed propitious to bring together many of the major figures in the chromosome world. Hopefully, the new facts that they would impart to each other would lead to the new ideas that will transform speculative hypotheses into solid facts. In the task of assembling this Symposium, I have been aided by advice from John Cairns, Joseph Gall, Charles Laird, Matthew Meselson, Mary Lou Pardue, and Charles Thomas. Unfortunately, all the names they suggested could not be accommodated on the final program which quickly grew to a length where new additions could only be made with the knowledge that the audience would most certainly lose its capacity for still another fact.

This meeting opened the evening of May 31 continuing on to noon of June 6. Over 240 people were in attendance, including the largest group ever of European scientists seen at Cold Spring Harbor. That we could accommodate so many from abroad results from the strong financial support provided by the National Institutes of Health, The National Science Foundation, and the United States Atomic Energy Commission.

J.D. Watson

THURSDAY, May 31

Opening Remarks: David S. Hogness, Stanford University Medical School, Palo Alto, California

FRIDAY MORNING, June 1

Chairman: D. Von Wettstein, University of Copenhagen, Denmark

W.L. Fangman, T.D. Petes, C.S. Newlon and B.E. Byers, Department of Genetics, University of Washington, Seattle: "Yeast Chromosomal DNA: Size, Structure and Replication."

J. Marmur, D. Cryer, C. Goldthwaite and J. Blamire, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York: "Studies on Nuclear and Mitochondrial DNA of *Saccharomyces cerevisiae*."

B. Byers and L. Goetsch, Department of Genetics, University of Washington, Seattle: "Spindle Plaque Duplication and Integration of the Yeast Cell Cycle."

R. Kavenoff and B.H. Zimm, Chemistry Department, University of California at San Diego, La Jolla: "On the Nature of 'Chromosome-Sized' DNA Molecules."

D.E. Comings and T.A. Okada, Department of Medical Genetics, City of Hope National Medical Center, Duarte, California: "Some Aspects of Chromosome Structure in Eukaryotes."

G.H. Jones, Department of Genetics, University of Birmingham, Birmingham, England: "Modified Synaptonemal Complexes in Spermatocytes of *Stethophyma Grossum*.

P.B. Moens, Department of Biology, York University, Downsview, Toronto, Canada: "Fine Structure Characteristics of Meiotic Prophase Chromosomes."

FRIDAY EVENING, June 1

Chairman: John Cairns, Imperial Cancer Research Fund, London, England.

D.E. Pettijohn, E. Miles and R. Hecht, Department of Biophysics and Genetics, University of Colorado Medical Center. Denver: "RNA Molecules Bound to the Folded Bacterial Genome Stabilize DNA Folds and Segregate Regions of Supercoiling."

A. Worcel, E. Burgi, L. Carlson and J. Robinton, Department of Biochemical Sciences, Princeton University, Princeton, New Jersey: "Properties of a Membrane Attached Form of the Folded Chromosome of *E. coli*."

H. Delius and A. Worcel, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York and Department of Biochemical Sciences, Princeton University, Princeton, New Jersey: "Electron Microscopic Visualization of the Folded Chromosome of *E. coli*."

L.S. Lerman, Vanderbilt University: "The Condensation of DNA *in vitro* and its Relation to the Structure of Chromosomes."

J.F. Pardon, B.M. Richards and R.I. Cotter: "The Role of Histones in Chromosome Structure."

E.J. DuPraw, Stanford University School of Medicine, Palo Alto, California: "Quantitative Constraints in the Arrangement of Human DNA."

P.M. Kraemer, L.L. Deaven, H.H. Crissman, J.A. Steinkamp and D.F. Petersen, Cellular and Molecular Radiobiology Group, Los Alamos Scientific Laboratory, Los Alamos, New Mexico: "On the Nature of Heteroploidy."

SATURDAY MORNING, June 2

Chairman: A.D. Hershey, Carnegie Institution of Washington, Cold Spring Harbor, New York

N.P. Salzman, National Institutes of Health, Bethesda, Maryland: "Replication of SV40 DNA."

D.R. Wolstenholme, K. Koike and P. Cochran-Fouts, Department of Biology, University of Utah, Salt Lake City: "Replication Forms of Mitochondrial DNA from Rat Tissues and Evidence for Discontinuous Replication."

L.I. Grossman*, H. Kasamatsu*, L. Matsumoto†, L. Piko*†, D.L. Robberson‡, R. Watson*, and J. Vinograd*, *Div. Chemistry and Biology, CalTech, Pasadena; †Dev. Biol. Lab., VA Hospital, Sepulveda, Calif.; ‡Imperial Cancer Research Fund, London: "The Replication and the Structure of Mitochondrial DNA."

H.G. Callan, Zoology Department, The University, St. Andrews, Scotland: "DNA Replication in the Chromosomes of Eukaryotes."

A.B. Blumenthal, H.J. Kriegstein and D.S. Hogness, Department of Biochemistry, Stanford University School of Medicine, Stanford, California: "The Units of DNA Replication in Drosophila Chromosomes."

J.H. Taylor, Institute of Molecular Biophysics, Florida State University, Tallahassee: "Functional Subunits of Chromosomal DNA from Higher Eukaryotes."

J.A. Huberman, H. Horwitz, R. Minkoff and M.A. Waqar, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Discontinuous Replication of Eukaryotic DNA."

I. Balazs, E.H. Brown, R.J. Parker and C.L. Schildkraut, Division of Biology (Cell Biology), Albert Einstein College of Medicine, Bronx, New York: "DNA Replication in Synchronized Cultured Mammalian Cells."

H. Weintraub, MRC Laboratory of Molecular Biology, Cambridge, England: "The Assembly of Newly Replicated DNA into Chromatin."

SATURDAY AFTERNOON, June 2

Chairman: Herman W. Lewis, National Science Foundation, Washington, D.C.

B.H. Judd and M.W. Young, Department of Zoology, University of Texas at Austin: "An Examination of the One Cistron:One Chromomere Concept."

B. Hochman, Department of Zoology, University of Tennessee, Knoxville: "Analysis of a Whole Chromosome in Drosophila."

G. LeFevre, Jr., Department of Biology, California State University, Northridge: "A Cytogenetic Analysis of Irradiated X Chromosomes of *Drosophila Melanogaster*."

V. Sorsa, Department of Genetics, University Helsinki, Finland: "Organization of Chromomeres."

D.M. Prescott and K.G. Murti, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: "Chromosome Structure and the Genetic Apparatus of *Stylonychia*."

G.M. Hewitt, School of Biological Sciences, University of East Anglia, Norwich, England: "The Integration of Supernumerary Chromosome Material into the Orthopteran Genome."

H. Rees and J. Hutchinson, Department of Agricultural Botany, University College of Wales, Great Britian: "Nuclear DNA Variation Due to B Chromosomes."

SUNDAY MORNING, June 3

Chairman: Matthew Meselson, Harvard University, Cambridge, Massachusetts

E.H. Davidson and R.J. Britten, Division of Biology, California Institute of Technology, Pasadena: "Sequence Organization of Eukaryotic DNA."

J. Bonner, W. Garrard, J. Gottesfeld, D. Holmes, J.S. Sevall and M. Wilkes, Division of Biology, California Institute of Technology, Pasadena: "Structure of the Eukaryote Chromosome."

C.D. Laird, W.Y. Chooi, E. Cohen, E. Dickson, N. Hutchinson, M. Lamb, D. Nash, S. Olson and S. Turner, Department of Zoology, University of Washington, Seattle: "Nucleotide Sequences in Drosophila Chromosomes."

J.E. Hearst, Department of Chemistry, University of California, Berkeley: "The Chromosome of *Drosophila Melanogaster*."

C.A. Thomas, R.E. Pyeritz, D.A. Wilson, B.M. Dancis, C.S. Lee, M.D. Bick, H.L. Huang and B.H. Zimm, Department of Biological Chemistry, Harvard Medical School, Cambridge, Massachusetts: "Cyclodromes and Palindromes in Chromosomes."

F.H. Schachat and D.S. Hogness, Department of Biochemistry, Stanford University School of Medicine, Stanford, California: "The Relationship between Isolated Thomas Circles and Satellite DNAs in Drosophila Chromosomes."

M. Botchan and G. McKenna, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "The Distribution of Tandemly Repeated DNA in the Mouse Genome as Determined by the Use of Restriction Endonucleases."

R. Appels, D. Brutlag, E. Goldring and J. Peacock, Division of Plant Industry, Commonwealth Scientific & Industrial Research Organization, Canberra, Australia: "Organization of Repeated DNA in Drosophila Chromosomes."

SUNDAY EVENING, June 3

Chairman: Peter Walker, University of Edinburgh, Scotland

G.T. Rudkin, Department of Genetics, University of Nijmegen, The Netherlands, and The Institute for Cancer Research, Philadelphia, Pennsylvania: "Repetitive DNA in Polytene Chromosomes."

J.G. Gall, Department of Biology, Yale University, New Haven, Connecticut: "Repetitive DNA in *Drosophila*."

M. Blumenfeld, Department of Genetics, University of Wisconsin, Madison: "The Evolution of Satellite DNA in *Drosophila Virilis*."

E.M. Southern and G. Roizes, MRC Mammalian Genome Unit, University of Edinburgh, Scotland: "Action of a Restriction Endonuclease on Higher Organism DNA."

M. Bobrow and K. Madan, Genetics Laboratory, Department of Biochemistry, Oxford, England: "Banding of Human Chromosomes with Acridine Orange."

B. Weisblum, Pharmacology Department, University of Wisconsin Medical School, Madison: "Fluorescent Probes of Chromosomal DNA Structure *in situ*: Three Classes of Acridines."

MONDAY MORNING, June 4

Chairman: Barbara McClintock, Carnegie Institution of Washington, Cold Spring Harbor, New York

S.G. Clarkson and M.L. Birnstiel, Institute fur Molekularbiologie II, der Universitat Zurich, Switzerland: "Clustered Arrangement of +RNA Genes of *Xenopus laevis*."

T.A. Grigliatti*, B.N. White*, G.M. Tener*, T.C. Kaufman†, J.J. Holden† and D.T. Suzuki†, Departments of Biochemistry* and Zoology†, The University of British Columbia, Canada: "Studies on the Transfer RNA Genes of *Drosophila melanogaster*."

M.L. Pardue, Massachusetts Institute of Technology, Cambridge: "Localization of Repeated DNA Sequences in *Xenopus* Chromosomes."

F. Ritossa, Institute of Genetics, University of Bari, Bari, Italy: "Magnification of rRNA Genes in *Drosophila*."

K.D. Tartof, Institute for Cancer Research, Philadelphia: "Fluctuation of Ribosomal RNA Gene Redundancy and the Evolution of Redundant Genes."

D.D. Brown, K. Sugimoto and D. Carroll, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: "The Organization and Evolution of Ribosomal and 5S DNAs in *Xenopus laevis* and *Xenopus mulleri*."

G.P. Smith, Laboratory of Genetics, University of Wisconsin, Madison: "Evolution of Homogeneous and Heterogeneous Multigene Families by Unequal Crossover."

A. Lima-de-Faria, Institute of Molecular Cytogenetics, University of Lund, Lund, Sweden: "Localization of Gene Amplification for 28S and 18S Ribosomal RNA in Specific Chromomeres."

MONDAY EVENING, June 4

Chairman: Gordon Lark, University of Utah, Salt Lake City

S. Mizutani, Chil-Yong Kang and H.M. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: "RNA-directed DNA Polymerase Activity in Avian Viruses and Cells."

G.P. Tocchini-Valentini*, V. Mahdavi†, R.D. Brown* and M. Crippa†, *CNR Laboratory of Cell Biology, Rome; †Department of Animal Biology, University of Geneva: "Synthesis of Amplified rDNA."

D. Hourcade, D. Dressler and J. Wolfson, Biological Laboratories, Harvard University, Cambridge, Massachusetts: "On the Mechanism of Ribosomal DNA Amplification."

M. Kimura and T. Ohta, National Institute of Genetics, Mishima, Japan: "Gene Pool of Higher Organisms as a Product of Evolution."

S. Ohno, Department of Biology, City of Hope Medical Center, Duarte, California: "Conservation of Ancient Linkage Groups in Evolution."

M. Hsu, H. Kung and N. Davidson, Department of Chemistry, California Institute of Technology, Pasadena: "A Proposed Method for Mapping Poly-A Sequences in RNA Molecules by Electron Microscopy — Applications to Sindbis Virus RNA."

M.W. Berns, Department of Developmental and Cell Biology, University of California, Irvine: "Chromosome Microdissection by Laser."

TUESDAY MORNING, June 5

Chairman: Noboru Sueoka, University of Colorado, Boulder

U. Grossbach, Max-Planck-Institut fur Biologie, Tubingen, Germany: "Chromosome Puffs and Gene Expression in Polytene Cells."

B. Daneholt and H. Hosick, Department of Histology, Karolinska Institutet, Stockholm, Sweden: "A Transcript of Giant Size in a Balbiani Ring."

B. Lambert, Department of Histology, Karolinska Institutet, Stockholm, Sweden: "Repeated Nucleotide Sequences in a Single Puff of *Chironomus tentans* Polytene Chromosomes."

H.D. Berendes, C. Alonso, P.J. Helmsing, H.J. Leenders and J. Derksen, University of Nijmegen, The Netherlands: "Structural and Functional Units in the Genome of *Drosophila hydei.*"

M. Ashburner, Department of Genetics, University of Cambridge, England: "Control of Transcription (Puffing Activity) in Polytene Chromosomes of *Drosophila melanogaster.*"

O. Hess, Institut fur Allegmeine Biologie, University of Dusseldorf, Germany: "Local Structural Variation of the Y Chromosome of *Drosophila hydei* and their Correlation to Genetic Activity."

W. Hennig and I. Hennig, Max-Planck-Institut fur Biologie, Tubingen, Germany: "Structure and Function of the Y Chromosome of *Drosophila hydei.*"

TUESDAY EVENING, June 5

Chairman: Franklin W. Stahl, University of Oregon, Eugene

P.M. Lizardi and D.D. Brown, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: "Studies on Silk Fibroin mRNA and its Genes."

J.O. Bishop and K.B. Freeman, Department of Genetics, University of Edinburgh, Scotland: "A study of DNA Sequences in the Region of the Hemoglobin Genes."

M. Grunstein*, S. Levy*, P. Schedl† and L.H. Kedes*, Departments of Medicine* and Biochemistry†, Stanford School of Medicine, and VA Hospital*, Palo Alto, California: "Biochemical and Sequence Analysis of Purified Messenger RNAs for Histones."

O. Smithies, Laboratory of Genetics, University of Wisconsin, Madison: "Immunoglobulin Genes: Tandemly or Laterally Duplicated?"

T. Delovitch and C. Baglioni, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Immunization Genes: A Test of Somatic vs. Germ Line Hypothesis by RNA/DNA Hybridization."

P. Leder, H. Aviv, J. Gielen, Y. Ikawa*, S. Packman, D. Swan and J. Ross, Laboratory of Molecular Genetics, NICHD and Viral Leukemia & Lymphoma Branch, NCI*, NIH, Bethesda, Maryland: "The Regulated Expression of Globin and Immunoglobulin Genes."

WEDNESDAY MORNING, June 6

Chairman: Cedric I. Davern, University of California, Santa Cruz

B.J. McCarthy, D.S. Doenecke, C. Johnson, D.S. Nasser and J. Nishiura, Department of Biochemistry, University of California Medical Center, San Francisco: "Transcription and Chromatin Structure."

R. Axel, H. Cedar, G. Felsenfeld, NIAMDD-NIH, Bethesda, Maryland: "Chromatin Template Activity and Chromatin Structure."

V.G. Allfrey, G. Vidali, E.M. Johnson, A. Inoue and J. Karn, Rockefeller University, New York, New York: "Relationships between Nuclear Protein Phosphorylation and Gene Activation."

A.J. Louie, Imperial Cancer Research Fund, London: "Enzymatic Modifications and their Possible Roles in Regulating the Binding of Basic Proteins to DNA and Chromosomal Structure."

S.C.R. Elgin, Division of Biology, California Institute of Technology, Pasadena: "Nonhistone Chromosomal Proteins of *Drosophila.*"

E. Stubblefield, Department of Biology, University of Texas at Houston, M.D. Anderson Hospital and Tumor Institute, Houston: "Fractionation of Mammalian Chromosomes by Isopyknic Centrifugation."

WEDNESDAY AFTERNOON, June 6

Chairman: Jun-Ichi Tomizawa, National Institutes of Health, Bethesda, Maryland

W. Gilbert, N. Maizels and A. Maxam, Department of Biochemistry and Molecular Biology and Committee on Biophysics, Harvard University, Cambridge, Massachusetts: "Sequences of Controlling Regions of the Lactose Operon of *E. coli.*"

G.P. Georgiev, A.J. Varshavsky, R.B. Church and A.P. Ryskov, Institute of Molecular Biology, Acad. Sci., Moscow, USSR: "On the Structural Organization of Transcriptional Unit in Animal Chromosomes."

J. Paul, P. Harrison, G. Birnie, A. Hell, B. Young, R.S. Gilmour, C. Drewienkiewicz and R. Williamson, Beatson Institute for Cancer Research, Glasgow, Scotland: "The transcriptional Unit in Eukaryotes."

W. Jelinek, G. Molloy, M. Salditt, R. Wall and J.E. Darnell, Department of Biological Sciences, Columbia University, New York, New York: "Studies on the Structure of HnRNA and the Biogenesis of mRNA."

M. Crippa, D. Dina and I. Meza, Department of Animal Biology, University of Geneva, Switzerland: "Unique and Repeated Sequences in Messenger RNAs."

J.E. Edstrom and R. Tanguay, Department of Histology, Karolinska Institutet, Stockholm, Sweden: "Chromosome Products in *Chironomus tentans* Salivary Gland Cells."

H.F. Lodish, R.A. Firtel, A. Jacobson, J. Tuchman, T. Alton, B. Young and L. Baxter, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Transcription and Structure of the Genome of the Eukaryotic Cellular Slime Mold *Dictyostelium discoideum.*"

THURSDAY MORNING, June 7

Chairman: Hans Ris, University of Wisconsin, Madison

T.E. Martin and H. Swift, Department of Biology, University of Chicago: "Structure of RNA: Protein Complexes from the Nucleus of Eukaryotic Cells."

B.A. Hamkalo and O.L. Miller, Jr., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee; and A.H. Bakken, Biology Department, Yale University, New Haven, Connecticut: "Ultrastructure of Active Eukaryotic Genomes."

A. Gierer, Max-Planck-Institut fur Virusforschung, Tubingen, Germany: "Combinatorial Models of Gene Regulation in Higher Organisms."

SUMMARY: Hewson Swift, University of Chicago
"Organization of Genetic Material in Eukaryotes: Progress and Prospects."

CONTROL OF PROLIFERATION IN ANIMAL CELLS

Arranged by
B. Clarkson, Sloan-Kettering Institute for Cancer Research and R. Baserga, Temple University
Attended by 112 Participants

SUNDAY EVENING, MAY 20

OPENING ADDRESS: Renato Baserga, Temple University

MONDAY MORNING, MAY 21

Chairman: Vittorio Defendi, The Wistar Institute

S. Hakomori, C. Gahmberg and R. Laine, Departments of Pathobiology and Microbiology, University of Washington, Seattle, Washington: "Surface Carbohydrate Changes that Induce 'Contact Inhibition'."

D.R. Critchley, K.A. Chandrabose, J.M. Graham and I.A. Macpherson, Imperial Cancer Research Fund Laboratories, London: "Cell Density Dependent Glycolipid Synthesis in NIL2 Hamster Cells."

O. Renkonen, A. Luukkonen, J. Brotherus and L. Kaariainen, Department of Biochemistry, University of Helsinki: "Composition and Turnover of Membrane Lipids in Semliki Forest Virus (SFV) and in the Host Cells."

P.W. Robbins, G.C. Wickus, B. Wolf, C. Hirschberg, T. Sasaki, P. Branton and K. Keegstra, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Biochemistry of the Transformed Cell Surface."

W.J. Grimes, Department of Biochemistry, College of Medicine, University of Arizona, Tucson: "Biological and Biochemical Characterization of Surface Changes in Normal, MSV and SV40 Transformed, and Spontaneously Transformed Clones on Balb/c Cells."

R.O. Brady, Developmental and Metabolic Neurology Branch, NINDS, National Institutes of Health, Bethesda, Maryland: "Alterations in the Pattern and Synthesis of Gangliosides in Tumorigenic Virus-transformed Cells."

MONDAY EVENING, MAY 21

Chairman: Bayard Clarkson, Memorial Sloan-Kettering Cancer Center

R.R. Burk, Imperial Cancer Research Fund Laboratories, London "Some Properties of a Migration Factor from a Transformed Cell Line."

R.W. Holley, The Salk Institute, San Diego, California: "Serum Factors and Growth Control Mechanisms."

H. Eagle, Department of Cell Biology, Albert Einstein College of Medicine, New York City: "Effect of Environmental pH on the Growth and Function of Cultured Mammalian Cells."

A. Vogel(a), P. Hough(b), J. Oey(c) and R. Pollack(a); (a) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (b) Brookhaven National Laboratories, Upton, New York, (c) Universitat, Konstanz, Germany: "Two Classes of Revertants Isolated from SV40-transformed 3T3 Cells."

H. Temin, G.L. Smith, McArdle Laboratory, University of Wisconsin, Madison; N.C. Dulak, Department of Biochemistry, University of Kansas Medical Center, Kansas City: "Control of Multiplication of Normal and Virus-transformed Chicken Embryo Fibroblasts by Purified Multiplication-stimulating Activity with Nonsuppressible Insulin-like Activity."

H. Rubin and D. Fodge, Department of Molecular Biology, University of California, Berkeley: "Glucose Catabolism and the Regulation of DNA Synthesis in Chick Embryo Cells."

TUESDAY MORNING, MAY 22

Chairman: Howard M. Temin, University of Wisconsin

L.F. Lamerton and N.M. Blackett, Department of Biophysics, Institute of Cancer Research, Sutton, Surrey, England: "A Comparison of Proliferative Characteristics of Bone Marrow and Tumours, and Response to Cytotoxic Agents."

M. Tubiana, E. Frindel, H. Croizat and F. Vassort, Institut de Radiobiologie Clinique, Villejuif, France: "Study of Some of the Factors which Influence the Proliferation and the Differentiation of Hematopoietic Stem Cells."

B. Clarkson, Memorial Sloan-Kettering Cancer Center, New York City: "The Survival Value of the Dormant State in Neoplastic and Normal Cell Populations."

W.K. Sinclair, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois: "Modification of Cell Cycle Response of Synchronous Mammalian Cells to Ionizing Radiation by Inhibitors of Repair."

G.P. Wheeler, Biochemistry Department, Southern Research Institute, Birmingham, Alabama: "Biochemistry and Cell Kinetics as Aids in Selecting Combinations of Agents for Cancer Therapy."

J. Folkman, Harvard Medical School, Boston, Massachusetts: "Self-regulation of Growth in Three Dimensions."

TUESDAY EVENING, MAY 22

Chairman: Renato Baserga, Temple University

P.A. Marks, R.A. Rifkind, A. Bank, L. Cantor and D. Singer, Columbia University, College of Physicians & Surgeons, New York City: "Erythroid Cell Proliferation and Differentiation: Action of Erythropoietin."

A.S. Gordon, J. LoBue and T.N. Fredrickson, Department of Biology, New York University, New York City, and the Department of Pathobiology, University of Connecticut, Storrs: "Erythropoietin and its Significance in the Pathophysiology of Murine Erythroleukemia."

D. Metcalf, Walter and Eliza Hall Institute, Melbourne, Australia: "Regulatory Control of Normal and Leukemic Granulocytic Cells."

J.E. Till, G.B. Price and E.A. McCulloch, The Ontario Cancer Institute, Toronto, Canada: "Factors Affecting Granulopoietic Colony Formation by Human Marrow Cells."

L. Sachs, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: "Control of Growth and Differentiation in Normal Hematopoietic and Leukemic Cells."

J.J. Trentin, J.M. Rauchwerger and M.T. Gallagher, Division of Experimental Biology, Baylor College of Medicine, Houston, Texas: "Regulation of Hemopoietic Stem Cell Differentiation and Proliferation by Hemopoietic Inductive Micro-environments (HIM)."

WEDNESDAY MORNING, MAY 23

Chairman: Harry Eagle, Albert Einstein College of Medicine

C. Basilico, Department of Pathology, New York University School of Medicine, New York City: "Host Cell Control of Viral Transformation."

R. Risser and R. Pollack, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Biological Analysis of Clones of SV40-infected Mouse Cells."

V. Defendi and K. Hirai, Wistar Institute, Philadelphia, Pennsylvania: "Changes of Regulation of Host DNA Synthesis and Viral DNA Integration in SV40 Infected Cells."

H. Green, Department of Biology, Massachusetts Institute of Technology, Cambridge: "RNA Synthesis during the Transition from Resting to Growing State."

B. Ozanne and M. Lurye, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Revertants of Virus Transformed Mouse Cells."

A.B. Pardee, L. Jimenez De Asua and E. Rozengurt, Department of Biochemical Sciences, Princeton University, Princeton, New Jersey, and Imperial Cancer Research Fund Laboratories, London: "Functional Membrane Changes and Cell Growth: Significance and Mechanism."

WEDNESDAY EVENING, MAY 23

Chairman: Harry Rubin, University of California

M. Edidin and A. Weiss, Department of Biology, The Johns Hopkins University, Baltimore, Maryland: "Intermixing of Heterokaryon Plasma Membrane Antigens after Fusion of Cells of Various Established Lines."

P. Satir and B. Satir, Department of Physiology-Anatomy, University of California, Berkeley: "Design and Function of Site-specific Particle Arrays in the Cell Membrane."

S. Roth, A. Patteson, D. White and K. Dorsey, Department of Biology, The Johns Hopkins University, Baltimore, Maryland: "Growth Control in Malignant Cells Induced by Cell Surface Glycosylation."

G.L. Nicolson, Cancer Council and Electron Microscopy Laboratories, Armand Hammer Center for Cancer Biology, The Salk Institute for Biological Studies, San Diego, California: "Changes in the Display of D-Galactopyranosyl-like Residues on Normal and Transformed Cell Surfaces."

M. Inbar and L. Sachs, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: "Fluidity of the Surface Membrane and the Control of Growth."

J. Shoham and L. Sachs, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: "Differences in Lectin Agglutinability of Normal and Transformed Cells in Interphase and Mitosis."

THURSDAY MORNING, MAY 24

Chairman: Gerald M. Edelman, The Rockefeller University

J. Voorhees, M. Stawiski and E. Duell, Department of Dermatology, University of Michigan, Ann Arbor; M. Haddox and N. Goldberg, Department of Pharmacology, University of Minnesota, Minneapolis: "Imbalanced Cyclic AMP and Cyclic GMP Levels in the Rapidly Dividing, Incompletely Differentiated Epithelium of Psoriasis."

J.R. Sheppard, Dight Institute for Human Genetics, University of Minnesota, Minneapolis: "Cyclic AMP and Cell Division."

K.N. Prasad and S. Kumar, Department of Radiology, University of Colorado Medical Center, Denver: "Cyclic AMP and the Differentiation of Mouse Neuroblastoma Cells in Culture."

N.D. Goldberg, M.K. Haddox, E. Dunham, C. Lopez and J.W. Hadden, University of Minnesota Medical School, Minneapolis: "Evidence for Opposing Influences of Cyclic GMP and Cyclic AMP in the Regulation of Cell Proliferation and other Biological Processes."

J.P. Durham, R. Baserga and F.R. Butcher, Department of Biochemistry, Glasgow University, Scotland; Department of Pathology, Temple University, Philadelphia, Pennsylvania; and Division of Biological Sciences, Brown University, Providence, Rhode Island: "Lack of Correlation between Catecholamine Analog Effects on Cyclic Adenosine 3′5′ Monophosphate and Adenyl Cyclase and the Stimulation of DNA Synthesis, α-Amylase Secretion and Glycogen Depletion in Mouse Parotid Gland."

I. Pastan, G.S. Johnson, W.B. Anderson, M. Willingham and R. Carchman, Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland: "Cyclic AMP, Fibroblasts and Cellular Transformation."

THURSDAY EVENING, MAY 24

Chairman: Howard Green, Massachusetts Institute of Technology

M.C. Raff and S. DePetris, Medical Research Council Neuroimmunology Project, Zoology Department, University College London, London: "Surface Distribution of Membrane Macromolecules."

C.W. Stackpole, U. Hammerling, M.E. Lamm*, M.P. Lardis and J. Lumley-Frank, Sloan-Kettering Institute for Cancer Research, and *Department of Pathology, New York University School of Medicine, New York City: "Effect of Ferritin and Other Visual Markers on Hybrid Antibody-induced Topographical Displacement of Cell Surface Components."

F. Melchers and J. Andersson, Basel Institute for Immunology, Basel, Switzerland: "Proliferation of Immunoglobulin-forming B-cells and Their Differentiation into Immunoglobulin-secreting Cells after Mitogenic Stimulation."

G.M. Edelman, The Rockefeller University, New York City: "Surface Alterations and Mitogenesis in Lymphocytes."

J.L. Fahey, Department of Microbiology & Immunology, University of California, Los Angeles: "Control of Proliferation and Protein Synthesis in Antibody Forming Cells."

E. Diener, MRC Transplantation Unit and Department of Pathology, University of Alberta, Edmonton, Alberta, Canada: "Regulation of the Immune Response at the Single Cell Level."

M.D. Hollenberg and P. Cuatrecasas, Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland: "Hormone Receptors and Membrane Glycoproteins during *in vitro* Transformation of Lymphocytes."

FRIDAY MORNING, MAY 25

Chairman: Gordon Tomkins, University of California Medical Center

R.A. Tobey, L.R. Gurley, C.E. Hildebrand, R.L. Ratliff and R.A. Walters, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico: "Sequential Biochemical Events in Preparation for DNA Replication and Mitosis."

H.L. Cooper, Cell Biology Section, NIDR, National Institutes of Health, Bethesda, Maryland: "Studies of Poly-A-bearing RNA in Resting and Growing Human Lymphocytes."

G.J. Todaro, M.M. Lieber, R. Benveniste and D.L. Livingston, Viral Leukemia & Lymphoma Branch, National Cancer Institute, Bethesda, Maryland: "Endogenous Type C Virus Secretion: The Expression of a Normally Repressed Cell Function."

S. Kornfeld and W. Eider, Washington University School of Medicine, St. Louis, Missouri: "Inhibition of DNA and Protein Synthesis in Lymphocytes by the Phytoagglutinins of *Ricinus communis* and *Abrus precatorius*."

P.N. Rao and R.T. Johnson, Department of Developmental Therapeutics, University of Texas at Houston, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, and the Department of Zoology, University of Cambridge, England: "Regulation of Cell Cycle in Somatic Cell Hybrids."

G.C. Mueller and S. Seki, McArdle Laboratory, University of Wisconsin, Madison: "Dependence of DNA Replicase Activity on RNA and Protein Synthesis."

J. Short, N.B. Armstrong, R.A. Mitchell, M.A. Kolitsky, R. Zemel and I. Lieberman, Department of Anatomy & Cell Biology, University of Pittsburgh Medical School, and Veterans Administration Hospital, Pittsburgh, Pennsylvania: "On the Control of Nuclear DNA Synthesis in Liver."

FRIDAY EVENING, MAY 25

Chairman: Paul A. Marks, Columbia University

V.G. Allfrey, G. Vidali, E.M. Johnson, A. Inoue and J. Karn, The Rockefeller University, New York City: "The Role of Nuclear Phosphoproteins in Control of Transcription."

L. Zardi, Jung-Chung Lin, R.O. Petersen and R. Baserga, Department of Pathology, Temple University School of Medicine, Philadelphia, Pennsylvania: "Specificity of Antibodies of Non-histone Chromosomal Proteins of Fibroblasts in Culture."

G.D. Birnie, G. Threlfall, A.J. MacGillivray and J. Paul, The Beatson Institute for Cancer Research, Glasgow, Scotland: "Factors Controlling Genetic Expression in Growing and Non-growing Cells."

T.W. Borun, W.K. Paik, H.W. Lee, D. Pearson and D. Marks, Fels Research Institute and Department of Biochemistry. Temple University School of Medicine, Philadelphia, Pennsylvania: "Histone Methylation and Phosphorylation during the HeLa S-3 Cell Cycle."

R. Chalkley, R. Balhorn and V. Jackson, Department of Biochemistry, School of Medicine, University of Iowa, Iowa City: "Histone Deposition and Phosphorylation during Chromosome Replication."

R.R. Klevecz, L.N. Kapp and J.A. Remington, Department of Biology, City of Hope National Medical Center, Duarte, California: "Discontinuous DNA Synthesis and Periodic Gene Expression."

SATURDAY MORNING, MAY 26

Chairman: Arthur Pardee, Imperial Cancer Research Fund Laboratories

M.H. Makman, B. Dvorkin and E. Keehn, Departments of Biochemistry and Pharmacology, Albert Einstein College of Medicine, New York City: "Hormonal Regulation of Cyclic AMP in Aging and in Virus-transformed Human Fibroblasts and Comparison with other Cultured Cells."

F. Bresciani, E. Nola, V. Sica and G.A. Puca, Istituzioni di Patologia Generale, University of Naples Medical School: "Early Stages in Estrogen Control of Cell Proliferation."

E.V. Jensen, S. Mohla, P.I. Brecher and E.R. DeSombre, Ben May Laboratory for Cancer Research, University of Chicago: "Receptor Transformation — A Key Step in Estrogen Action."

B.W. O'Malley, L. Chan, J.P. Comstock and A.R. Means, Baylor College of Medicine, Houston, Texas: "Estrogen-mediated Differentiation of Chick Oviduct Target Cells."

H.A. Armelin, K. Nishikawa and G.H. Sato, Department of Biology, University of California at San Diego: "Control of Mammalian Cell Growth in Culture: The Action of Protein and Steroid Hormones as Effector Substances."

D.D. Cunningham, C.R. Thrash and R.D. Glynn, Department of Medical Microbiology, College of Medicine, University of California, Irvine: "Initiation of Division of Density-inhibited Fibroblasts by Glucocorticoids."

G.M. Tomkins, R. Kram, P. Mamont, V. Daniel, G. Litwack and H.R. Bourne, Department of Biochemistry and Biophysics, University of California, San Francisco: "Mechanisms of Growth Control in Cultured Cells."

SATURDAY AFTERNOON, MAY 26

Chairman: Max Burger, Bio-Center of the University of Basel

A.C. Allison, Clinical Research Centre, Harrow, Middlesex, England: "Plasma Membrane Interactions in Relation to Control of Cell Proliferation."

R.D. Estensen, J.W. Hadden, M.K. Haddox and N.D. Goldberg, University of Minnesota Medical School, Minneapolis: "Tumor Promoter (Phorbal Myristate Acetate): Effects on Membrane Structure and Function in Mouse 3T3 Cells and Human Lymphocytes."

C.P. Stanners, L.H. Thompson and J.L. Harkins, Ontario Cancer Institute, Toronto, Canada: "Studies on a Mammalian Cell Mutant with a Temperature-sensitive Leucyl t...IA Synthetase."

T.S. Argyris, Department of Pathology, Upstate Medical Center, SUNY, Syracuse: "Stimulators, Enzyme Induction and the Control of Liver Growth."

Y.J. Topper and B.K. Vonderhaar, National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, Maryland: "Proliferation in Development."

R.L. Davidson* and M.D. Bick†, *Genetics Division, Children's Hospital Medical Center & Department of Microbiology and Molecular Genetics, and †Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: "A Mutant Mammalian Cell which Requires Bromodeoxyuridine for Growth."

SUNDAY MORNING, MAY 27

Chairman: Vincent G. Allfrey, The Rockefeller University

A. Vaheri, E. Ruoslahti and T. Hovi, Department of Virology and Department of Serology and Bacteriology, University of Helsinki: "Cell Surface and Growth Control of Chick Fibroblasts in Culture."

M.M. Burger, Department of Biochemistry, Bio-Center of the University of Basel, Basel, Switzerland: "A Surface Alteration Brought about by Protease Treatment and Leading to Temporary Loss of Growth Control."

K. Dano, G. Kellerman, J. Loeb, D. Loskutoff, L. Ossowski, A. Piperno, J. Quigley, D. Rifkin, A. Tobia, J. Unkeless and E. Reich, The Rockefeller University, New York, N.Y.: "Studies of Fibrinolysin T."

H.P. Schnebli, Friedrich Miescher-Institut, Basel, Switzerland: "On the Mechanism of Growth Inhibition of Tumor Cells by Protease Inhibitors."

I.N. Chou, P.H. Black and R. Roblin, Departments of Medicine and Microbiology and Molecular Genetics, Harvard Medical School, Boston: "Effects of Protease Inhibitors on Growth of SV3T3 and 3T3 Cells."

SUMMARY: Michael Stoker, Imperial Cancer Research Fund

FIFTH ANNUAL TUMOR VIRUS MEETING

SIMIAN VIRUS 40, POLYOMA AND ADENOVIRUSES

Arranged by
P. Sharp, Cold Spring Harbor Laboratory
Attended by 149 Participants

SUNDAY, AUGUST 12

Defective Genomes

M. Martin, L.D. Gelb and J.B. Milstien, Laboratory of Biology of Viruses, NIAID, NIH, Bethesda, Maryland, K.K. Takemoto, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, Maryland, and D. Nathans, Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland: "Characterization of 'Heavy' and 'Light' SV40-like Particles Isolated from Human Brain."

G.C. Fareed* and M.A. Martin†, *Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts and †NIAID, NIH, Bethesda, Maryland: "Specific Genetic Reassortment in an SV40-like Virus of Human Origin."

G. Khoury, G. Fareed and M. Martin, Laboratory of Biology of Viruses, NIAID, NIH, Bethesda, Maryland and D. Nathans, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: "The Characterization of Circular SV40 DNA Molecules Containing Three R_1 Sites and Triplication of a Specific One-third of the Viral Genome."

T.N.H. Lee, W.W. Brockman and D. Nathans, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: "SV40 Variants Containing DNA with Deletions and/or Substitutions of Cell DNA."

J.E. Mertz and Paul Berg, Stanford Medical Center: "Isolation and Characterization of Defective SV40 Genomes."

M. Fried and Don Robberson, Imperial Cancer Research Fund, London, England: "Isolation and Characterization of Polyoma Defective DNA."

S. Rozenblatt, S. Lavi, N. Frenkel, M.F. Singer and E. Winocour, Department of Genetics, Weizmann Institute of Science. Rehovot. Israel: "Substituted SV40 DNA."

N. Frenkel, S. Rozenblatt, S. Lavi and E. Winocour, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: "Comparison of Host Sequences in Different Populations of SV40 Substituted DNA."

G. Frenkel and Ernest Winocour, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: "Degradation and Re-utilization of Viral DNA during SV40 Infection of Permissive Cells."

MONDAY MORNING, AUGUST 13

Transcription

R. Dhar, B.S. Zain, S.M. Weissman and J. Pan, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: "Comparison of Sequence RNA Transcribed in Infected Cells and by *E. coli* RNA Polymerase from a Segment of SV40 DNA."

Y. Aloni, Department of Genetics, The Weizmann Institute of Science, Rehovot, Israel: "Non-selective Transcription of SV40 DNA in Productively Infected Cells."

C.T. Patch, A.M. Lewis Jr. and A.S. Levine, National Institutes of Health, Bethesda, Marlyand: "Evidence that the Early Templates of SV40 Are Not Contiguous."

P. May*, E. May*, D. Hayward* and R. Weil†, *Institut de Recherches sur le Cancer, Villejuif, France, †Department of Molecular Biology, University of Geneva, Geneva, Switzerland: "A. Isolation of 'early 19 S' SV40 RNA from Polysomes. B. Evidence for SV40-induced Stimulation of Overall Cellular RNA Synthesis."

S. Manteuil, G. Danglo and M. Girard, Service de Physiologie des Virus, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: "Simian Virus 40 Transcriptive Intermediates."

R. Weil*, C. Salomon*, E. May† and P. May†, *Department of Molecular Biology, University of Geneva, Geneva, Switzerland, †Institut de Recherches sur le Cancer, Villejuif, France: "Early Events of the Lytic Infection with Polyoma (Py) Virus."

B. Carter, G. Khoury and J. Rose, Laboratory of Biology of Viruses, NIH, Bethesda, Marlyand: "AAV RNA Transcription."

S.M. Astrin, The Institute for Cancer Research, The Fox Chase Center for Cancer and Medical Sciences, Philadelphia: "*In Vitro* Transcription of the SV40 Sequences in SV3T3 Chromatin."

M. Yaniv, O. Croissant and B. Lescure, Departments of Molecular Biology and Virology, The Pasteur Institute, Paris, France: "Transcription of Polyoma DNA with *E. coli* RNA Polymerase."

MONDAY EVENING, AUGUST 13

Proteins

S.I. Reed and G.R. Stark, Department of Biochemistry, Stanford University, California: "X and Y, Two New SV40-specific Antigens."

M-E. Mirault and G.R. Stark, Department of Biochemistry, Stanford University, California: "Purification of SV40-specific X Antigen from Polyribosomal Fractions of SV40-transformed Hamster Cells."

M. Osborn and K. Weber, Cold Spring Harbor Laboratory, Cold Spring Harbor: "T-Antigen in SV40 Infected Cells."

P.C. van der Vliet and A.J. Levine, Department of Biochemistry, Princeton University, Princeton, New Jersey: "DNA Binding Proteins Specific for Adenovirus Infected Cells."

T. Friedmann, Department of Pediatrics, University of California, San Diego: "The Relatedness of Polyoma Proteins."

D.M. Pett, M.K. Estes and J.S. Pagano, Departments of Bacteriology & Immunology and Medicine, University of North Carolina School of Medicine, Chapel Hill: "Characterization of Histone-like Proteins of SV40 Virus."

P. Rouget, D. Blangy, A. Parodi and F. Cuzin, Department de Biologie Moleculaire, Institut Pasteur, Paris: "Specificity of Polyoma Virus Associated Endonuclease."

R.D. MacDonald and T. Gurney Jr., Department of Molecular Biology, University of California, Berkeley: "Characterization of SV40 DNA-Protein Complexes in Lytically Infected Cells."

D.A. Goldstein, M.R. Hall and W. Meinke, Department of Microbiology, Scripps Clinic and Research Foundation, La Jolla, California: "Nucleoprotein Complexes Containing Replicating Polyoma and SV40 DNA."

TUESDAY MORNING, AUGUST 14

Transcription: Genome Mapping Using Restriction Endonucleases

J. Tal and H.J. Raskas, Institute for Molecular Virology, St. Louis University School of Medicine, St. Louis: "Isolation and Characterization of Cytoplasmic Adenovirus 2 RNA Synthesized Late in Productive Infection."

M. Brunner and H.J. Raskas, Institute for Molecular Virology, St. Louis University School of Medicine, St. Louis: "Characterization and Fate of Nuclear Viral RNA Synthesized 18 Hours after Productive Infection with Adenovirus 2."

U. Pettersson, J. Sambrook, P.A. Sharp, C. Tibbetts, K. Johansson and L. Philipson, Cold Spring Harbor Laboratory and the Department of Microbiology, Uppsala University, Sweden: "Transcription of Adenovirus Type 2 DNA during Lytic Infection."

S.L. Bachenheimer, P. Hoffman and J.E. Darnell, Department of Biological Sciences, Columbia University, New York, N.Y.: "Transcription of the AD-2 Genome in Transformed Rat Cells."

R.I. Fox and S.G. Baum, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y.: "Post-transcriptional Block to Adenovirus Replication in Monkey Cells."

Y. Sugino, K Tsukamoto, D. Igarashi and Y. Niiyama, Biological Research Laboratories, Central Research Division, Takeda Chemical Ind., Ltd.: "Temperature Effect on Host Restriction of Viral Gene Expression."

E-S. Huang and J.S. Pagano, Departments of Bacteriology & Immunology and Medicine, University of North Carolina School of Medicine, Chapel Hill: "Physical Mapping of the SV40 Genome with Restriction Endonuclease Z."

B. Griffin, M. Fried and D. Robberson, Imperial Cancer Research Fund, London, England: "Restriction Enzyme Cleavage of Polyoma DNAs."

J.E. Germond, V.M. Vogt and B. Hirt, Swiss Institute for Experimental Cancer Research, Lausanne: "Activity of Single-strand-specific Nuclease S_1 on Polyoma DNA."

C. Mulder, H. Delius, P.A. Sharp and U. Pettersson, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Specific Fragmentation of Adenovirus DNA by Two Restriction Endonucleases."

TUESDAY AFTERNOON, AUGUST 14

Genetics

C.H.S. Young, P.E. Austin and J.F. Williams, Institute of Virology, University of Glasgow, Scotland: "Current Genetic Studies with Type 5 Adenovirus."

T. Grodzicker, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Host Range Mutants of $Ad2^{+}ND_1$."

J.A. Robb, University of California, San Diego, La Jolla; P. Tegtmeyer, Case-Western University, Cleveland; A. Ishikawa, NIH, Tokyo; G.R. Stark, Stanford University; and H.L. Ozer. Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: "Antigenic Phenotypes of SV40 Temperature-sensitive Mutants."

K. Yoshiike, A. Furuno and S. Uchida, Department of Enteroviruses, National Institute of Health, Tokyo, Japan: "Rescue of Defective SV40 DNA from a Transformed Mouse 3T3 Cell Line."

L.C. Norkin and T.L. Benjamin, Department of Microbiology, University of Massachusetts, Amherst, and Department of Pathology, Harvard Medical School, Boston: "Correlation between Induction of Cellular DNA Synthesis and Abortive Transformation by Polyoma Virus."

W. Doerfler and L. Philipson, Institute of Genetics, University of Cologne, Germany and Wallenberg Laboratory, University of Uppsala, Sweden: "Isolation and Characterization of the Endonuclease from KB Cells Infected with Adenovirus Type 2."

H. Burger and W. Doerfler, The Rockefeller University, New York, N.Y. and Institute of Genetics, University of Cologne, Germany: "Fast Sedimenting Adenovirus DNA—An Integrated Form of Viral DNA?"

P.C. Champe, C. Laughlin and W.A. Strohl, Department of Microbiology, Rutgers Medical School, New Brunswick, New Jersey: "Specificity and Mode of Action of a Viral Inhibitory Factor Obtained from Adenovirus-induced Hamster Tumor Cells."

M.A. Jerkofsky and F. Rapp, Department of Microbiology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania: "Stimulation of Adenovirus Replication in Simian Cells by Pre-treatment with IUdR."

WEDNESDAY MORNING, AUGUST 15

DNA Replication

F.L. Graham and A.J. van der Eb, Laboratory for Physiological Chemistry, State University of Leiden, The Netherlands: "Studies on the Biological Activity of Adenovirus Type 5 DNA."

L.V. Crawford, C. Syrett and A. Wilde, Imperial Cancer Research Fund, London, England: "Replication of Polyoma DNA."

M.S. Horwitz and C. Brayton, Department of Microbiology, Albert Einstein College of Medicine, New York, N.Y.: "The Origin of DNA Replication in Adenovirus Type 2."

W.P. Cheevers, R.B. Sadoff and J. Kowalski, Cancer Research Laboratory, University of Western Ontario, London, Canada: "RNA-linked Nascent Strands in Replicating Polyoma DNA: A Non-messenger DNA-dependent Transcription Requirement in Viral DNA Replication?"

K. Raska Jr. and K.K. Biron, CMDNJ, Rutgers Medical School, Piscataway, New Jersey: "Inhibition of Adenovirus Replication in Fibroblastic Cells by Dibutyryl-cAMP."

J. Ferguson and R. Davis, Department of Biochemistry, Stanford Medical Center, Stanford, California: "A Method for Studying Small Amounts of Homology between DNAs Applied to SV40 and Polyoma DNAs."

M. Botchan and Gillies McKenna, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Integration of SV40 in Transformed Cells."

A.H. Fried, Institut fur Virusforschung, Deutsches Krebsforschungzentrum, Heidelberg, Germany: "Heat Induced Strand Separation of the Cellular DNA Molecules Containing Integrated SV40 DNA in SV40 Transformed Cells."

B. Rosenwirth, S. Tjia and W. Doerfler, Institute of Genetics, University of Cologne, Germany: "Incomplete Particles of Adenovirus Type 2."

J. Lebowitz, Department of Biology, Syracuse University, Syracuse, New York and N.P. Salzman, Laboratory of the Biology of Viruses, NIH, Bethesda, Maryland: "Unpaired Bases in Superhelical SV40 DNA: Carbodiimide Modification."

WEDNESDAY AFTERNOON, AUGUST 15

Transformed Cells

R. Risser and R. Pollack, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.: "Wide Range of SV40 Transformants Found by Non-selective Scan of Infected Cells."

S.J. Pancake and P.T. Mora, NIH, Bethesda, Maryland: "Cell Surface Antigens Associated with Transformation by SV40 as Detected by Cytolytic and Absorption Techniques."

A. Vogel and R. Pollack, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.: "Susceptibility of Revertants of SV3T3 Cells to Retransformation by SV40 and MSV."

K.D. Noonan and G. Albrecht-Buhler, Department of Biochemistry, The University of Florida, Gainesville and Friedrich Miescher-Institut, Basel, Switzerland: "A Possible Mechanism of Monolayer Formation."

F. Kelly, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.: "Ctyochalasin B-resistant SV40 Transformed Cells."

A. Schultz, V. McFarland and P.T. Mora, National Cancer Institute, NIH, Bethesda, Maryland: "Phenotypic Expression of Transformation in Mouse Fibroblasts."

P.H. Fishman and R.L. Quarles, Developmental and Metabolic Neurology Branch, IR, NINDS, NIH, Bethesda, Maryland: "Membrane Glycoproteins of Normal and SV40 Transformed Mouse Cells."

C.D. Scher, D. Stathakos and H.N. Antoniades, Children's Medical Center and Center for Blood Research, Boston: "Characterization of Serum Factors Inducing Cell Division in Balb/3T3."

P.K. Horan, J. Lehman*, A. Romero and J.H. Jett, *Department of Pathology, University of Colorado Medical Center, Denver, Colorado; Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico: "Polyploid Formation Resulting from SV40 Infection."

HERPESVIRUSES MEETING

Arranged by
P. Spear, Rockefeller University and A. Nahmias, Emory University
Attended by 103 Participants

FRIDAY EVENING, AUGUST 17

Structure and Biochemistry of Herpesviruses

Chairman: P. Spear, Rockefeller University, New York

B. Roizman, D. Furlong, W. Gibson, J. Heine and R.W. Honess, Department of Microbiology, University of Chicago, Chicago, Illinois: "The Properties and Topology of the Herpes Simplex Virion."

E.K. Wagner, K.K. Tewari and R.C. Warner, Department of Molecular Biology and Biochemistry, University of California, Irvine: "The Molecular Size of the Herpes Simplex Virus Genome."

Y. Becker, Z. Frenkel, A. Ben-Zeev and U. Olshevsky, Laboratory for Molecular Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: "Studies on Peptides Bound to Herpes Simplex Virions DNA with an RNA Polymerase Activity."

S. Silverstein, N. Frenkel, S. Bachenheimer and B. Roizman, Department of Microbiology, University of Chicago, Chicago, Illinois: "Control of Transcription and of RNA Abundance in HEp-2 Cells Infected with Herpes Simplex Virus."

R.W. Honess and B. Roizman, Department of Microbiology, University of Chicago, Chicago, Illinois: "Patterns of Synthesis of Structural and Non-structural Herpesvirus Proteins—Evidence for Translational Regulation."

E-S. Huang and J.S. Pagano, Departments of Medicine and Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina: "Human Cytomegaloviruses: Characterization of Viral DNA and Immunologic Relations."

A. Adams, T. Lindahl and G. Klein, Departments of Tumor Biology and Chemistry, Karolinska Institutet, Stockholm, Sweden: "Linear Association between Cellular DNA and Epstein-Barr Virus (EBV) DNA in a Human Lymphoblastoid Cell Line."

SATURDAY MORNING, AUGUST 18

Genetics of Herpesviruses

Chairman: G. Klein, Karolinska Institute, Stockholm

P.A. Schaffer, J. Esparza and M. Benyesh-Melnick, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: "Functional Interactions between Temperature-sensitive Mutants of Herpes Simplex Virus Types 1 and 2."

I.W. Halliburton and M.C. Timbury, Department of Microbiology, University of Leeds and Institute of Virology, University of Glasgow: "Temperature-sensitive Mutants of Herpes Simplex Virus Type 2."

R.J. Courtney, D.H. Harris, D.R. Bone and M. Benyesh-Melnick, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: "Studies of Herpes Simplex Virus Type 1 (HSV-1)-Induced Polypeptides through the Use of Temperature-sensitive (ts) Mutants."

Herpesviruses Associated with Human Tumors
I. Epstein-Barr Virus

Chairman: G Klein, Karolinska Institute, Stockholm

Y. Kawai and M. Nonoyama, Department of Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois: "The Sensitivity of DNA-DNA Reassociation Kinetics for EBV-DNA."

J. Levine, E. Kieff and G. Klein, University of Chicago and the Karolinska Institute: "Relatedness of Burkitt Herpes Viral DNA to Infectious Mononucleosis DNA."

J.S. Pagano, M. Nonoyama and C.-H. Huang, Departments of Medicine and Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina: "Testing of Human Tumors for Epstein-Barr Viral DNA."

H. Wolf and H. zur Hausen, Institut fur Klinische Virologie, Erlangen, Germany: "Presence of EB Viral DNA in Epithelial Nasopharyngeal Carcinoma Cells."

SATURDAY EVENING, AUGUST 18

Herpesviruses Associated with Human Tumors
I. Epstein-Barr Virus (continued)

Chairman: F. Rapp, College of Medicine, Pennsylvania State University, Hershey

G. Miller, Departments of Pediatrics and Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut: "Release of Infectious Epstein-Barr Virus by Single Cell Clones of Human and Non-human Primate Lymphoblastoid Lines."

G. Klein, A. Okot Nyormoi, A. Adams and L. Dombos, Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden: "Sensitivity to Superinfection with EBV Concentrates and to Induction by IUdR in Established Lymphoblastoid Lines and Their Hybrids, Derived from Cell Fusion."

J.H. Joncas, J. Boucher, A. Boudreault and M. Granger-Julien, Institute of Microbiology and Hygiene of Montreal (University of Quebec), Montreal, Canada and Department of Microbiology and Department of Pediatrics, University of Montreal, Montreal, Canada: "Interferon Response to EBV Activation by Bromodeoxyuridine and Hydrocortisone."

G. de-The, D. Ablashi and A. Liabeuf, International Agency for Research on Cancer, Lyon, France: "EBV Nuclear Antigen in NPC Biopsies."

B. Reedman and G. Klein, Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden: "Detection of an EBV-determined Nuclear Antigen (EBNA) by Anticomplementary Fluorescence."

M.A. Epstein, Department of Pathology, University of Bristol Medical School, Bristol, United Kingdom: "Reticuloproliferative Disease in an EB Virus-inoculated Owl Monkey, with the Virus in Cultured Cells from the Lesion."

T. Shope, D. Dechairo and G. Miller, Departments of Pediatrics and Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut: "Malignant Lymphoma in Cottontop Marmosets following Inoculation of Epstein-Barr Virus."

SUNDAY MORNING, AUGUST 19

Herpesviruses Associated with Human Tumors

II. Herpes Simplex Virus

Chairman: A. Nahmias, Emory University, Atlanta

D. Davis and W. Munyon, Department of Medical Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York: "Properties of Cells Biochemically Transformed by HSV-1 and HSV-2."

R. Duff and F. Rapp, Department of Microbiology, The M.S. Hershey Medical Center of the Pennsylvania State University, Hershey, Pennsylvania: "The Transforming Potential of Herpes Simplex Virus Type 1."

B.R. McAusland and B. Garfinkle, Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: "Transformation of Cultured Cells by Non-irradiated Herpes Simplex Type 1 and Type 2."

A. Hollinshead, Department of Medicine, The George Washington University, Washington, D.C. and G. Tarro, Department of Viral Oncology, University of Naples, Naples, Italy: "Separation and Analysis of Herpesvirus Genetic Markers."

T. Lehner, E.J. Shillitoe, J.M.A. Wilton and L. Ivanyi, Department of Oral Immunology and Microbiology, Guy's Hospital Medical School, London, England: "Cell-mediated Immunity and Antibodies to Herpesvirus Hominis Type 1 in Oral Carcinoma and Precancerous Lesions."

E. Adam, J.L. Melnick and W.E. Rawls, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: "Differences in Type 2 Herpesvirus Infections between Breast Cancer and Cervical Cancer Patients with the Same Sexual Attributes."

H.E. Kaufman and Y. Centifanto, Department of Ophthalmology, University of Florida College of Medicine, Gainesville, Florida: "Virus Chemotherapy."

SUNDAY AFTERNOON, AUGUST 19

Herpesviruses Associated with Non-Human Tumors

Chairman: L. Melendez, Harvard Medical School, Boston

M. Mizell, L. Charbonnet, N. Moustoukas and C. Bahn, Laboratory of Tumor Cell Biology, Tulane University, New Orleans, Louisiana: "*In Vitro* Induction of Lucke Tumor Herpesvirus (LTHV) and Early Detection of LTHV Antigens by Indirect Immunofluorescence."

L.S. Kucera, Department of Microbiology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: "Oncogenic Properties of a Continuous Line of Lucke Tumor Cells."

G.D. Hsiung and C.K.Y. Fong, Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut and Veterans Administration Hospital, West Haven, Connecticut: "Persistent Infection and Transforming Capacity of Guinea Pig Herpesvirus."

K. Nazerian and T. Lindahl, Departments of Tumor Biology and Chemistry, Karolinska Institutet, Stockholm, Sweden: "DNA of Marek's Disease Virus (MDV) in Virus-induced Tumors."

P.A. Long and L.F. Velicer, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan: "Production and Purification of a Soluble Antigen from Marek's Disease Herpesvirus Infected Cells."

L.A. Falk, L.G. Wolfe and F. Deinhardt, Department of Mircobiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois: "The Natural History of Herpesvirus Saimiri Infections in Squirrel Monkeys."

R. Laufs and L.V. Melendez, Hygiene-Institut der Universitat Gottingen, West Germany: "Latent Infection of Monkeys with Herpesvirus Saimiri."

MONDAY MORNING, AUGUST 20

Latency and Immunology of Herpesviruses

Chairman: J. Stevens, University of California, Los Angeles

J.G. Stevens, M.L. Cook and F.B. Knotts, Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles: "Latent Herpetic Infection of the Peripheral and Central Nervous Systems."

J.R. Baringer and P. Swoveland, Neurology Research Laboratory, Veterans Administration Hospital and University of California, San Francisco: "Latent Herpes Simplex Virus Infection in Man and Animals."

T.C. Merigan, L.E. Rasmussen, D.A. Stevens and G.W. Jordan, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, California: "Lymphocyte Interferon Production and Transformation after Herpes Simplex Infection in Humans."

F.A. Ennis, Department of Medicine, Boston University Medical Center, Boston, Massachusetts: "The Effect of Antibody and Sensitized Spleen Cells on Herpes Simplex Infection."

P. Roane, Department of Microbiology, College of Medicine, Howard University, Washington, D.C.: "IgA, IgG, and IgM Antibodies in Human Sera Reactive with Membrane Antigens of Intact Herpesvirus Infected Cells."

P.A. Brunell and A. Gershon, Department of Pediatrics, New York University School of Medicine, New York: "The Antibody Response to Membrane Antigen during the Various Stages of Varicella-Zoster Infections."

BACTERIOPHAGE MEETINGS

LYTIC SECTION

Arranged by
C. Anderson, Cold Spring Harbor Laboratory

Attended by 90 Participants

THURSDAY, AUGUST 23

M. Vallee, Biochimie Medicale, Faculte de Medicine, Marseille, Fran. . "Further Studies on the Immunity Function of Bacteriophage T_4."

D.P. Snustad, S.K. Harlander, K.A. Parson, H.R. Warner, D.J. Tutas, J. Wehner and J.F. Koerner, University of Minnesota: "Mutants of Bacteriophage T4 Deficient in the Ability to Induce Nuclear Disruption."

L.W. Black, Biochemistry Department, University of Maryland Medical School: "Restriction of Phage T4 Internal Protein I Mutants by a Strain of *E. coli*."

T. Homyk and Jon Weil, Vanderbilt University, Nashville, Tennessee: "Deletion Analysis in Phage T4."

A. Rodriguez and Jon Weil, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "Mutants in a New Non-essential Gene of T4 are Lethal in a Mutant of *Escherichia coli*."

FRIDAY MORNING, AUGUST 24

M. Fuke, Laboratory of Molecular Biology, NIAMDD, NIH, Bethesda, Maryland: "Cleavage of RNA-bacteriophage RNAs into Large Fragments for Determining Their Complete Base Sequences."

R. Kramer, M. Rosenberg and J. Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "Terminal Sequences of *in Vivo* T7 Early RNAs."

F. Hagen, J. Hopper, G. Ko and T. Young, Department of Biochemistry, University of Washington, Seattle: "*In Vitro* Translation of *in Vivo* T7 mRNA."

T. Young, Department of Biochemistry, University of Washington, Seattle: "Early T4 mRNA Synthesis in Polarity-suppressing (SuA) Strains of *E. coli*."

D. Taylor and A. Guha, Department of Microbiology, Erindale College, University of Toronto, Toronto, Canada: "Asymmetric Transcription during Bacteriophage F1 Development in *Clostridium* Sporogenes."

L. Snyder, R. Maier and D. Montgomery, Michigan State University, East Lansing: "Genetic Experiments Showing That the Host RNA Polymerase Is Directly Required for T4 DNA Replication."

K.V. Chace, J.R. Johnson and D.H. Hall, Duke University Medical Center, Durham, North Carolina: "Alterations in Gene Expression by Folate Analog Resistant Mutants of T4."

J.D. Karam and M.G. Bowles, Biochemistry Department, Medical University of South Carolina, Charleston: "R9: An Overproducer of T4 'Early' Proteins."

L.J. Heere, Department of Biochemistry, Medical University of South Carolina, Charleston: "Temporal Expression of the T4 rII Gene."

R. Horvitz, Department of Biology, Harvard Biological Laboratories, Cambridge: "Polypeptide Bound to the Host RNA Polymerase Is Specified by T4 Control Gene 33."

T. Fox and J. Pero, Biological Laboratories, Harvard University, Cambridge: "A Complex of RNA Polymerase with Five Proteins Synthesized after Infection of *B. subtilis* with Phage Spo1."

D. Ratner, Department of Biochemistry and Molecular Biology, Harvard Biological Laboratories, Cambridge: "Affinity Binding of Phage Proteins to Immobilized *E. coli* RNA Polymerase."

SATURDAY MORNING, AUGUST 25

M. van Montagu, Laboratory of Histology and Genetics, State University of Ghent, Belgium: "Studies with Conditional Lethal Mutants of RNA Bacteriophage MS2."

W.M. Huang and J.M. Buchanan, Department of Biology, Massachusetts Institute of Technology, Cambridge: "T4 Early Proteins Which Bind to DNA."

D. Rosenthal and P.J. Reid, Department of Biochemistry, University of Connecticut Health Center, Farmington: "Rifampicin Resistant DNA and RNA Synthesis in Phage T4 Infected *Escherichia coli*."

M.G. Wovcha, C.-S. Chiu and P. Tomich, Department of Biological Chemistry, University of Michigan, Ann Arbor: "Direct Role in T4 DNA Synthesis of a Phage-induced Enzyme Forming a Deoxyribonucleotide."

P.K. Tomich, C.-S. Chiu, M. Wovcha and G.R. Greenberg, Department of Biological Chemistry, University of Michigan, Ann Arbor: "Activity of T4 Phage-induced Early Enzymes *in Vivo*."

L.T. Dunham and A.R. Price, Department of Biochemistry, University of Michigan, Ann Arbor: "Properties and Role of the dTTPase-dUTPase Induced by *Bacillus subtilis* Phage ϕe."

SATURDAY EVENING, AUGUST 25

N.V. Hamlett and Hillard Berger, Department of Biology, Johns Hopkins University, Baltimore, Maryland: "A Mutant of Bacteriophage T4 Deficient in Recombination and UV-repair."

Y.-C. Yeh and J.-R. Wu, University of Arkansas, Little Rock: "A Gene Controls on Phenotypic Expression of DNA Arrested Synthesis in Gene 59 Mutants of Bacteriophage T4."

J.R. Christensen and D.F. Figurski, Department of Microbiology, University of Rochester, Rochester, New York: "Phenotype of D_0 Mutants of Phage T1."

D.H. Hall and L.A. Goscin, Duke University Medical Center, Durham, North Carolina: "Degradation and Utilization of Bacterial DNA by Hydroxyurea-sensitive Mutants of T4."

D. Mace, L. Moran, J. Goldberg and B. Alberts, Department of Biochemical Sciences, Princeton University, Princeton, New Jersey: "T4 Bacteriophage Replication Gene Products."

J. Chao, L. Chao and J. Speyer, Biological Sciences Group, Genetics and Cell Biology Section, University of Connecticut, Storrs: "Host DNA Enzymes Affect T4 Head Morphology."

SUNDAY MORNING, AUGUST 26

E. Linney, Department of Microbiology, State University of New York, Stony Brook and M. Hayashi, Department of Biology, University of California, San Diego, La Jolla: "Rifampicin Sensitive Synthesis of the ΦX-174 Gene A Product 'A'."

T. Henry and Rolf Knippers, Friedrich-Miescher-Lab of the Max-Planck-Society, Tubingen, West Germany: "Isolation and *in Vitro* Function of the ΦX-174 Gene A Product—A Highly Specific DNA Endonuclease."

C. Hours and D.T. Denhardt, Department of Biochemistry, McGill University, Montreal, Quebec, Canada: "A Linear Double-stranded Intermediate in ΦX-RF Replication."

L.B. Dumas and C.A. Miller, Department of Biological Sciences, Northwestern University, Evanston, Illinois: "Bacteriophage ΦX-174 DNA Replication in Temperature-sensitive Host DNA Synthesis Mutants."

E.G. Kranias and Lawrence B. Dumas, Department of Biological Sciences, Northwestern University, Evanston, Illinois: "Bacteriophage ΦX-174 DNA Replication in a DNAC Temperature-sensitive Mutant of *Escherichia coli* C."

F.D. Funk, Department of Microbiology, The Pennsylvania State University, College of Medicine, Hershey: "Nonproductive Asymmetric DNA Synthesis by Mutant Infections of ΦX-174."

E.J. Siden and Masaki Hayashi, Department of Biology, University of California, San Diego, La Jolla: "The Role of the Gene B Product in Bacteriophage ϕX-174 Development."

SUNDAY AFTERNOON, AUGUST 26

J.L. Woolford and R.E. Webster, Duke University Medical Center, Durham, North Carolina: "Alterations in Phospholipid Synthesis in f1 Wild Type and *Amber* Mutant Infected Cells."

G.J. Bourguignon, T.K. Sweeney and H. Delius, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Electron Microscopic Studies on the Replication of T5 Phage DNA."

S.M. Berget, R.F. Drong and H.R. Warner, Department of Biochemistry, University of Minnesota, St. Paul: "Isolation and Characterization of a Mutant of Bacteriophage T5 Defective in the Ability to Induce Deoxynucleotide Kinase."

G. Chinnadurai and D.J. McCorquodale, Institute for Molecular Biology, University of Texas at Dallas, Dallas: "Regulation of Expression of Late T5 Genes."

J. Beaudoin and A. Niveleau, Departement de Microbiologie et d'Immunologie, Universite de Montreal and Centre Hospitalier Universitaire, Universite de Sherbrooke, Sherbrooke, Quebec, Canada: "Analysis of Phage M13 Populations with an Electronic Curvemeter System."

R.N.H. Konings, Laboratory of the Molecular Biology, University of Nijmegen, Nijmegen, The Netherlands: "Synthesis of Phage M13 Specific Proteins in a DNA Dependent Cell-free System."

MONDAY, AUGUST 27

C.M. Kerr and P.D. Sadowski, Department of Medical Genetics, University of Toronto, Canada: "Maturation of Bacteriophage T7 DNA. Assay of the Gene 19 Protein *in Vitro*."

L. Onorato and M.K. Showe, Haverford College, Haverford, Pennsylvania: "*In Vitro* Studies of the Gene 21 Dependent Proteolysis of Purified 22 Protein of T4."

U.K. Laemmli, J.A. Wagner, N. Teaff and G. d'Ambrosia, Department of Biochemical Sciences, Princeton University, Princeton, New Jersey: "T4 Head Assembly: Packaging DNA into Preformed Heads."

J. Goldstein and S.P. Champe, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey: "*In Vitro* Formation of a T4 Internal Peptide."

B. Terzaghi and E. Terzaghi, Biology Board, University of California, Santa Cruz: "Gene(s) for the T4 Collar."

Y. Kikuchi and Jonathan King, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts: "Collagenase Sensitive Sites Necessary for T4 Tail Tube Assembly."

M.K. Singh and E. Terzaghi, Biology Board, University of California, Santa Cruz: "Amino Acid Side Groups Involved in T4 Tail Fiber Assembly/Attachment."

LYSOGENIC SECTION

Arranged by
E. Bade, Cold Spring Harbor Laboratory

Attended by 104 Participants

WEDNESDAY, AUGUST 29

D. Henderson and J. Weil, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "A New Class of Bacterial Mutants Affecting Lambda Morphogenesis."

S. Emmons, J. Thomas, V. McCosham and R. Baldwin, Department of Biochemistry, Stanford University, Stanford, California: "Production of Tandem Duplications in Phage Lambda."

L. Randall-Hazelbauer and M. Schwartz, Unite de Genetique Moleculaire, Departement de Biologie Moleculaire, Institut Pasteur, Paris, France: "Isolation of Phage Lambda Receptor from *Escherichia Coli*."

H. Greer, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts: "Properties of Gene KIL of Lambda."

D.A. Grosenbaugh and D.L. Wulff, Department of Molecular Biology and Biochemistry, University of California, Irvine: "Lysogenization of *E. coli* by λc171*cin*-1."

THURSDAY MORNING, AUGUST 30

E. Cassuto, P. Yot and R. Wiegand, Departments of Internal Medicine, Molecular Biophysics and Biochemistry and Human Genetics, Yale University, New Haven, Connecticut: "Isolation and Transcription of Fragments Derived from the Attachment Site of λ"

B. Allet and J. Manley, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "DNA Fragments Cleaved from the λImmunity Region by Various Restriction Enzymes."

E.G. Bade and H. Delius, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "On the Structure of Mu-1 DNA."

W. Wackernagel and C. Radding, Departments of Medicine and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "Recombination of Phage λDNA *in Vitro*: Formation of Biologically Active Joint Molecules by the Sequential Action of λExonuclease and 32 Protein."

M. Syvanen, Department of Biochemistry, Stanford University Medical Center, Stanford, California: "*In Vitro* Genetic Recombination of Bacteriophage Lambda."

THURSDAY EVENING, AUGUST 30

G. Pruss, R. Goldstein, J. Lengyel and R. Calendar, Department of Molecular Biology, University of California, Berkeley: "*In Vitro* Head Morphogenesis of Phage P2 and Its Satellite Phage P4."

J. King and S. Casjens, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Head Forming 'Enzyme' in P22 Assembly."

J. Jarvik and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Genetic Determination of the Ordered Events in Phage P22 Assembly."

J. Edelman and J. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Analysis of P22 Late Functions by Gene Dosage Experiments."

B.K. Tye, J.A. Huberman and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: "On the Circular Permutation of the Phage P22 Chromosome."

M.M. Susskind and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Inactivation of λRepressor by P22 Antirepressor."

FRIDAY MORNING, AUGUST 31

C.J. Panchal and A. Guha, Microbiology Department, Erindale College, University of Toronto, Toronto, Canada: "Divergent Orientation of Transcription from the Arginine Gene ECBH Cluster of *Escherichia coli*."

P. Ray and M. Pearson, Department of Medical Genetics, University of Toronto, Toronto, Canada: "Transcription of the Late Genes of λ."

S. Nakanishi, S. Adhya, M. Gottesman and I. Pastan, Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland: "Activation of Transcription at Specific Promoters by Glycerol."

N. Kleckner, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Lambda $N^{-}CRO^{-}$, Plasmid Formation, and x-O-P Transcription."

K. Krell, LGCB, NIMH, Bethesda, Maryland and E.D. Jacobson, Department of Biology, Georgetown University, Washington, D.C.: "Formation of Antisense Message during Biotin Escape Synthesis."

S. Adhya, M.E. Gottesman, B. de Crombrugghe and D.L. Court, Laboratory of Molecular Biology, NCI, NIH, Bethesda, Maryland: "Termination Factor *RHO* and Polarity—Release of Polarity by λ*N*-Function."

C. Dambly and J.-P. Lecocq, Departement de Biologie Moleculaire, Universite Libre de Bruxelles, Belgium: "Interference of Bacterial Mutations with the Expression of Viral Regulatory Genes."

FRIDAY EVENING, AUGUST 31

H. Drexler, Department of Microbiology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina and J.R. Christensen, Department of Microbiology, University of Rochester Medical Center, Rochester, New York: "The Transduction of λpfu by Coliphage T1."

J.L. Rosner, NIH, Bethesda, Maryland: "Specialized Transduction of *pro* Genes by Coliphage P1."

N. Sternberg and R. Weisberg, NICHD, NIH, Bethesda, Maryland: "Packaging of DNA by λ."

E.G. Bade and L. Buzzo, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Preferential Generalized Transduction of Some *E. coli* K-12 Markers by Temperate Bacteriophage Mu-1."

M.M. Howe and David Zipser, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Possible Transduction by the 'Split Ends' of Bacteriophage Mu-1."

SATURDAY MORNING, SEPTEMBER 1

J. Geisselsoder, Department of Molecular Biology, University of California, Berkeley: "Some Properties of A P2 DNA - Membrane Complex."
R. McMacken, H. Moerck and R. Boyce, Department of Biochemistry, University of Florida, Gainesville: "Nicking of Superhelical λDNA and Other Studies on DNA Replication in dna- Hosts."
S. Takahashi and R.B. Inman, Biophysics Laboratory and Biochemistry Department, University of Wisconsin, Madison: "The Role of Gene O and P Products in λDNA Synthesis."
M.M. Gottesman, M.E. Gottesman, S. Gottesman and M. Gellert, NIH, NIAMDD and NCI, Bethesda, Maryland: "Heteroduplex Mapping of λ Reverse."
S. Hilliker and D. Botstein, Massachusetts Institute of Technology, Cambridge: "Comparison of P22 and λ Replication and Control by Means of λ *IMM* P22 Hybrid Phages."
W. Schroder, E.G. Bade and H. Delius, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "*E. coli* DNA Sequences in Replicating Intermediates of Mu-1 DNA."

SATURDAY EVENING, SEPTEMBER 1

H.A. Nash, NIMH, Bethesda, Maryland: "Integrative Recombination."
S. Gottesman and Max Gottesman, NCI, NIH, Bethesda, Maryland: "Elements of Site-specific Recombination in λ."
S. Chung, L. Green and H. Echols, Department of Molecular Biology, University of California, Berkeley: "Control of the INT Reaction: Constitutivity in a Lysogen and Mutants with a Specific Defect in Integrative Recombination."
K. Shimada and A. Campbell, Department of Biological Sciences, Stanford University, Stanford, California: "INT-Constitutive Mutants of Bacteriophage Lambda."
W. Schroder, University of Cologne, Germany and Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; and P. van de Putte, Medical Biological Laboratory, Rijswijk, The Netherlands: "Genetical and Physical Study on Prophage Mu Excision."
A.I. Bukhari, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: "Excision of Mu DNA from the Host Chromosome."

SUNDAY, SEPTEMBER 2

P. Tavernier, K. Barta and J. Zissler, Department of Microbiology, University of Minnesota, Minneapolis: "New Recombination Genes of Bacteriophage Lambda."
J. Lehman and R. Roehrdanz, McArdle Laboratory, University of Wisconsin, Madison: "A b2-specified Inhibitor of Site-specific Recombination."
R. Gayda, S. Hua and A. Markovitz, Department of Microbiology, University of Chicago, Illinois: "Increased N Gene Function Due to a Bacterial Mutation, *lon*- (*cap*R9)."
J. Schell, M. Holsters, N. van Larebeke, I. Zanen, R. Schilperoort, H. Teuchy and M. van Montagu, Laboratory for Genetics and Laboratory for Histology and Genetics, State University of Ghent, Belgium and Laboratory for Biochemistry, University of Leiden, The Netherlands: "A Cryptic Prophage Associated with a Circular DNA Plasmid in *Agrobacterium Tumefaciens*."
A.L. Taylor and N.S. Gonzalez, University of Colorado Medical Center, Denver: "Genetic Instability of Strains Dilysogenic for Phage Mu-1."

RIBOSOMES MEETING

Arranged by
P. Lengyel, Yale University, M. Nomura, University of Wisconsin, and
A. Tissieres, University of Geneva

Attended by 169 Participants

TUESDAY, SEPTEMBER 4

Introduction: Alfred Tissieres, University of Geneva, Switzerland

WEDNESDAY MORNING, SEPTEMBER 5

Structure of Procaryotic Ribosomes. Part 1

Chairman: Pierre Francois Spahr, University of Geneva, Switzerland

B. Wittman-Liebold, Max-Planck-Institut fur Molekulare Genetik, Berlin-Dahlem, Germany: "Studies on the Primary Structure of *E. coli* Ribosomal Proteins."
H.G. Wittmann, Max-Planck-Institut fur Molekulare Genetik, Berlin-Dahlem, Germany: "Mutationally Altered Proteins in *E. coli* Ribosomes."

K. Higo and L. Kahan, Departments of Biochemistry and Genetics, and the Institute for Enzyme Research, University of Wisconsin, Madison: "Structural Correspondence between *E. coli* (E) and *B. stearothermophilus* (B) 30S Ribosomal Proteins."

P. Fellner and J.P. Ebel, Laboratorie de Chimie Biologique, Faculte des Sciences, Strasbourg, France: "The Primary Structure of High Molecular Weight Ribosomal RNA."

R.A. Zimmerman, G.A. Mackie and P. Spierer, Departement de Biologie Moleculaire, Universite de Geneve, Geneva, Switzerland: "Location and Structure of Protein Binding Sites on 16S and 23S Ribosomal RNAs."

A.E. Dahlberg, Brown University, Providence, Rhode Island and J.E. Dahlberg, University of Wisconsin, Madison: "Multiple Conformations of 30S Ribosomal Subunits and 16S rRNA in *E. coli*."

R. Monier and J. Feunteun, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: "5S RNA and Its Interaction with Ribosomal Proteins."

V.A. Erdmann, J.R. Horne, O. Pongs, M. Sprinzl* and J. Zimmerman, Max-Planck-Institut fur Molekulare Genetik, Berlin-Dahlem and *Experimentelle Medezin, Gottingen, Germany: "5S RNA: Its Structure and Function."

H. Noll, Department of Biological Science, Northwestern University, Evanston, Illinois.

WEDNESDAY EVENING, SEPTEMBER 5

Structure of Procaryotic Ribosomes. Part 2

Chairman: Alexander Rich, Massachusetts Institute of Technology, Cambridge

K.E. van Holde, Department of Biochemistry and Biophysics, Oregon State University and W.E. Hill, Department of Chemistry, University of Montana: "General Physical Properties of Ribosomes."

J.A. Lake and D.D. Sabatini, Department of Cell Biology, New York University School of Medicine, New York: "Structural Studies on Ribosome Crystals Isolated from Hypothermic Chicken Embryos."

A.D. Wolfe, P. Dessen, D. Pantaloni, T. Godefroy-Colburn, M. Graffe, J. Dondon and M. Grunberg-Manago, Institut Biologie Physico-chimique, Paris, France and L. Enzymologie, 91190 Gif-sur-Yvette, France: "Studies on the Reversible Association of Ribosomes."

G.R. Craven and L. Changchien, Laboratory of Molecular Biology, University of Wisconsin, Madison: "Chemical Approaches to the Determination of Ribosome Architecture."

L.C. Lutter, U. Bode, H. Zeichhardt, G. Stoffler and C.G. Kurland, Wallenberg Laboratory, Uppsala University, Sweden and M-P-I fur Molekulare Genetik, Berlin, Germany: "Ribosomal Protein Neighborhoods."

R.R. Traut*, T-T. Sun*. A. Bollen* and L. Kahan†, *Department of Biological Chemistry, School of Medicine, University of California, Davis; †Institute of Enzyme Research, University of Wisconsin, Madison: "Crosslinking of *E. coli* 30S Ribosomal Proteins."

P.B. Moore and D.M. Engelman, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut and B.P. Schoenborn, Department of Biology, Brookhaven National Laboratory, Upton, New York: "On the Distribution of RNA and Protein in the Ribosomal Subunits of *E. coli*."

C.R. Cantor, K.H. Huang, L.S. Gennis and R. Fairclough, Departments of Chemistry and Biological Sciences, Columbia University, New York: "Fluorescence Techniques for Studying Ribosome Structure."

A. Rich, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts: "What the Structure of Transfer RNA Tells Us about the Ribosome."

THURSDAY MORNING. SEPTEMBER 6

Functional Role of Molecular Components. Part 1

Chairman: Severo Ochoa, New York University

W.A. Held, W.R. Gette and M. Nomura, Institute for Enzyme Research and Departments of Biochemistry and Genetics, University of Wisconsin, Madison: "30S Ribosomal Components Affecting the Specificity of Initiation of Protein Synthesis in the Translation of R17 RNA."

M.L. Goldberg and J.A. Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "Cistron Specificity of 30s Ribosomes Heterologously Reconstituted with Components from *E. coli* and *B. stearothermophilus*."

W. Szer and S. Leffler, Department of Biochemistry, New York University School of Medicine, New York: "Ribosomal Specificity in the Translation of Phage RNAs."

A. Bollen*, L. Kahan†, J.W.B. Hershey*, A. Cozzone* and R.R. Traut*, *Department of Biological Chemistry, School of Medicine, University of California, Davis; †Institute for Enzyme Research, University of Wisconsin, Madison: "Identification of the Binding Site for Initiation Factor If-2 on the *E. coli* 30S Ribosome by Crosslinking with Bis-suberimidate."

M. Pellegrini, H. Oen, D. Eliat* and C.R. Cantor, Departments of Chemistry and Biological Sciences, Columbia University, New York. *Permanent address, The Hebrew University: "Affinity Labeling of the Ribosomal P Site."

E. Kuechler, A.P. Czernilofsky, K. Bauer, E. Collatz and G. Stoffler, Institut fur Biochemie, University of Vienna, Austria and Max-Planck-Institut fur Molekulare Genetik, Berlin, Germany: "Affinity Label for the tRNA Binding Sites of *E. coli* Ribosomes."

H. Matthaei, L. Bispink, F. Beesk, M. Doedens, U. Ucer and E. Bermek, Max-Planck-Institut fur Experimentelle Medizin, Gottingen, Germany: "Affinity Labeling of Ribosomal Binding Sites."

O. Pongs, R. Bald and V.A. Erdmann, Max-Planck-Institut fur Molekulare Genetik, Berlin-Dahlem, Germany: "Affinity-Labeling of Bacterial Ribosomes with Antibiotic Analogs."

D. Elson, A. Zamir, R. Miskin, L. Ginzburg, N. Sonenberg, M. Wilchek and P. Spitnik-Elson, Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel: "Studies of *E. coli* Ribosomal Proteins."

P.I. Schrier, J.A. Maassen and W. Moller, Department of Physiological Chemistry, State University, Leiden, The Netherlands: "Ribosomal Components Involved in Elongation Factor EF-G and EF-T Dependent Hydrolysis of GTP."

N. Brot, R. Marcel, A.H. Lockwood*, U. Maitra†, W.P. Tate‡, C.T. Caskey‡, and H. Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey, *Princeton University, Princeton, New Jersey, †Albert Einstein College of Medicine, Bronx, New York and ‡Baylor College of Medicine, Houston, Texas: "The Requirement for Ribosomal Proteins L_7 and L_{12} in Protein Synthesis."

J.P.G. Ballesta and D. Vazquez, Inst. Biologia Celular, Madrid, Spain: "Chemical Alteration of Ribosomal Functions and Proteins."

J. Petre, Y. Pollack and H. Inouye, Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel: "Structural Relationship between Ribosomal Protein S1 and Interference Factor i."

THURSDAY EVENING, SEPTEMBER 6

A. Functional Role of Molecular Components. Part 2

Chairman: Marianne Grunberg-Manago, Institut de Biologie Physico-Chimique, Paris

G. Stoffler, Max-Planck-Institut fur Molekulare Genetik, Berlin-Dahlem: "Immunochemical Approaches to the Study of Ribosome Structure and Function."

J.C. Lelong, R. Stoffler, A. Bollen, M. Lazar and F. Gros, Pasteur Institute, Paris and Max-Planck-Institut of Molecular Genetics, Berlin, Germany: "30S Proteins as Elements of the Ribosomal Decoding Sites."

W.P. Tate and C.T. Caskey, Department of Medicine, Section of Medical Genetics, Baylor College of Medicine, Houston, Texas: "Polypeptide Chain Termination."

H.F. Noller, Thimann Laboratories, University of California, Santa Cruz: "Sites of Chemical Modification of 16S Ribosomal RNA."

B. Biosynthesis of Procaryotic Ribosomes. Part 1

Chairman: Joel Flaks, University of Pennsylvania, Philadelphia

S.R. Jaskunas, F. Engbaek and M. Nomura, Institute for Enzyme Research, University of Wisconsin, Madison: "Amber Mutants of a Ribosomal Protein Operon in *E. coli*."

B. Jarry, C. Vola and R. Rosset, C.N.R.S., Centre de Biologie Moleculaire, Marseille, France: "Ribosomal RNA Genes in *Escherichia coli*."

S. Osawa, A. Muto, R. Takata and A. Kimura, Hiroshima University, Japan: "Mapping of Ribosomal Protein Genes and Regulation of rRNA Synthesis in *B. subtilis*."

I. Smith, Department of Microbiology, The Public Health Research Institute of the City of New York, Inc., New York: "Genetics of Ribosomes in *Bacillus subtilis*."

P.S. Sypherd, R.E. Bryant, T. Fujisawa and K. Dimmitt, Department of Medical Microbiology, University of California, Irvine: "Ribosome Assembly Mutants of *E. coli*."

FRIDAY MORNING, SEPTEMBER 7

Biosynthesis of Procaryotic Ribosomes. Part 2

Chairman: Klaus Weber, Harvard University, Cambridge and Cold Spring Harbor Laboratory

J. Gallant, T. Laffler and B. Harada, Genetics Department, University of Washington, Seattle.

R. Block and W.A. Haseltine, Department of Biophysics, Harvard University, Cambridge, Massachusetts: "Synthesis of Guanosine Tetra- and Penta-Phosphate."

N.O. Kjeldgaard, F. Skou Pedersen and E. Lund, Department of Molecular Biology, University of Aarhus, Denmark: "tRNA Dependent *in vitro* Synthesis of Guanosinetetra and Pentaphosphates."

J. Sy and Y. Ogawa, The Rockefeller University, New York: "Nonribosomal Synthesis of Magic Spot Compounds.

E. Hamel and M. Cashel, LMG, NICHD, National Institutes of Health, Bethesda, Maryland: "The Interaction of pppGpp with GTP-requiring Protein Synthetic Factors of *Escherichia coli*."

A. Travers and R. Cukier-Kahn, MRC Laboratory of Molecular Biology, Cambridge, England: "Effect of Hi-Protein on rRNA Synthesis *in vitro*."

N.R. Pace, M.L. Sogin and J. Cuneo, National Jewish Hospital and Research Center and University of Colorado School of Medicine, Denver: "Precursors of 5S Ribosomal RNA in *Bacillus subtilis*."

N. Nikolaev, L. Silengo, C.N. Kwan, S. Gotoh and D. Schlessinger, Department of Microbiology, Washington University School of Medicine, St. Louis: "Enzymatic Processing of RNA in *E. coli* and HeLa."

J.H. Alix, D.H. Hayes, F. Hayes and M. Vasseur, Laboratorie de Chimie Cellulaire, Institut de Biologie Physico-Chimique, Paris, France: "Metabolism of Ribosomal Protein and RNA during Ribosome Biosynthesis."

P. Thammana and J. Davies, Department of Biochemistry, University of Wisconsin, Madison: "Methylation of 16S RNA during Ribosome Assembly."

L. Lindahl, University Institute of Microbiology, Oster Farimagsgade 2A, Copenhagen, Denmark: "*In Vivo* Assembly of *E. coli* Ribosomes."

FRIDAY EVENING, SEPTEMBER 7

A. Biosynthesis of Procaryotic Ribosomes. Part 3

Chairman: N.O. Kjeldgaard, University of Aarhus, Denmark

H. Bremer and P. Dennis, Institute for Molecular Biology, University of Texas at Dallas: "Control of the Synthesis Rate of Ribosomal Protein in *E. coli*."

P.P. Dennis and R. Young, University of Texas at Dallas and Institute for Enzyme Research, University of Wisconsin, Madison: "Control of the Synthesis of Ribosomal Proteins in *Escherichia coli* B/r."

E. Kaltschmidt, L. Kahan and M. Nomura, The Institute for Enzyme Research and Department of Biochemistry and Genetics, University of Wisconsin, Madison: "*In vitro* Synthesis of Ribosomal Proteins."

B. Effects of Antibiotics and Other Agents on Ribosomes

Chairman: Fritz Lipmann, Rockefeller University, New York

R.T. Garvin, R. Rosset and L. Gorini, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston: "Ribosomal Assembly Influenced by Growth in the Presence of Streptomycin."

R.S. Zitomer, G.L. Kreider, W. Wind, C.J. Fetterolf and J.G. Flaks, Department of Biochemistry, University of Pennsylvania Medical School, Philadelphia: "The Influence of Mutations at the Streptomycin Locus on 30S Subunit Conformation."

B. Brownstein, D. Viceps and G. Kreider, Department of Biology, Temple University, Philadelphia, Pennsylvania: "Effects of Streptomycin on Streptomycin-dependent Strains and Revertants from Dependence: An Attempt to Correlate *in vitro* Findings with Effects on Growing Cells."

P.F. Sparling, Y. Ikeya and G.C. Foster, Departments of Medicine and Bacteriology, University of North Carolina School of Medicine, Chapel Hill: "Erythromycin Dependent Mutant of *E. coli*."

B. Weisblum, Department of Pharmacology, University of Wisconsin Medical School, Madison: "Inducible Methylation of 23S rRNA in *Staphylococcus Aureus* Associated with Resistance to the Macrolide Lincosamide and Streptogramin B-type Antibiotics."

P.-C. Tai, B.J. Wallace and B.D. Davis, Bacterial Physiology Unit, Harvard Medical School: "Interactions of Various Antibiotics with Ribosomes in Different Stages of Their Cycle."

SATURDAY MORNING, SEPTEMBER 8

A. Effect of Phages and Colicins on Ribosomes

Chairman: Peter Lengyel, Yale University, New Haven, Connecticut

K. Jakes and N.D. Zinder, The Rockefeller University, New York: "Mechanism of Action of Colicin E3 and Its Immunity Protein."

J. Sidikaro and M. Nomura, Institute for Enzyme Research, University of Wisconsin, Madison: "Inactivation of Ribosomes by Colicin E3 and Isolation of E3-immunity Substance."

B. Structure of Eukaryotic Ribosomes

Chairman: Jonathan Warner, Albert Einstein College of Medicine, Bronx, New York

I.G. Wool, Department of Biochemistry, University of Chicago: "Structure and Function of Eukaryotic Ribosomes."

G.A. Howard, J.A. Traugh and R.R. Traut, Department of Biological Chemistry, School of Medicine, University of California, Davis: "Number, Molecular Weights and Stoichiometry of Ribosomal Proteins from Rabbit Reticulocytes."

H. Gould, Department of Biophysics, King's College, London: "Description of Eukaryotic Ribosomal Protein."

R.A. Cox and Elizabeth Godwin, National Institute for Medical Research, Mill Hill, London: "Unusual Features of the Major Ribonucleic Acid Species of the Larger Subribosomal Particle of Eukaryotes."

I. Sundkvist and T. Staehelin, Basel Institute for Immunology, Basel, Switzerland: "Structural and Functional Aspects of Free 40S Ribosome Subunits in Mammalian Cells."

A. Krystosek and D. Kabat, Department of Biochemistry, University of Oregon Medical School, Portland, Oregon: "Phosphorylation of Ribosomal Proteins in Eukaryotes."

M.G. Hamilton, Memorial Sloan-Kettering Cancer Center, New York: "Studies of the Structure of Rat Liver Ribosomes."

SATURDAY AFTERNOON, SEPTEMBER 8

Biosynthesis of Eukaryotic Ribosomes

Chairman: Joseph Gall, Yale University, New Haven, Connecticut

J.R. Warner, Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: "The Assembly of Ribosomes in Eukaryotes."

B.E.H. Maden, M. Salim and J.S. Robertson, Department of Biochemistry, University of Glasgow, Glasgow, Scotland: "Progress in the Structural Analysis of Mammalian 45S RNA."

T.L. Helser and C.S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California, Irvine: "Mutations in Yeast that Affect the Ribosome."

P.K. Wellauer and I.B. Dawid, Carnegie Institution of Washington, Department of Embryology, Baltimore, Maryland: "Secondary Structure Maps of rRNA Molecules as Determined by Electron Microscopy."

B.B. Spear, Department of Biology, Yale University, New Haven, Connecticut: "Differential Replication of Ribosomal Genes in Eukaryotes."

T. Honjo and R.H. Reeder, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: "Repression of *Xenopus Mulleri* Ribosomal RNA Synthesis in Interspecies Hybrids between *X. Laevis* and *X. Mulleri*."

R.L. Hallberg, Division of Biology, Cornell University, Ithaca, New York: "Ribosomal Protein Synthesis in Interspecific Hybrids of *Xenopus*."

SUNDAY, SEPTEMBER 9

A. Biosynthesis of Organelle Ribosomes

Chairman: David Luck, Rockefeller University, New York

D. Luck, Rockefeller University, New York: (Title to be Announced)

G. Schlanger, N. Ohta, M. Inouye and R. Sager, Biology Department, Hunter College, New York: "Mutations of Chloroplast Genes Alter Chloroplast Ribosomal Activity and Proteins in Chlamydomonas."

J.E. Boynton, E.H. Harris, N.W. Gillham, M.F. Conde and W.G. Burton*, Duke University and University of Notre Dame*: "Do Non-Mendelian Antibiotic Resistant Mutations Affect both Chloroplast and Mitochondrial Ribosomes?"

L. Bogorad, J.N. Davidson, L.J. Mets and M.R. Hanson, Biological Laboratories, Harvard University, Cambridge, Massachusetts: "Genes Which Affect Proteins of Chloroplast Ribosomes."

B. Concluding Remarks

Chairman: Masayasu Nomura, University of Wisconsin, Madison

W. Gilbert, Harvard University, Cambridge

These meetings were partially supported by grants from the National Science Foundation, American Cancer Society, National Cancer Institute and Robertson Research Fund.

Abstracts of the meetings were mailed to all who subscribe to the Laboratory's Abstracting Service.

IN-HOUSE SEMINARS

Cold Spring Harbor in-house seminars were initiated to be 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 provided a necessary opportunity for the graduate students and post-graduate staff to develop their skills in defending, organizing, and presenting their research. In addition to those listed below, seminars were given by many of the people who are involved in research at this laboratory.

September

15th. Dr. Dan Kolakofsky, Department of Molecular Biology, University of Geneva, Switzerland: "Sendai RNA."

October

13th. Dr. Arthur H. Lockwood, Albert Einstein College of Medicine, Bronx, New York: "The Mechanism of Initiation of Protein Synthesis in *E. coli*."

17th. Dr. Donna Lindstrom, University of California, San Diego, California: "Polyoma Transcription."

27th. Dr. John Newbold, University of North Carolina, Chapel Hill, North Carolina: "Mapping of SV40 RNA Species from Lytically Infected and Transformed Cells with the Aid of Restriction Enzymes."

November

1st. Dr. John Atkins, Trinity College, Dublin: "External Suppressors Specific for Frameshift Mutants, and Suppressors Which Suppress both Frameshift and UGA Mutants."

3rd. Dr. L.V. Melendez, Primate Research Center, Harvard Medical School, Southborough, Massachusetts: "Oncogenic Herpes Viruses of Monkeys."

10th. Dr. Larry Chasin, Biological Sciences Department, Columbia University, New York, New York: "Mutagenesis and Linkage in Cultured Chinese Hamster Cells."

16th. Dr. Tom Broker, Department of Chemistry, California Institute of Technology, Pasadena, California: "An Electron Microscopic Analysis of Pathways for T4 DNA Recombination."

17th. Dr. Paul Schendel, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin: "Initiation of DNA Synthesis *in vitro*."

20th. Dr. Ferruccio Ritossa, University of Bari, Italy: "The Process of rDNA Magnification in *Drosophila*."

December

1st. Dr. Herbert Boyer, Department of Microbiology, University of California, San Francisco Medical Center, San Francisco, California: "R-factor Controlled Restriction and Modification of DNA: Enzymes and Substrates."

4th. Dr. Stewart Austin, Department of Microbiology, Harvard Medical School, Boston, Massachusetts: "Cell Free Synthesis of *E. coli* RNA Polymerase Subunits."

8th. Dr. Janet Butel, Baylor College of Medicine, Texas Medical Center, Houston, Texas: "Infectious DNA in SV40 Transformed Cells."

15th. Dr. Warren Gibbs, Department of Molecular Biology, University of California at Berkeley, California: "Satellite Bacteriophage P_4: P_2 Makes What P_4 Takes."

29th. Dr. James Darnell, Department of Biological Sciences, Columbia University, New York, New York: "The Origin of mRNA in Mammalian Cells."

January

5th. Dr. Abraham Hsie, Oak Ridge National Laboratory, Oak Ridge, Tennessee: "Control of Phenotypic Expression by Cyclic AMP and Hormones in Mammalian Cells."

10th. Dr. Clark Tibbetts, Department of Microbiology, The Wallenberg Laboratory, Uppsala, Sweden: "Approaches to Physical Gene Mapping of Adenoviruses: Formamide and Thermochromatography."

19th. Dr. Stuart Aaronson, Viral Carcinogenisis Branch, National Cancer Institute, Bethesda, Maryland: "Biology of Mammalian RNA Tumor Viruses."
Dr. Ed. Ziff, MRC Laboratory of Molecular Biology, Cambridge, England: "Determination of Nucleotide Sequences in DNA."

23rd. Dr. Fred Blattner, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin: "Sequencing of λ Transcription Products."

26th. Dr. Ted Gurney, Department of Molecular Biology, University of California at Berkeley, California: "Bouyant Densities and Polymerase Activities of Some Isolated Nuclei."

February

13th. Dr. Dan Rifkin, Rockefeller University, New York, New York: "An Enzyme Associated with Oncogenic Transformation."

16th. Dr. Peter Carlson, Department of Biology, Brookhaven National Laboratory, Upton, New York: "The Somatic Cell Genetics of Higher Plants."

19th. Dr. D. Bottstein, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts: "On the Many Ways One Virus Can Prevent Another Virus from Growing."

March

2nd. Dr. Rolf Sternglanz, Department of Biochemistry, SUNY at Stony Brook. New York: "Studies on the Mechanism of DNA Chain Growth in *Bacillus Subtilis.*"

7th. Dr. Tom Maniatas, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: "The Structure of λ Operators."

9th. Dr. Aaron Shatkin, Hoffman-LaRoche Institute, Nutley, New Jersey: "Structure and Function of Reovirus."

15th. Dr. John Watkins, Department of Pathology, University of Oxford, England: "*In situ* Hybridization Studies with SV40 Transformed Rabbit Cells."

16th. Dr. J. Kallos, Department of Microbiology, Columbia University, New York, New York: "Is the Site of Action of Cyclic AMP Intracellular or at the Cell Surface?"

April

6th. Dr. Kenneth F. Watson, Robert Koch Institute, Berlin, Germany: "The Properties of Reverse Transcriptase and its Potential Role in the Replication of Viral RNA."

11th. Dr. Larry Gold, University of Colorado, Boulder, Colorado: "The T_4 Development *in vitro.*"

17th. Dr. Paul Hough, Department of Physics, Brookhaven National Laboratory, Upton, New York: " Λ λ ."

24th. Dr. George Stark, Stanford University, Stanford, California: "X-Antigen of SV40".
Dr. Alvin Markovitz, University of Chicago, Chicago, Illinois: "The *lon* Syndrome: Cell Division, UV Sensitivity and Operon Expression."

May

3rd. Dr. U.K. Laemmli, Department of Biological Chemistry, Princeton University, Princeton, New Jersey: "Packaging of DNA during T4 Assembly."

7th. Dr. Ronald Davis, Department of Biochemistry, Stanford University, Stanford, California: "*In vitro* Joining of DNA Molecules."

8th. Dr. Olke C. Uhlenbeck, Department of Biochemistry, University of Illinois, Urbana, Illinois: "Stepwise Synthesis of Oligoribonucleotides and Binding Them to Nucleic Acids."

July

20th. Dr. Walter Mangel, University of California at Berkeley, California: "*In vitro* Studies of Initiation of Transcription by *E. coli* RNA Polymerase with T7 DNA."

UNDERGRADUATE RESEARCH PARTICIPATION PROGRAM

Summer 1973

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 147 students have completed the course.

The objectives of the program are (1) greater understanding of the fundamental principles of biology; (2) increased awareness of major problem areas under investigation; (3) increased awareness of physical and intellectual tools for modern research and the pertinence of this information to future training; and (4) personal acquaintance with research, research workers, and centers for study.

The following students, listed with topics of research and Laboratory sponsors, were enrolled in the program, sponsored this year with Laboratory funds.

James B. Breitmeyer, University of California, Santa Cruz *Supervisor:* Richard Roberts	Purification and characterization of a new restriction endonuclease from *Hemophilus aegyptius*
Robert Heimer, Columbia University *Supervisor:* Peter Greenaway	ATP-dependent DNA methylases and endonucleases in chicken embryos
Helen Hollingsworth, Brown University *Supervisor:* Phillip Sharp	Mapping of *Hemophilus parainfluenzae* fragments of adeno 2 DNA
James F. Jackson, Jr., Princeton University *Supervisor:* R.F. Gesteland	Isolation and sequencing of a tyrosine suppressor tRNA from yeast
Angus P. McIntyre, Harvard University *Supervisor:* A.I. Bukhari	Study of an unusual growth-factor-requiring mutant of *Escherichia coli*
Bernard H. Shen, Harvard University *Supervisor:* Peter Greenaway	A comparative study on DNA methylases in various rabbit tissues
T. Kevin Sweeney, Cornell University *Supervisor:* Hajo Delius	Partial denaturation of phage T5 DNA
Nina F. Tabachnik, Yale University *Supervisor:* Richard Roberts	Purification and characterization of a second new restriction endonuclease from *Hemophilus aegyptius*
Paula Traktman, Radcliffe College *Supervisors:* Martha Howe & David Zipser	Mutants of bacteriophage Mu-1 defective in lysogenization
Mariana Wolfner, Cornell University *Supervisor:* R.F. Gesteland	*In vitro* protein synthesis in a wheat germ system using natural messages

NATURE STUDY COURSES

The Children's Nature Study Program was designed to organize young people who want to learn more about the out-of-doors and to help them learn the answers, or how to discover the answers, to many questions that arise from everyday observation of nature. One of the

primary functions of the courses is to stimulate an interest in nature by observation and the study of the flora, fauna and geology of the area around Cold Spring Harbor.

The Laboratory provided a headquarters building (Jones Laboratory) and much of the laboratory and field equipment. Some courses were held at Uplands Farm this year. A total of 374 students participated in the 1973 summer program.

The Huntington Federal Savings and Loan Association donated the scholarships for 16 children. The Three Harbors Garden Club also contributed three scholarships.

As a service to the community, two free courses were offered high school students to study the ecology of Cold Spring Harbor. Mr. Richard Rosenman directed these programs which cooperated with the Town of Huntington project for the study of the inner harbor. This project now in process is being partially supported with Laboratory funds, space and equipment.

STAFF

Thomas Stock, M. Ed., Earth Science Teacher, Centereach, N.Y.; Program Director
Leslee Cattrall, University of Chicago
Mimi Cattrall, Huntington High School graduate
Don Feeney, Brown University
Tom Hedberg, Syracuse University
Frances Jeppesen, M. S., Oxford University; Cold Spring Harbor Laboratory
Fred Maasch, M. Ed., Biology Teacher at Islip High School
Richard Rosenman, M. A., Biology Teacher at Cold Spring Harbor High School
Debby Taylor, University of New Hampshire

COURSES

General Nature Study
Advanced Nature Study
Elementary Geology
Bird Study
Seashore Life
Geology
Animals with Backbones
Icthyology - Herpetology
Ecology of the Estuary
Fresh Water Life
Insect Study
Plant-Insect Relationships
Plant Ecology
Geology of Long Island
Marine Biology
Animal Ecology

NATURE STUDY WORKSHOP FOR TEACHERS

The 18th annual Workshop in Nature Study was offered during the summer of 1973 and was attended by 22 elementary and secondary school teachers. The program was designed to familiarize them with the natural environment of the Long Island area and those aspects of the environment which affect the animals and plants living there. The course consisted of field trips to ponds, streams, seashore, woodlands, fields and other natural habitats to collect and study first-hand the flora and fauna of the locale, with indoor laboratory work-time divided between lectures and practical work. The experiences of the course are designed to help teachers in their classroom science activities.

The instructors for the course were Otto Heck, Assistant Professor of Biology at Trenton State College, New Jersey, and Thomas Stock, M.Ed., Director of the Children's Nature Study Program and a science teacher at Centereach High School.

A scholarship for this course was given by the North Country Garden Club of Long Island.

LABORATORY STAFF

November 1973

Director
J. D. Watson

Administrative Director
William R. Udry

Research Scientists
Bernard Allet
Carl Anderson
Bal Apte
Ahmad Bukhari
Hajo Delius
Francoise Falcoz-Kelly
Raymond Gesteland
Robert Goldman
Peter Greenaway
Terri Grodzicker
Paul Hough
Walter Keller
Carel Mulder
Robert Pollack
Richard Roberts
Joseph Sambrook
Phillip Sharp
Klaus Weber
Mary Weber
David Zipser

Postdoctoral Fellows
John Arrand
John Atkins
Michael Botchan
Jeremy Bruenn
Jane Flint
Martha Howe
James Lewis
Barry Shineberg

Graduate Students
Elias Lazarides
James Manley
Bradford Ozanne
Rex Risser
Arthur Vogel

Research Assistants
Susanne Arelt
Janet Arrand
Peter Baum
Paula Curtin
James Granfortuna
Ronni Greene
Paula Grisafi
Dreania LeVine
Heather Lund
Maria Lurye
Bernadine Miller
Phyllis Myers
Linda Rawluk
Tashmeem Razzaki
Donald Ruthig
John Scott
Ruth Solem
Nabi Sullivan
Carole Thomason
Ingrid Wendel

Supporting Staff
Bruce DeTroy
Agnes Fisher
Maria Hedges
Betty Jacques
Robert Lancaster
John Maroney
Helen Parker
Wanda Sann
Barbara Smith
Judy Smith
Linda Stanley
Madeline Szadkowski
Robert Yaffe

Genetics Research Unit,
Carnegie Institution of Washington
Alfred Hershey, Director
Agnes Fisher
Barbara McClintock

Publications Department
Gloria Danielson
Nancy Ford
Judy Gordon

Library
Susan Gensel, Librarian
Larry Delbert
Laura Hyman

Administrative Staff
William Keen, Comptroller
Maryalice Gladding, Personnel
Barbara Eggers
William Genovese
Charlene McPherson
Agnes Meyer
Dorothy Nilsson
Eileen Oates

Building & Grounds Department
Jack Richards, Superintendent
Charles Anderson
Robert Anderson
John Bach
Alastair Brown
Carol Caldarelli
Donald Caldarelli
Barbara Cuff
Mario DaSilva
Carmen Dewan
Zebedee Harris
Douglas Haskett
Robert Klipera
Julius Kulmayer
William Leyden
Alfred Nickel
James Stanley
Hans Trede
William VanHouten

E. Carleton MacDowell — 1887-1973

Dr. E. Carleton MacDowell died November 7 at Huntington Hospital after a brief illness. He was 86. His wife, the former Charlotte Gannett, had died in 1940.

MacDowell was a member of the Department of Genetics of Carnegie Institution and its predecessor, the Station for Experimental Evolution, from 1914 until 1952 and continued his work at the Cold Spring Harbor Laboratory until his retirement in 1955.

Graduated from Swarthmore College in 1909, he earned a doctorate in zoology at Harvard in 1912 and taught biology at Dartmouth and Yale before moving here. Like many scientists of later generations, he first became acquainted with Cold Spring Harbor as a student in courses given at the Laboratory.

When he came here in 1914, he set out to test the popular idea that alcoholism causes heritable mental degeneracy. He used rats because their learning behavior could be tested in a maze. Concerning this work, Davenport commented in 1915 that "experimental modification of germ plasm . . . may be said to be the loftiest aim of the experimental evolutionist." By 1919 (see the Carnegie Annual Report for that year) the results did seem to bear out the popular idea, at least to Davenport's satisfaction. However, MacDowell cautioned that "much detailed study will be required before any generalization can be drawn." His scrupulosity, characteristic of the man, was justified, for by 1921 it appeared that at least some of the effects of alcohol on future generations were due to selective survival of the more hardy young while carried by their alcoholic mothers. The offspring, apparently, were superior physical specimens but were not at their best in puzzle boxes. (Modification of genes by chemical treatment was not demonstrated until the effects of mustard gas were studied by the more precise genetic methods available during the Second World War.)

MacDowell extended this work in many ways, chiefly in mice, studying several aspects of growth and reproduction, and the physiological effects of alcohol on these processes. Later his interest shifted to mouse genetics, and most of his efforts after 1927 were devoted to pioneer work on inheritance in, and resistance to, leukemic cells. This work anticipated several preoccupations of biologists today: cellular inheritance, tissue specificity, and cancer.

MacDowell was Secretary of the Long Island Biological Association from 1944 until 1969. He also served as officer of the local branches of the American Red Cross. During the First World War he did relief and reconstruction work in France with the American Friends Service Committee. He maintained a lifelong interest in the theatre and served on the advisory board of the Huntington Township Theatre Group. For many years he led square dance groups, often in summer on the lawn at the Laboratory. In recent years, he devoted many hours to natural landscaping at his home, Seagull Hill, on Titus Lane in Cold Spring Harbor. — *A.D. Hershey*

FINANCIAL STATEMENT

Balance Sheet
Year ended October 31, 1973
with comparative figures for 1972

ASSETS

	1973	1972
Current funds:		
Unrestricted:		
Cash	$ 309,669	$ 144,220
Accounts Receivable	52,993	44,059
Inventory	68,800	71,984
Prepaid Expenses	60,627	16,253
Total unrestricted	492,089	276,516
Restricted:		
Cash	68,510	116,781
Grants receivable	284,878	878,033
Total restricted	353,388	994,814
Total current funds	845,477	1,271,330
Plant funds:		
Unexpended cash	333,819	294,998
Land and improvements	186,839	138,297
Buildings	1,416,114	1,100,203
Furniture, fixtures and equipment	280,443	165,199
Construction in progress	17,629	72,814
	2,234,844	1,771,511
Less allowance for depreciation	372,898	273,124
Total plant funds	$1,861,946	$1,498,387

LIABILITIES AND FUND BALANCES

Current funds:		
Unrestricted:		
Accounts payable	124,423	59,928
Accrued expenses	113,851	41,151
Fund balance	253,815	175,437
Total unrestricted	492,089	276,516
Restricted		
Fund balance	353,388	994,814
Total current funds	845,477	1,271,330
Plant funds:		
Fund balance	$1,861,946	$1,498,387

Statement of Current Revenues, Expenditures and Transfers
Year ended October 31, 1973
with comparative figures for 1972

	1973	1972
Revenues:		
Expendable grants	$1,575,458	$1,463,526
Indirect cost allowance on grants	569,832	470,874
Contributions	83,266	125,287
Robertson Research Fund contribution	155,827	—
Summer programs	117,303	113,876
Laboratory rental	18,244	21,272
Investment income	38,278	15,913
Book sales	213,856	175,850
Dining hall	84,241	75,625
Rooms and apartments	106,002	79,615
Other sources	2,810	2,625
Total revenues	2,965,117	2,544,463
Expenditures:		
Research*	1,427,716	1,334,052
Summer programs*	191,818	146,205
Library	69,240	39,730
Operation and maintenance of physical plant	372,366	274,069
General and administrative	294,280	210,708
Scholarships	3,120	1,452
Book sales*	128,384	129,933
Dining hall*	116,265	77,598
	2,603,189	2,213,747
Transfers:		
To unexpended plant funds	282,766	318,573
To invested in plant	165,447	13,420
	448,213	331,993
Total expenditures and transfers	3,051,402	2,545,740
Excess of expenditures and transfers over revenues	$ (86,285)	$ (1,277)

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

NOTE: The financial statements, certified by our independent auditors, Peat, Marwick, Mitchell Co., were not completed at the time of this printing. Copies of the statements will be 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, 1972 – October 31, 1973

Grantor	*Investigator or Program*	*Total Award*	*Duration of Grant*
NEW GRANTS			
National Science Foundation	Dr. Pollack/Dr. Goldman	$ 115,600	5/1/73-10/31/75
	Cell Proliferation Meeting	10,000	5/15/73-4/30/74
National Institutes of Health	General Research Support	68,693	1/1/73-12/31/73
American Cancer Society	Herpesvirus Meeting	4,000	8/1/73-8/31/73
	Cell Proliferation Meeting	11,000	6/1/73-7/31/73
Jane Coffin Childs Memorial Fund for Cancer Research	Dr. Bukhari	32,000	12/1/72-11/30/74
Grass Foundation	Neurobiology Training	5,000	6/1/73-9/30/73
CONTINUING GRANTS			
National Science Foundation	Dr. Zipser	75,000	9/15/70-9/30/73
	Symposium Support	5,000	5/1/73-4/30/74
	Dr. Zipser	83,200	1/1/72-12/31/73
	Dr. Gesteland	121,000	9/1/71-8/31/74
National Institutes of Health	Dr. Zipser	190,000	1/1/70-12/31/73
	Dr. Zipser	92,000	5/1/70-4/30/75
	Dr. Gesteland	80.000	1/1/69-12/31/73
	Dr. Pollack	338,620	1/1/70-12/31/73
	Symposium Support	69,300	4/1/69-3/31/74
	Dr. Watson	7,500,000	1/1/72-12/31/76
American Cancer Society	Dr. Sambrook	100,000	12/1/70-11/30/75
	Dr. Cairns	107,569	1/1/69-3/31/73
Jane Coffin Childs Memorial Fund for Cancer Research	Dr. Jeppesen	17,688	9/1/71-8/31/73
Alfred P. Sloan Foundation	Dr. Watson	450,000	10/1/70-9/30/75
Volkswagen Foundation	Dr. Watson	60,000	1/1/71-12/31/75
Atomic Energy Commission	Symposium Support	8,000	5/31/73-6/7/73

SPONSORS
of the Cold Spring Harbor Laboratory

Abbott Laboratories
Bristol Meyers Company
E. I. duPont de Nemours Company
Foundation for Microbiology
International Business Machines Company
The Merck Company Foundation
Russell and Janet Doubleday Fund
Schering Foundation, Inc.
G. D. Searle and Company
The Upjohn Company

FRIENDS
of the Cold Spring Harbor Laboratory

Prof. Seymour Benzer
Kallol K. Bose
David Botstein
Victor Bruce
Dr. John Cairns
Alan Campbell
Dr. Ernst Caspari
Sewell P. Champe
Ciba-Geigy Corporation
Eloise E. Clark
Richard F. Coons
Dr. and Mrs. Max Delbruck
Henry Drexler
Dr. and Mrs. John L. Duffy
Barbara Felberbaum
Flow Labs, Inc.
Dr. Naomi Franklin
Mark and Valerie Furth
Dr. Martin Gellert
Prof. H. Bentley Glass
Dr. Joseph S. Gots
Dr. Maurice Green
Prof. Donald M. Green
Dr. Samson R. Gross
Dr. I. C. Gunsalus
Newman and Helen N. Guttman
Dr. Philip and Zlata Hartman
Robert and Margot Haselkorn
Dr. Alexander Hollaender
W. J. Kauzmann
Dr. R. F. Kimball
Eugene and Phoebe LeBoy
Dr. Peter Lengyel
Dr. Robert V. Lewis
Dr. Margaret Lieb
The Lilly Research Laboratories
Mr. and Mrs. Robert Lindsay
Mrs. Mary A. Littauer
Mr. and Mrs. John H. Livingston
Dr. S. E. Luria
Dr. Julius Marmur
Jerome S. Miller
Morgan Guaranty Trust Company
Dr. Gisela Mosig
Dr. and Mrs. Stuart Mudd
Hans Noll
Masayasu Nomura
Meihan Nonoyama
Dr. Clarence P. Oliver
Mr. and Mrs. Robert P. Olney
Mr. and Mrs. Walter H. Page
Dr. David and Dorothy Perkins
Pfizer, Inc.
Mrs. Collier Platt
Mrs. Frank C. Powell
Reader's Digest
Mr. and Mrs. Willis L.M. Reese
Jose L. Reissig
Mr. and Mrs. John T. Ricks
Peter N. Rosenthal
Dr. Gordon Sato
Dr. Carl Schildkraut
Hiroto Shimojo, M.D.
Dr. and Mrs. Walter A. Shropshire, Jr.
Alice Shure
Dr. Benjamin V. Siegel
Smith, Kline and French Foundation
Dr. and Mrs. James N. Spuhler
E. R. Squibb and Sons
Dr. P. R. Srinivasan
Prof. S. G. Stephens
Sterling Drug, Inc.
F. William Studier
Mr. and Mrs. William R. Udry
Dr. H. E. Umbarger
M. van Montagu
Dr. David Vazquez
Dr. and Mrs. Felix Wasserman
Charles A. Werner
Reed and Sue Wickner
Dr. B. S. Wildi
Henry Wingate
William A. Woodcock
Dr. Charles Yanofsky

CONTRIBUTORS

to the Cold Spring Harbor Laboratory

David Apirion
Dr. Rolf Benzinger
Kostia Bergman
Mr. and Mrs. John Busby
M. Combs
Jack B. Friedman
Prof. Felix Haurowitz
Dr. and Mrs. Mahlon B. Hoagland
Mr. Irving D. Jakobsen
Dr. Kenneth J. Kerwin
Mrs. Jesse Knight
Dr. Julia E. Lever
Dr. and Mrs. Monroe L. Levin
Alan Liss
Members of Systems Engineering, PRD Electronics, Inc.
Mr. and Mrs. Glen Lyle
Leila L. McKnight
Dr. Karl Maramorosch
Dr. and Mrs. Leo M. Meyer
Jerome Meyerson
Dr. John H. Ray
Mr. and Mrs. Samuel B. Rogers
Mr. and Mrs. James Rousmaniere
Prof. Donald Scott, Jr.
Mrs. James C. Skiff
Dr. Rolf Sternglanz
Mr. and Mrs. Richard Storrs
Dr. S. R. Suskind
Mr. and Mrs. D. B. Tenney
Mrs. E. W. Taylor
Dr. and Mrs. Samuel Teich
Dr. Charles A. Thomas, Jr.
Mrs. Martin Travis

LONG ISLAND BIOLOGICAL ASSOCIATION

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 to concentrate its efforts on obtaining financial support from the community for the Laboratory's work.

OFFICERS

Chairman	Edward Pulling
President	Walter Page
Treasurer	James Eisenman
Secretary	Mrs. Ward C. Campbell
Asst. Sec'y - Treas.	William R. Udry

DIRECTORS

Class of '73
Duncan Cox
Arthur Crocker
James Eisenman
Nevil Ford
Angus McIntyre
Robert Olney
Mrs. Sara Redmond

Class of '74
Dr. Oakes Ames
Mrs. Rolla Campbell
Davis Haines
Ralph Maffei
Walter Page
Gerard Piel

Class of '75
Mrs. Ward C. Campbell
Norris Darrell
Joseph Eggert
Mrs. William A. Flanigan
Walter Rothschild
Mrs. Franz Schneider
Dudley Stoddard

Class of '76
Mrs. Charles O. Ames
Mrs. Robert Cummings
Dr. Bentley Glass
Edward Kozlik
Edward Pulling
Dr. J. D. Watson

CONTRIBUTORS

Mr. & Mrs. David M. Abbot
Mr. & Mrs. John R. Abbot
Sarah W. Abbot
Mr. & Mrs. Thomas Ackerman
Mr. & Mrs. W. H. Alston
Mr. Amyas Ames
Mrs. Charles E. Ames
Mr. & Mrs. Charles O. Ames
Dr. & Mrs. Oakes Ames
Mrs. William H. Ames
Mrs. Hoyt Ammidon
Mrs. Henry H. Anderson
Mr. & Mrs. J. S. Armentrout, Jr.
Mrs. Robert W. Ayer
Mr. & Mrs. Gilbert A. Ball
Elizabeth King Balliett
Mr. & Mrs. George C. Barclay
Dr. James C. Barnett
Mr. & Mrs. Edmund Bartlett
Mrs. E. Farrar Bateson
Mr. & Mrs. Robert E. Belknap
Mrs. Nicholas Biddle
Mrs. B. DeWitt Bleecker
Rev. & Mrs. Lyman Bleecker
Mr. & Mrs. Allen Boorstein
Mr. & Mrs. William Brengle
Dr. & Mrs. Arik Brissenden
Norma and James Bryan
Mr. Louis H. Buck
Elizabeth Long Burr
Mr. & Mrs. S. R. Callaway
Dr. & Mrs. Rolla Campbell, Jr.
Mr. & Mrs. Ward C. Campbell
Mrs. William Carl
Mr. & Mrs. Thomas Cattrall, Jr.
John C. Cattus
Dr. Frank C. Ciafone
Mr. Charles M. Clark, Jr.
David C. Clark
Dr. & Mrs. Bayard Clarkson
Mr. Michael Collins
Mr. & Mrs. Patrick Collins
Dr. Crispin Cooke
Mr. Richard F. Coons
Mr. & Mrs. Howard Corning, Jr.
Mr. & Mrs. Robert L. Corwin
Mr. Duncan B. Cox
Edward V. Cox, Jr.
Mr. & Mrs. Charles L. Craig
Mr. & Mrs. Miner Crary, Jr.
Mr. & Mrs. Arthur M. Crocker
Mr. George A. Crocker
Mr. Jarvis Cromwell
Mr. & Mrs. Robert Cummings
Mr. & Mrs. Curtis Cushman
Dr. & Mrs. Paul Cushman, Jr.
Mr. & Mrs. Norris Darrell, Jr.
Mr. & Mrs. Donald Darroch
Mr. F. Trubee Davison
Mr. John Dayton, Jr.
Mr. & Mrs. Jay Debow
Mr. Raymond de Clairville
Mr. Robert F. De Graff
Mary L. Del Gatto
Mrs. William Denby
Mrs. Richard Derby
Mr. & Mrs. Douglas Despard
Mrs. Alvin Devereux
Joseph C. Dey, Jr.
Mr. David J. Dowd
Mr. & Mrs. C. L. Drasher, Jr.
Dr. & Mrs. Lester Dubnick
Mr. & Mrs. H. Du Bosque
Mr. E. P. Dunlaevy
Mr. R. L. Duvall
Mrs. Walter K. Earle
Dr. J. D. Ebert
Mrs. William Edgar
Mr. & Mrs. James Eisenman
Dr. & Mrs. Albert Eisenstein
Mr. Ashton G. Eldredge
Mr. Henri Eyl
Mrs. Julia F. Fairchild
Mr. & Mrs. J. D. Fairchild
Mr. Moulton H. Farnham
Mr. & Mrs. W. Allston Flagg
Joan P. Flanigan
Mr. & Mrs. Howard R. Ford
Mr. & Mrs. Nevil Ford
Mr. & Mrs. Joseph C. Fox
James A. Fowler, Jr.
Mr. & Mrs. George Franklin
Mr. D. Kent Gale
Mr. & Mrs. Clarence Galston
Mr. Craig M. Garretson
Mr. Charles S. Gay
Mrs. William C. Gay
Louis F. Geissler, Jr.
Mrs. R. R. Gillespie
Mr. & Mrs. Neil Gilman, Jr.
Dr. & Mrs. H. B. Glass
Dr. & Mrs. Dulaney Glen
Mr. & Mrs. Bernard J. Gloisten
Mr. & Mrs. Joseph Goeschl
Mr. C. Fitzhugh Gordon
Mr. William R. Grant
Mr. & Mrs. Richard G. Green
Guilford Foundation, Inc.
Mrs. Joanna J. Hadden
Mr. & Mrs. Lawrence J. Hahn
Mr. C. Davis Haines
Catherine A. R. Hamlen
Mrs. Paul L. Hammond
Mr. Henry U. Harris
Herbert A. Harris
Dr. & Mrs. C. Hartenstein
R. Graham Heiner
Huyler C. Held
Mr. James E. Hellier
Dr. William W. Heroy
Dr. Roger M. Herriot
Mr. & Mrs. W. A. Heuslein
Mr. & Mrs. Herman Heydt, Jr.
Mariana and Charles S. Hoppin
Mr. & Mrs. George Hossfeld
Mr. & Mrs. Philip G. Hull
Mr. & Mrs. John C. Ingersoll
Mr. & Mrs. David Ingraham
Valdemar F. Jacobsen
Walter B. James Fund
Mrs. Nelson D. Jay
Robert D. Jay
Mrs. B. Brewster Jennings
Mrs. Ward Johnson, Jr.
Mr. & Mrs. John Juda, Jr.
Mr. & Mrs. Morris I. Karpen
Mr. Robert Winthrop Kean
Kidder, Peabody & Company
Mr. James. G. King
Mrs. Francis S. Kinney
Mr. John P. Kipp
Mrs. Marguerite Knight
Mr. & Mrs. Kenneth Knowles
Robert P. & Angela P. Koenig Fund
Mr. & Mrs. Edward Kozlik
Mr. & Mrs. Paul Kramer
Mr. & Mrs. Edward Lamont
Mr. Victor R. Larsen
Mr. Orin T. Leach
Mrs. R. J. LeBoeuf, Jr.
Mrs. Burton J. Lee
Rev. & Mrs. T. Carleton Lee
Mr. & Mrs. George Lindsay, Jr.
Mr. & Mrs. Robert Lindsay
Mr. Bernard Lippman
Mr. John H. Livingston
Mr. & Mrs. Laurence M. Lomb
Edward Townsend Look
Mr. & Mrs. Ray Luhnow, Jr.
David L. Luke III
Mr. & Mrs. John MacKay, Sr.
Dr. Robert L. McCollom
Mr. & Mrs. James McCurdy
Miss Diana McIlvaine
Mrs. Donald McInnes
Mr. & Mrs. Angus McIntyre
Mr. & Mrs. Henry R. Macy
Ralph J. Maffei
Mrs. John R. Mahoney
Mrs. Norman J. Marsh
Mr. & Mrs. John M. Martin
Mr. William J. Martin, Jr.
Mrs. Walter G. Mattern
Mr. John D. Maxwell
Dr. Ernest Mayr
Mr. & Mrs. K. B. Middendorf
Mr. & Mrs. William C. Miller
Mr. William H. Miller
Mr. & Mrs. Dudley H. Mills
Mr. & Mrs. G. G. Montgomery, Jr.
Mrs. William G. Moore
Mr. H. S. Morgan
Mr. & Mrs. George Morgese
Mr. Grinnell Morris
Dorothy E. Muma
Edith S. N. Muma
Mr. & Mrs. John R. Muma
Mr. & Mrs. Francis W. Murray III
Nassau Savings & Loan Association
Mr. & Mrs. Quigg Newton
Mrs. George Nichols
Mrs. Hoffman Nickerson
Mr. & Mrs. John W. Nields
Mr. & Mrs. William Niven
Mr. Lawrence W. Northam
Charles J. Nourse
Miss Juliet L. Nourse
Mr. & Mrs. Charles Noyes
Mrs. David C. Noyes, Jr.
Mr. & Mrs. Robert P. Olney
Mr. & Mrs. George O'Neill

Craig and Charlotte Osborn
Mr. & Mrs. Arthur Page, Jr.
Dr. & Mrs. John H. Page
Mr. & Mrs. Walter H. Page
Mr. & Mrs. F.W. Pain
Partners of Paine, Webber, Jackson & Curtis
Mrs. Carleton H. Palmer
Mr. & Mrs. Dudley F. Parker
Mr. Henry S. Parker
Mr. & Mrs. S. D. Parkinson
Mr. & Mrs. William Parsons, Jr.
Mr. & Mrs. Charles F. Paul
Mr. & Mrs. William H. Peck
Mrs. Paul G. Pennoyer
Dr. Richard N. Pierson
Collier and Louise Platt
Mr. & Mrs. Thomas C. Platt
Mr. & Mrs. Francis T. P. Plimpton
Mr. Martin M. Pollak
Mrs. Arthur W. Pope
Mr. & Mrs. Edward E. Post
Mr. A. J. Powers, Jr.
Mr. & Mrs. William Preston
Public Welfare Foundation, Inc.
Dr. & Mrs. Edward Pulling
Mr. Thomas L. Pulling
Mr. & Mrs. James T. Pyle
Dr. John Ray
Robert M. Reiss, M.D.
Mrs. Gordon S. Rentschler
Mr. & Mrs. Bernard Reverdin
Mr. & Mrs. John T. Ricks
Mr. & Mrs. James A. Rousmaniere
Mr. John K. Roosevelt
Mrs. Quentin Roosevelt
Mrs. Charles W. Root
Mrs. Reginald P. Rose
Mr. & Mrs. W. N. Rothschild, Jr.
Mrs. Frank A. Royce
Mrs. John Rutherfurd
Mr. & Mrs. B. Salzberg
Patricia Pulling Sands
Mr. & Mrs. William Sansom
Mrs. Theodore F. Savage
Mr. & Mrs. C. Schiefflin
Mr. John M. Schiff
Dr. Carl J. Schmidlapp
Mrs. Franz Schneider
Mr. & Mrs. David R. Schwarz
Mr. & Mrs. F. E. Seymour
Mr. Dale Sharp
Mr. Paul C. Sheeline
Mr. Leon G. Sherburne, Jr.
Dr. & Mrs. Paul Siminoff
Mrs. James C. Skiff
Mr. & Mrs. Bruno Skoggard
Mr. & Mrs. Robert Smails
S. Abbot Smith
Mr. & Mrs. Theo G. Smith
Mr. William S. Smoot
Mr. Theodore E. Stebbins
Mrs. J. Rich Steers
Mr. Dudley Stoddard
Mr. & Mrs. Joseph S. Stout
Mr. & Mrs. T. C. Streibert
Dr. Janice M. Studholme
Charles J. Symington
Mrs. Alyce S. Takami
Mrs. Elizabeth Williams Taylor
Mrs. Henry C. Taylor
Mr. & Mrs. Frederick Taylor
Mr. Daniel G. Tenny, Jr.
Mr. & Mrs. Philip H. Theopold
Mr. & Mrs. Ernest Turner
Mr. & Mrs. William R. Udry
Mr. & Mrs. William T. Uhl
Union Federal Savings Bank
Mr. Joseph A. Usellis
Dr. & Mrs. T. A. Vandersall
Mrs. Oliver A. Vietor
Mr. & Mrs. Eric A. von Raits
Mr. & Mrs. Colton P. Wagner
Mr. Charles J. Waldmann
Mr. Charles J. Waldmann, Jr.
Mr. & Mrs. Thomas F. Walker
Mr. & Mrs. Ethelbert Warfield
Bradford A. and Nancy J. Warner
Mr. & Mrs. H. L. Warner, Jr.
Edward and Isabella Watts
Miss Isabella Watts
Mr. Arthur D. Weekes, Jr.
Mrs. F. Carrington Weems
Mr. & Mrs. John Weghorn
Mrs. David Weld
Mr. & Mrs. Taggart Whipple
Mrs. Alex White
Whitehall Foundation, Inc.
Mr. Theodore S. Wickersham
Mr. & Mrs. Henry S. Wingate
Mrs. Willis D. Wood
William A. Woodcock
Mrs. Ford Wright
Mr. & Mrs. Woodhull B. Young

View of Cold Spring Harbor after ice storm – Photo by Hajo Delius
Montage, photos by R. Yaffe, R. Pollack