COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1976

COLD SPRING HARBOR LABORATORY

COLD SPRING HARBOR, NEW YORK

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COLD SPRING HARBOR, LONG ISLAND, NEW YORK

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DIRECTOR'S REPORT

The fascination that the DNA molecule holds for so many has never, until recently, held the connotation of flirting with disaster. I, for one, have never given a moment's thought to whether my passion about the nature of the gene might be misplaced, much less a major danger to others or even to the future of mankind itself. Yet hardly a day now passes that I do not see the term "recombinant DNA" in some news article, and I am constantly being asked questions about the possible dangers of DNA research when I am in the company of students or even with our nonscientific friends in the Cold Spring Harbor community. Answers to such questions are never simple, and now even less so. The vision of the hysterics has so peopled biological laboratories with monsters and super bugs that I often feel the discussion has descended to the realm of a surrealistic nightmare from which we will most surely soon awaken.

I am afraid, however, that it is not a nightmare from which we suffer, but a massive miscalculation in which we cried wolf without having seen or even heard one. Although from childhood we have been told that this is a silly way to proceed, we thought we were acting wisely because it was not an ordinary wolf, which we could fight away with a club, that we feared, but a creature so formidable that it could eat us up before we could even cry for help. The "super wolf" in this case is, of course, the new types of DNA molecules ("recombinant DNA") that can now be made in test tubes. The tool that has made such creatures possible is a newly discovered class of ordinary enzymes (the restriction enzymes) which make specific cuts in DNA molecules in a way that allows their genetic information to be specially rearranged. For example, the end product might be a new DNA molecule consisting of sections derived from both bacterial and human cells. Even more important, the means now exist to put these hybrid molecules back into bacteria, thereby creating new forms of bacteria capable of synthesizing human proteins together with their normal bacterial products.

If all goes as we now suspect it will, we should be able to produce, for example, human insulin in virtually inexhaustible quantities, as well as scores of other medically useful human proteins that are now in short supply. Naturally, many first-rate scientists have begun to do research in this area, for not only can we foresee a variety of important applied benefits, but, in addition, the recombinant DNA technology provides a simple means to work out the detailed structure of the chromosomes of higher organisms, thereby opening up for the first time the possibility of, for example, detailed molecular characterization of the genes involved in the immunological response. This is a practical breakthrough of vast importance, and we can anticipate that in the next several years there will be a rash of discoveries which without "recombinant DNA" might not have been achieved within this century.

Simultaneously, however, with the arrival of this new technology, some of us began to wonder whether it might also have unexpectedly bad consequences, such as through the creation of new types of organisms never yet subjected to the pressures of evolution and which might have disease-causing potentials that we do not now have to face. In particular, we worried about the creation of bacteria selectively tailored to be resistant to all known antibiotics or the insertion of the genes of tumor viruses into bacteria known to multiply in humans. Such discussions, first initiated at the 1973 Gordon Conference on Nucleic Acids, were followed in April 1974 by the proposal by a number of molecular biologists, of which I was one, that there be a formal moratorium on these two types of experimentation until an international meeting could be held to discuss whether such experimentation did, in fact, pose any plausible public health danger. It is important to stress that in making this proposal we did not urge any restrictions on most other forms of recombinant DNA research, as we believed these other forms of research would be just as safe as experiments we now conduct routinely with many forms of viruses and bacteria.

Because those of us who signed the moratorium proposal were respected scientists, not known for environmental or political kookism, we were taken seriously, and eleven months later some one hundred and

fifty people came together at the Asilomar Conference Center in California to make further proposals. This course of action, however, was far from what I had anticipated when we gathered together at MIT to urge the limited moratorium. Instead of coming together to discuss whether the moratorium should be continued, the debate focused on how widely it should be extended. Committees had already been at work drafting regulations under which recombinant DNA research could be done. Much to my dismay, the category of experiments thought to need regulations had become greatly expanded, with the general operational rule being that while your own experimental system was safe, there was no reason to assume your neighbor's was. From the start I felt most out of tune, and almost no one took seriously my opening argument that since there was no effective way to regulate experimentation with the known pathogenic bacteria and viruses, how could we come up with any logical rules for assuring protection from dangers that we could quantitate even less well.

As we know all too well today, the result of the meeting was a near unanimous consensus that we must act as if virtually all forms of recombinant DNA technology were potentially dangerous and work to minimize the possibility of bad consequences by employing a variety of devices for the physical containment of the new bacteria. There was also agreement that the specific bacteria (*E. coli* strain K12) to be used for such experiments should be especially tailored genetically so that even if they did escape into the outside world, they would have no chance to colonize the human digestive tract. All in all, most everyone, including the press, thought the affair was a smashing success, and the world of molecular genetics would gain infinite kudos for the deliberate self-restraint with which it had moved ahead. To be sure, there were mimeographed sheets in the back of the hall that spit out nonsense about recombinant DNA leading to the enslavement of the masses by genetic engineering, but this was regarded as scientifically absurd and surely never to be taken seriously by any sensible person.

I, however, left Asilomar feeling very uneasy. My view that the whole affair was a hasty rush into unjustified bureaucratic roadblocks that would set back the course of legitimate science was considered by some to be eccentric irresponsibility. Yet I did not then, nor do I now, believe all recombinant DNA research is necessarily totally safe. The future automatically entails uncertainty, and the days of all of us are marked by unexpected occurrences that might suddenly do us in. Obviously, no sane person rushes in directions where he anticipates harm to himself or others, and we would be deluding ourselves if we were to proceed as if in a risk-free world. Instead, our sanity demands that we try to find means to order actions in terms of the magnitude of their potential risk. In particular, when no quantitation is possible because we have never faced a particular situation before, we must not assume the worst. If we did, we would quickly be suffocated by the inaction of unlimited prudent behavior. There are just too many awful scenarios about living that can be written if we let our imaginations loose, and anyone who would attempt to warn his neighbors of all the awful things that might happen tomorrow would soon be without an audience.

This is most surely the case for those scientists who work with viruses that multiply in humans. Every day new mutations occur that may convey to progeny viruses unexpected new properties, and so we can never say that the apparent safety of yesterday will extend into the future. On the other hand, we do know from past experience that when most viruses and bacteria are adapted to grow under laboratory conditions, they lose any pathogenicity for humans, and that, for example, there is no reason to assume a scientist who works with laboratory strains of polio or one of the adenoviruses faces greater occupational hazards than say a truck driver or plumber. In the last analysis, the only way to evaluate the need for regulatory controls is to look at the past and see whether a particular occupation poses unusual risks.

With most forms of recombinant DNA research, however, there is no laboratory past to guide us. And when we had some facts to ponder over, I fear we did not use them well. Much too late, I have come to believe that the two types of experiments that we urged a halt to under the original moratorium pose no real threat to the general public. Much too superficially, we came to the conclusion that it would be foolhardy, to say the least, to make bacteria which are resistant to many classes of antibiotics or are capable of synthesizing a deadly poison that they normally do not make. Instead, we should have focused upon the more important fact that most, if not all, bacterial species are not clean entities which never exchange DNA with each other in nature. Instead, they are continually subject to injection of foreign bacterial DNA as a result of infection by bacteriophages or plasmids whose host ranges frequently encompass more than one bacterial genera (e.g., *Vibrio* and *Escherichia*). Thus, if through recombinant DNA technology we were to make an *E. coli* strain that, say, makes the cholera toxin, we would very likely be repeating what nature has done many times in the past. Of course, it might be a lethal bug for any host it might infect, but since as far as we know it doesn't presently exist in nature, there is every reason to believe that even if it did escape from the laboratory it would not have any positive selective advantage and so would not pose any major public health threat. To be sure, you can argue that such novel killers would be just the thing for CIA or Mafia types, but I fail to see why they could not continue to pursue Castro with our ordinary preexisting bacterial

pathogens.

Even less convincing, especially in retrospect, were the arguments against putting the genes of tumor viruses into laboratory strains of bacteria. The argument here was that such cancer-gene-bearing bacteria might accidently colonize parts of the human body, opening up the chance that they might release cancer-causing DNA that might pass into our cells and initiate a cancer. Already by the time of Asilomar, however, we realized that many, if not all, of the so-called DNA tumor viruses were in fact ordinary animal viruses that routinely infect most of us early in life. By still unknown means, they remain latent in our bodies for the remainder of our lives, usually only expressing themselves as disease-causing agents under various forms of physical stress (e.g., herpesvirus-induced cold sores).

As a case in point, within our first five years of life almost all of us are infected with BK virus, a human papovavirus that can make cells cancerous. Yet despite the fact that this virus resides within us for most of our remaining years, it doesn't routinely lead to a multiplicity of tumors, and whether it is connected with any human cancer is completely open to conjecture. In any case, the danger we face from our intestinal bacteria acquiring a little oncogenic virus DNA must be negligible compared to that which we face every time we are infected with any of the innumerable DNA-containing viruses.

This leads to the question of why did many first-rate scientists become upset at the thought that Paul Berg and his students wanted to place the SV40 genome within a strain of *E. coli* (K12) known not to effectively colonize the human gut. Yet I and many others, including finally Paul himself, took the matter seriously enough to initiate the chain of events that inexorably led to the regulatory mood at Asilomar. The best answer that I can give for our intellectual sloppiness was suggested to me in Sacramento by Governor Jerry Brown, who, after listening to me talk about recombinant DNA, asked me whether we had gotten into this mess because of "liberal guilt." When I repeated this most perceptive comment to my Harvard molecular biologist friend Matt Meselson, he misunderstood me to say "left-wing guilt," trying to be generous to the motivation of our long-time acquaintances on the Boston left.

But the heart of our trouble comes not from the silly diatribes of "Science for the People," but from the fact that at Asilomar the molecular genetics establishment put the weight of their collective authority behind a set of guidelines that implied that we could honestly predict that one form of recombinant DNA experimentation might carry more potential danger than another. In contrast, I, for one, saw no way to decide whether work on *Drosophila* DNA, or yeast DNA, or mouse DNA should be more or less restricted, if at all, and so found the Asilomar experience an exercise in the theater of the absurd. Particularly misguided was the placing of work with human DNA in the highest potential risk category, thereby restricting it to biological-warfare-like facilities and insuring that almost no one in pure research could work with it. Yet this is a DNA to which our ordinary *E. coli* intestinal flora must be exposed constantly, for it is very hard to imagine that none of the human DNA released from the normal sloughing off of dead epithelial cells does not occasionally enter neighboring bacterial cells and become integrated into their DNA. Nor should we assume the lack of any genetic consequences from those "abnormal" transfers of human DNA that now can be so explicitly displayed in the many multicolored magazines of our sexually permissive era.

There was, however, no discussion at Asilomar of these hard, if not perennial, facts of life. Instead, the almost unanimous final consensus appeared to hinge solely on the oft-repeated speculation that human DNA might carry the genomes of the so-called RNA tumor viruses; and given no real understanding of why so many cells harbor such potential sources of trouble, the general paranoia which affects almost all of us about cancer carried the day. No enlightened liberal would want responsibility for a decision that had the slightest chance of increasing human cancer incidence. More to the point would have been the acceptance of the counter argument that as all vertebrate cells most likely carry many such genomes as normal genetic components, their real function must not be to cause cancer, and we should not panic about potential exposure to DNA sequences which we already possess.

Fortunately, there are now second thoughts about the matter, and there are efforts within NIH to downgrade the hysteria over test-tube-transferred human DNA. Given the total softness of the reasons for initially fearing it, however, it will not be easy to convince others that anything has really changed, and the whole effort to rehabilitate human DNA must be seen by many as an attempt to place personal ambition ahead of the public good.

So, to say the least, we are in a rotten mess. Not only do we face the prospect that the miserable set of guidelines will be enacted into formal laws, but by their very coming into existence it will be generally accepted as fact that such research carries a profound threat to human existence. It is as if we were to pass a law saying that purple witches carry much more potential danger than green witches. Then it would be hard to tell anyone that all you meant was that an imaginary purple witch might be more scary to your three-year-old than an imaginary green witch. No sensible nation would go to such trouble for witches that did not exist. Thus when any reputable person says that the guidelines should be given the force of law, he is in fact saying to the public that he is scared of DNA. It does not help for our "best

and brightest" to say to each other that they now know that Asilomar was a tactical mistake, and then to pop off to Washington to flatter our legislators into thinking they are acting most wisely in giving recombinant DNA more attention than they ever paid to the Pearl Harbor fiasco.

Instead of going back to the question which should have dominated the Asilomar gathering—Is there a reason for formal guidelines?—our congressmen are being asked to decide between two silly alternatives: either (1) to formalize the Asilomar nonsense at the level of the now current NIH guidelines, and in so doing create another regulatory bureaucracy that will not only cost piles of money but will legitimize a control over the direction of science not seen since the Middle Ages, or (2) to effectively stop all recombinant DNA research by taking seriously the doomsday scenarios of an odd coalition that ranges from environmental kooks, who want to save the world by banning not only all man-made additives but also evolution; through the Cavalieri-Chargaff monopole of disgruntled biochemists who have never been happy with many key triumphs of molecular biology and who now seem to suggest that we should all stop in our tracks; to a group of Boston pseudoleftists whose greatest triumph over the past decade was to announce how bright they were to isolate a pure gene for (β-galactosidase) and then to denounce themselves as wicked capitalistic geneticists in a farcical press conference that almost might have occurred during the Stalinist 1930s.

Then there is also Bob Sinsheimer, the Caltech molecular biologist, who has never been connected with even a trace of eccentricity, just the opposite! And if he is publicly scared about using *E. coli* in recombinant DNA research, must not we take him seriously and take an intermediate position. We should, of course, listen, but only in the context that good scientists are no different from good bankers and brokers, and that when they speak about the future they inherently have a slippery base. Only at the risk of great peril can it be assumed that when experts disagree, the truth must be somewhere in between. We shall always find some "expert" fraction who, all too overwhelmed by the already awful complexity of everyday life, will want to call a halt to anything new, arguing that we are already too close to doom to take any more chances with the future.

The trouble here is that almost everyone has his own pet list of irresponsible assaults on the human condition, and that unless there is some solid hint that real harm will emerge, we had best go along with the more optimistic alternative. Even if Sinsheimer's morbid fear becomes reality and research with recombinant DNA does in fact produce a potentially pathogenic *E. coli* strain, the worldwide consequences would very likely be minimal. Not only is *E. coli* remarkably uninfectious, but there are antibiotics presently available that should control the mean new variants. In fact, I do not know of any authority on infectious disease who takes the recombinant DNA doomsday scenario seriously.

So you might expect that our Congressmen would be deluged by angry molecular biologists who do not at all like the idea of being singled out as perpetrators of potential crime without the slightest trace of evidence and being tainted thereafter by perpetual suspicions of guilt. Instead, the dominant establishment comment these days is that the matter is already so out of control that our only concern should be to contain our Frankenstein before it hovers over all of biological research, and, for example, we have to obtain a "memorandum of understanding" every time we want to work with a new human cell line. This most defeatist mood assumes that some federal law restricting recombinant DNA research is bound to come, and we should focus our efforts on seeing to it that it takes precedence over local laws and so keep the youthful burghers of Berkeley from outperforming the mad antics of the Cambridge mayor. We should remember, however, that even a "reasonable" law that talks only about recombinant DNA is a perfect setup for a new round of local initiatives, say, for example, to protect New Haven from unregulated work at Yale with potential human tumor viruses. And if that happens, as with the recombinant DNA story, we are likely to find that our opposition is for the most part led not by individuals with any deep knowledge of or even fear of our work, but by persons who, for a myriad of reasons, do not like the fruits of science, if not of th intellect, and see us as the most vulnerable foe.

I thus believe that the ostrich-like approach of mos molecular biologists to the events of the past two year can only lead to much more restrictive action agains our freedom, not only to do good science, but to find th facts that may be necessary for the survival of our b now inherently advanced societies. In privat conversations with the vast majority of molecula biologists, I find almost universal agreement that th guidelines are a total farce but no one who feels equal t the task of telling the Senior Senator from Massachusett that the Emperor has no clothes. Equally bad, the cred bility of so many top figures within our national scien tific and health bureaucracy has been tied to a defens of the safety, and hence reasonableness, of the guide lines that, as with our Vietnam fiasco, no official o importance can be expected to admit that he has been naive fool. So it is not surprising that they are alread being propelled by the momentum of their past non sense to adopt their own form of the Cambodian escala tion by trying to have the American guidelines enforce throughout the world by a series of international agre ments that will make the Swiss have more examiners o DNA research than they will ever have for their banks

So by this stage I realize how much the rest of th world misses the almost automatic rejection by Charle

De Gaulle of any Anglo-American initiative. If he were still with us, the whole story could still have a happy ending. We could look forward to spending part of each year in France, not only eating the food we want to eat but also doing the science that we so associate with the best of our free societies.

Highlights of the year

Gift of Robertson House and creation of the Banbury Educational Center

Of immense importance to the future of this Laboratory was the gift last June by Mr. Charles S. Robertson of his marvelous Georgian-style house on Banbury Lane, in Lloyd Harbor, with its some forty-five acres of land. Most of this acreage is covered by an easement to the Nature Conservancy, which insures that it will remain in its current undeveloped state. Built in 1936 to serve as the home of Mr. Robertson and his wife, the late Marie H. Robertson, this most attractive house was designed by the noted architect Mott B. Schmidt and located on land that had belonged to the family of Mr. Robertson's mother, the Sammises, for most of the 300 years that have passed since the original settling of Huntington township in the middle of the 17th century. Along with the main house are two smaller dwellings, as well as a splendid garage, also the design of Mr. Schmidt, a swimming pool that looks out onto Cooper's Bluff, and a hard-surface tennis court.

The main house, to be named Robertson House, will serve as a residence for visitors attending either one of the major conferences held at the Laboratory itself or one of the special small meetings which we intend to hold on the Robertson property. Toward the latter goal, we are in the process of converting the garage into a modern conference building, which will contain not only a large conference room with a capacity of approximately thirty-five, but also a library, reception rooms, and office space for the staff that will be appointed to run the meetings held at the Banbury Lane property. For the design of the Conference Center, which we intend to call the Banbury Conference Center, we have again enlisted the services of the noted architects Moore, Grover & Harper of Essex, Connecticut. Their plans are now being executed by Mr. Jack Richards, our exceptionally talented contractor-in-residence, and we anticipate its first use in June 1977 for a lecture course on the synapse. Possession of this marvelous facility, with its well-defined size and limited dining facilities, will allow us to hold, throughout the year, tightly organized conferences of between twenty-five and thirty-five participants, a perfect complement to the usual one-hundred- to three-hundred-and-fifty-person gatherings that characterize our summer sessions. In theory, we should be overjoyed by the very large number of participants who come to our major meetings, but there are times when we wistfully remember the tiny phage meetings of the late '40s and early '50s and look forward to occasions when we shall not have to face the prospect of feeling ill at ease because we can't remember some of the names and faces from the previous summer.

Five-year renewal of Cancer Center Grant

The spirited pace at which we have been able to probe how viruses may make cells cancerous and our ability to explore seriously the biochemistry and molecular structure of the cancer cell reflect not only the excellence of

Banbury Conference Center under construction

our scientific staff but also their most efficient funding, largely through the vehicle of a single, very large, program-project type grant administered through the Cancer Center Program of the National Cancer Institute. This grant covers the salaries of virtually all our key scientists who work on cancer and provides most of the funds needed for both supplies and equipment. As it is most assuredly at the heart of our high-level functioning, the fact that our current five-year grant would end on December 13, 1976 necessarily made us apprehensive about the way in which our renewal application would be received by the Site Visit Committee, the Cancer Center Study Section, and finally by the National Cancer Advisory Board. Naturally, much effort, skillfully coordinated by Joe Sambrook, went into the preparation of the almost three-hundred-page grant proposal. By the time the Site Visit Committee arrived, we began to relax. We felt reassured by our ability to report several incisive new facts not known when the formal grant proposal was prepared several months before.

Happily, the final verdict was a reassuring vote of approval, with the National Cancer Advisory Board awarding us this past September direct costs of $1,590,000 for 1977, a substantial increase over the 1976 sum of $1,100,00. By the end of the year, however, the National Cancer Institute found that it had so overcommitted itself that it had to reduce by $100,000 the sum committed to us. Of course, we are still most pleased by the final increment, as it will allow us to bring the expenses of our present programs approximately in line with the level of our monetary intake and will give us the capability of markedly expanding our cell biology efforts. Thus we *can* set our sites still higher but we must never lose sight of the fact that the costs of scientific research invariably grow faster than the average inflationary rate. Even with a substantial infusion of Robertson Research funds, we found it necessary last year (for the first time in many years) to draw upon our very limited uncommitted cash reserve to keep us going.

Losses to scientific staff and new appointments

The fact that we are unable to offer permanent positions to most of our staff means that each year we must expect that several staff members, who we have come to regard as irreplaceable, will move on to positions that offer more long-term security. Leaving us in October for a permanent position at the University of Heidelberg was Walter Keller, who came to James lab in the fall of 1970 and subsequently played a major role in its development as a leading center for tumor virus molecular biology. Another major loss was the departure of Tom Maniatis in early 1977 for an Associate Professorship at Caltech. Tom came here in January 1975, on leave from Harvard, to study animal cell culture methods, intending to return within a year when Harvard's P3-type lab was to be completed. Tom subsequently became a casualty of the recombinant DNA debacle in Cambridge, and the stay of his group here began to acquire

Maryalice Gladding, surrounded by friends and family, at her retirement party

semipermanent status. Unfortunately, our luck could not continue indefinitely, and having come to believe that he could not count on the rapid return of Harvard's Biological Labs to a nonhysterical state, Tom decided to accept a most attractive offer from Caltech. While Tom was here, his research kept us in the center of recombinant DNA studies, and with great regret we accepted the inevitability of his departure.

Also leaving us were several talented Postdocs—Al Bothwell to join David Baltimore's group at the Massachusetts Institute of Technology; Sharon Endow for further postdoctoral research in Edinburgh; Sara Lavi to return to the Weizmann Institute; and Eugene Lukanidin to go back to the Molecular Biology Institute in Moscow. We also miss the talented protein chemist, Charles Miller, who spent his sabbatical year from Case Western Reserve Medical School in David Zipser's lab.

I also must note the retirement of Maryalice Gladding, who for twelve years served with dedication and skill as Executive Secretary in Nichols. First ably assisting John Cairns, then me, and finally Bill Udry, she gracefully, yet firmly, oversaw most of our administrative functions. Happily, her many close friends at the Laboratory were able to join in honoring her services at a special dinner held at the Robertson House just before Christmas, and later at a general staff luncheon held at Blackford Hall.

Newly appointed to the staff is Lan Bo Chen, who initially came as a Postdoc doing graduate work at MIT. As of January 1, 1977 he assumed the position of Senior Staff Investigator in charge of our Protein Chemistry section. Also promoted from the Senior Postdoc level to Staff Investigator positions were Louise Chow, Richard Gelinas, and Bill Topp.

An extremely talented supporting staff

The high quality of our scientific staff is known throughout the world. Less well appreciated, but equally important to our success, is the devoted excellence of our nonscientific personnel. For example, our library knows no equal for efficient functioning, and full credit for this must go to Susan Gensel, our most professional, yet cheerful, librarian.

Equally important has been the most imaginative work of Bruce De Troy, who heads our electronics and machine shop. Without his untiring efforts, our Neurobiology program would never have succeeded so well.

The feeling of gracious hospitality experienced by all who have stayed at Robertson House is owed in great part to the charm and diplomacy of Joan Cook, who has been in residence there with her two daughters. And given the potential chaos engendered by our constant level of new construction, the fact that our neighbors and visitors can still take delight in our grounds is a credit to the abilities of Hans Trede.

Mentioning these particular people is all too arbitrary, given the excellent work of so many here. I hope by singling our these few, the outside world can begin to appreciate the magnitude of our day-to-day activities, which each year become more and more like those of a small, high-quality university.

A very large and intellectually demanding Symposium on Immunology

This past June we were host to some three hundred and twenty-five immunologists who attended our 41st Symposium, entitled "Origins of Lymphocyte Diversity." There were some eighty-six formal presentations, as well as hours of informal discussion, most of which took place in small groups over meals or during the free afternoons. From the beginning one could sense this was not a gathering from which it would be possible to take home simple answers. How so many different antibodies are made has become an even more challenging problem than we could have predicted possible at the end of our 1967 Symposium. At the heart of our confusion is why so many classes of lymphocytes exist. Conceivably we may know how to think once we identify biological functions for the major histocompatibility proteins and the newly discovered immune-response (Ir) antigens. We were most fortunate in being able to call upon Niels Jerne to set the tone of the meeting with his incisive opening address and to have Gerry Edelman for his perceptive summary of the state of the field.

Once again, following a long tradition, many of our neighbors gave dinner parties for the speakers, and we wish to thank, in particular, Mrs. Charles O. Ames, Mr. and Mrs. Robert Cummings, Mr. and Mrs. Roderick Cushman, Mr. and Mrs. Norris W. Darrell, Jr., Mr. and Mrs. Clarence E. Galston, Mr. and Mrs. Angus P. McIntyre, Mr. and Mrs. Samuel D. Parkinson, Mr. and Mrs. Edward Everett Post, Mr. and Mrs. Richardson Pratt, Mr. and Mrs. Edward Pulling, Mr. and Mrs. Richard J. Weghorn, Mr. and Mrs. William A. Woodcock, Mr. and Mrs. George Lindsay, and Mr. and Mrs. Franz Schneider.

Most satisfying were the many letters received from speakers telling us that it was the most stimulating meeting they had attended in years.

The most comprehensive meeting yet held on the origins of human cancer

Several years ago, Howard Hiatt and I discussed the possibility of the Laboratory hosting a meeting designed specifically to give a broad overview of the various factors that may give rise to human cancer. This goal was realized in early September with a mammoth gathering at which some one hundred and twenty papers were presented. The topics ranged from pure epidemiology to public-policy considerations affecting the extent of human exposure to potential carcinogens.

Sir Richard Doll at Origins of Human Cancer meeting

Luckily we received a great deal of high-quality advice on how the program should be arranged, and John Cairns, Bruce Ames, and George Todaro deserve special mention for their help. Without the encouragement given by the National Cancer Institute, with its Director, Dick Rauscher, telling us to go ahead and think big, I doubt we would have had the courage to carry through on a meeting whose proceedings will require three large volumes to encompass.

We were most pleased when Sir Richard Doll graciously accepted our invitation to come from Oxford to deliver the opening address and were happy that we were able to provide such distinguished visitors as Sir and Lady Doll with the type of relaxing accommodations now available to us at Robertson House. Equally pleasing, the meeting provided the welcome opportunity to bring John and Elfie Cairns back to Cold Spring Harbor. John, given the assignment of summarizing the meeting, performed this almost superhuman task with his usual grace, clarity, and insight, and at the end we knew that this was a meeting that most definitely had been needed and one for which we could be rightfully proud.

A four-month-long summer program

Our "summer" commenced in early May with a large meeting on *Dictyostelium,* the first of ten meetings held this year on our grounds. It was followed by a most stimulating meeting on DNA Insertion Sequences, jumping genetic elements, the first of which was found by Barbara McClintock here some twenty years ago but whose universal significance has only come to be appreciated recently. Ahmad Bukhari was the principle organizer, a job much more complicated than initially conceived when he told us that he wanted to organize a small meeting of some forty-five to fifty people. The RNA Tumor Virus meeting came next, with over one hundred formal presentations, a semicatastrophe from which we learned our lesson and so arranged poster sessions for the August SV40-Adenovirus meeting. The middle of the summer saw a meeting on the Lactose Operon organized by Jeffrey Miller and Bill Reznikoff. Again the logistics were almost too much, not because of its size but because it overlapped our courses. Intellectually, it went very well and showed that *E. coli* was still very much alive.

The Robertson Research Fund maintains our freedom for innovation

The fact that we now have available the income from a sizable endowment fund established for the specified purpose of supporting research gives us the much needed flexibility to respond to new research advances by acquiring new instrumentation or by quickly committing funds to bring or keep talented younger scientists here. Over the past year, we committed approximately two-thirds of our Robertson funds toward the support of younger scientists, with the remainder going toward the completion of the Davenport lab renovation and toward the necessary complex equipment needed for the experimental work on the central nervous system in Jones lab.

Summer research in Neurobiology now a reality

When our summer courses in Neurobiology began in 1971, we knew they should be complimented by a program of pure research. But as long as we were limited to our facilities in McClintock lab, we also knew that having any research program would necessarily diminish our teaching efforts. The now completely renovated and fully equipped John D. Jones Laboratory allows us to take a big step forward toward our goal of making Cold Spring Harbor a leading center for Neurobiology research. This past summer Jones lab played two important roles: as the site of a marvelous new course on the central nervous system, which we believe to be the first of its kind; and as the center for a three-month research project on the synapse, which was ably organized by Rami Rahamimoff. Money needed for the operating expenses of the synapse program came from the Sloan Foundation grant, which was awarded to us last year to cover a three-year effort aimed at bringing together leading younger neurobiologists to share their experimental and theoretical know-how.

Vital to the success of all these efforts was our second year of support from the Marie H. Robertson Fund, a special fund established by the Banbury Foundation for the support of Neurobiology at Cold Spring Harbor. So far these funds have been used for the creation of new laboratory facilities and their equipment. These tasks should be finished by the fall of 1977, and we hope then

initiate, gradually, year-round efforts focusing on the ay nerve cells grow and form synapses.

ur publications list grows even more distinguished

his past year saw the publication of new additions to ur three main series: Volume 40 of the Symposia, a ven-hundred-and-fifty-page book on "The Synapse"; olume 4 of our Cell Proliferation series, a three-book t on "Cell Motility" which comprised over fifteen ndred pages; and "RNA Polymerase," a nine- ndred-page book in our Molecular Biology mono- ph series. All resulted from meetings held here, and cause of their uniformly high intellectual quality, they ould be well received.

Ve worry, however, that because of the widespread of copying machines they will not be bought in the nbers that are necessary to cover the truly large costs ociated with such large books. Our dilemma is two- l: The first problem arises from the fact that our etings are so large that we cannot issue reasonably d (200–400 pages) books without abandoning our ctice of publishing all papers presented by invited akers. The second aspect of our dilemma concerns desire to publish thoroughly attractive volumes ose appearance is appropriate to the high quality of nce they contain. Now we are trying to drastically er costs through the use of computer-assisted photo- procedures. But even this may not provide us with financial edge needed to protect us from having y percent of a book xeroxed rather than the com- e volume bought.

ven with all these serious problems, we are still ing out perhaps the highest quality advanced books iology and owe much to the deep and intelligent otion shown by Ms. Nancy Ford, who heads our lications department, and by her most competent , Annette Zaninovic, Roberta Salant, and Paul ge.

nstruction of the new Williams House

e of the more striking dwellings on the Lab grounds been Williams House, a whaling-period structure t to house persons connected with the whaling ac- ies long associated with Cold Spring Harbor. Heated y by fireplaces, it has served as summer housing for forty-five years it has belonged to the Lab. For many rs, we pondered whether it could be renovated to r-round use, only finally to conclude reluctantly t, never a firm dwelling to start, it had deteriorated ond repair, a conclusion rather dramatically con- ned when we found that we could topple the chim- ys with a push. So we have had our architects, Moore, over & Harper, draw up plans for a new Williams use, whose external features will almost duplicate se of the preexisting structure. The interior is another tter—five modern apartments, four of them duplex- and all with magnificent views of the harbor. The actual construction work became a reality when LIBA took on the financing, and as soon as last summer ended, a bulldozer arrived to knock down the by then thoroughly stripped and sad-looking building. The whole demolition task took less than two hours, allowing foundation work to start in October. The unexpectedly severe winter has set back our plans for a June 1 completion, and now July 4 appears to be the date when the first occupants will arrive.

LIBA fund drive for the new Williams House continues its vigorous course

Throughout its now fifty-three-year-old existence, the Long Island Biological Association (LIBA) has taken on the task of raising funds for the construction of many of our needed laboratories and residences. Their latest major effort is the financing of the new Williams House, and, toward this end, its Chairman, Edward Pulling, prepared an especially attractive fund-raising brochure showing the building as we hope it will soon look. Already more than seventy-five percent of the final $225,000 goal has been reached, a most remarkable achievement, and once again it is brought home to us how lucky we are to have the support of such marvelous neighbors.

Unfortunately, the untimely death of Dorcus Cummings this past fall deprived us of one of our most fervent supporters. Dorcus had been a member of LIBA for over twenty years and a Director for eight years. We will long remember her devotion to the work of the Laboratory and the extraordinary skill with which she contributed to the preservation of the beauty and ecology of the Cold Spring Harbor environment. A memorial fund has been established in her honor by her LIBA friends.

This year, two major scientific presentations were arranged for LIBA members. The first, on May 16, was by the noted science writer and lecturer, Isaac Asimov, who spoke on "The Moon—Threshold to Outer Space." It was a most entertaining occasion and concluded with dinner under a tent on the Airslie lawn. The second lecture was given by Professor Alexander Rich of the Massachusetts Institute of Technology at our Annual Meeting on December 4. His topic was the Viking mission to Mars and its search for any possible forms of life. Professor Rich brought with him many of the extraordinary photos taken from the surface of Mars, and everyone agreed that his presentation was one of the most fascinating ever presented before a LIBA audience.

Many of the LIBA Directors also served as hosts for a dinner party given at Airslie on Saturday, September 11 for the participants of our Origins of Human Cancer meeting.

Departing from the LIBA Board at the conclusion of their maximum terms were Bentley Glass and Edward Kozlik. Hoyt Ammidon, Jr., because of his move to Princeton, New Jersey, was forced to resign.

Directors newly elected at the annual meeting in December were: Walter N. Frank, Charles S. Gay, George J. Hossfeld, Jr., and William Parsons, Jr.

Our Board of Trustees has a very unique composition

Ours is the only major scientific organization whose Board of Trustees is composed about equally of leading citizens from the surrounding community and of noted scientists deeply conversant with its scientific activities. So there is no need for a scientific advisory board, and our Trustees, by themselves, can come quickly to many crucial decisions which need both financial and scientific input. Always a most important plus on our side is our Chairman, Harry Eagle, whose ability to lead the Board through complicated agendas with speed, accuracy, and humor is unsurpassed.

Because our By-laws limit Trustees to a maximum of six years continuous service, we must accept the loss of the valuable services of Dr. David Jacobus, Mrs. George Lindsay, and Mr. William Woodcock. All have generously dedicated themselves to our good on innumerable occasions, and we were happy to be able to use Robertson House for a special dinner after our October 26th Annual Meeting to express, in but a small way, our thanks for their help.

Newly elected to the Board at the Annual Meeting were Dr. Emilio Collado of Oyster Bay, former Executive Vice President of the Exxon Corporation; Mr. William Grant of Oyster Bay, President of the brokerage house Smith, Barney, Harris, Upham and Company, Inc.; and Mrs. Franz Schneider of Laurel Hollow, a long-time friend of the Laboratory and a former Director of LIBA.

Adequate future funding of high-level science must not be taken for granted

The uproar this year about recombinant DNA has kept us from facing up to a much more serious long-term issue—the fact that the amount of public monies now going toward research is not keeping pace with inflation, yet the number of institutions with large staffs of talented younger scientists is rising. So a crisis atmosphere already exists in many labs, and the number of excellent scientists who lack even minimally adequate funding is growing at an alarming pace. Unfortunately, I do not see any simple way out of this dilemma. I sense that the public is growing increasingly concerned that the results of science may be generating more social problems than they solve, and that this mistrust will not be easy to overcome. One must be aware that the high regard that the public once had for science has greatly diminished. Ten years ago, for example, it would have been highly unlikely that we could see the current impulsive rush of our legislators to legitimize by law the unfounded and desperate hopes of laetrile or to declare that, because we are not rats, saccharin will never cause cancer. As long as this mood predominates in the nation, we shall be lucky if we succeed in keeping our bad situation from getting even worse. As scientists, we shall have to spend more time educating the public, not only about our idealistic dreams, say to conquer the common cold or cancer, but also with regard to the old-fashioned idea on which I was brought up, that the pursuit of knowledge about the nature of life and about the universe in which it exists is a glorious endeavor that should be undertaken for its own sake. In this way, we shall not be so easily faulted, say for our failure to produce fusion before the price of oil leads to political, economic, and social chaos, or for our failure to yet conquer cancer and so set the stage for a counter-reaction in which scientifically unfounded benefits are attributed to the natural apricot and the rational conclusions of educated minds are ignored.

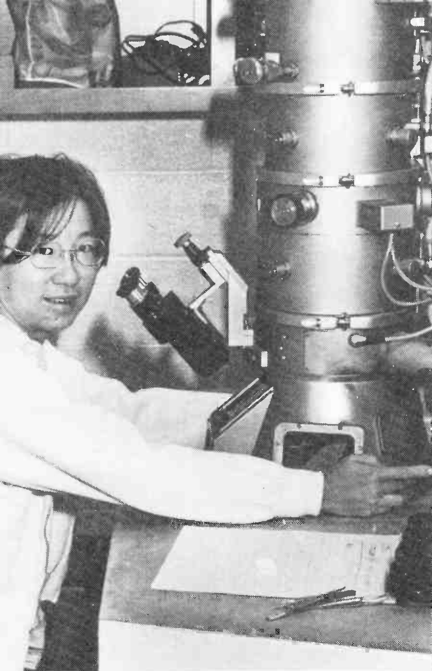
I am, of course, aware of the conventional wisdom that we can never sell the public on supporting science on a big scale solely as an intellectual endeavor, and there must be the promise of at least some worldly goodies before we can expect a commitment to the intellect equivalent to the cost of one of our modern nuclear submarines. If we must venture beyond our expertise, however, I suspect we are better equipped to do so as historians than as politicians. With more than a modicum of conviction, we can say that our society now moves because of the fruits of science, and that, were rational investigation to grind slowly to a halt, the meanness from which we have emerged over the past four hundred years would all too soon be back with us. Today we hear much revisionist talk about the wonders of the Victorian era and the gracefulness of the architecturally outlandish houses which dominated the sylvan landscapes of 19th-century America. The more relevant fact is that the life of the common man then was much more harsh than livable, and I have never met anyone over twenty-five who would want to sacrifice indoor plumbing or penicillin in a return to the so-called gracious life style of our Victorian past.

A very strong case can thus be made that we are needed for what we do best—to seek the laws of nature and to learn more of them. If we continue along this course, the chances are good that we may escape still further from the limitations of our evolutionary past. Naturally, our future paths cannot be delineated that simplistically, nor without caution. We must be prepared for the fact that some future options might not be tenable, and that we must then cry "stop" with all the strength we possess. We must think very carefully, however, before we take a negative tack, because if the concept of the scientist as the pessimist takes hold, then the scientist as the villain might well be the next item on the agenda.

Thus our main task at Cold Spring Harbor is to remain on the course that we have followed for the past

ty-six years—to do science on the highest possible l within the framework of openness and fair play ırd our peers whose objectives overlap ours. For if we can keep our ideals and self-confidence high, we can then look forward to the future with some degree of optimism.

June 30, 1977 *J.D. Watson*



YEAR-ROUND RESEARCH

MOLECULAR BIOLOGY OF TUMOR VIRUS

. SAMBROOK, G. Ballantyne, M. Botchan, A. Bothwell, G. Fey, D. Galloway, R. Greene, T. Grodzicker, J. Hassell, C. Jungreis, R. Kelch, W. Keller, S. Lavi, M. Lazarus, A. Lewis, E. Lukanidin, M. Mathews, R. McGuirk, R. Pashley, U. Pettersson, P. Reichel, J. Schneider, M. Sleigh, R. Tjian, A. Tolun, W. Topp, S. Weirich, I. Wendel, S. Young

During the last year work has progressed on several major projects:

1. physical mapping of adenovirus mutations and structural genes,
2. analysis of the genomes of defective adenovirus 2–SV40 hybrid viruses,
3. characterization of mutants, including amber and ochre nonsense mutants, of adenovirus 2–SV40 hybrids,
4. purification and characterization of SV40 T antigen and of adenovirus-SV40 fusion proteins which contain the C-terminal end of SV40 T antigen,
5. identification and mapping of small species of adenovirus mRNA and virus-associated RNAs,
6. analysis of field strains of type-C adenoviruses which appear to be recombinants of the standard serotypes,
7. isolation of deletion mutants of SV40, and
8. analysis of the structure of integrated viral DNA in transformed cells and revertants.

Physical mapping of mutations and structural genes of adenoviruses

Mutations

We have continued studies whose aim is to position many temperature-sensitive (ts) mutations and structural genes at physical locations on the viral genome and to thereby assign mutations to the genes for specific viral proteins. This, combined with physiological studies of ts mutants, helps clarify the function and role of these polypeptides in the viral replication cycle. The approach we have used has been described in detail previously; see J. Sambrook et al., *J. Mol. Biol.* 97:369 (1975) and T. Grodzicker et al., *Virology* (in press), wherein we reported the map positions of seven Ad5 mutants and four Ad2^{+}ND1 ts mutants.

In collaboration with Joe Weber, we have mapped the sites of the lesions in a set of Ad2 ts mutants. Over 100 wild-type recombinants were selected from 12 crosses between 4 Ad2 ts mutants and 3 Ad5 ts mutants. The DNA structures of about half of these have been determined with restriction endonucleases and have been compared with those of the parental genomes to deduce the locations of the ts mutations. All of the four Ad2 ts mutants mapped are defective in late functions and have been classified into separate complementation groups. The positions of the Ad5 ts mutations were determined previously [J. Sambrook et al., *J. Mol. Biol.* 97:369 (1975)] and have now been localized to more precise coordinates on the adenovirus physical map.

Structural genes

Many of the polypeptides expressed during lytic infection by Ad2 and Ad5 can be distinguished by their differing mobilities on SDS-polyacrylamide gels [T. Grodzicker et al., *Virology* (in press); J. Weber and J. Hassell (in preparation)]. By analyzing the polypeptides expressed by recombinants of known genetic constitution, we can locate the structural genes for several proteins on the physical map and assign ts mutations to specific genes. Analysis of the set of Ad2^{+}ND1/Ad5 recombinants allowed us to map the positions of the genes for hexon, 100K protein, and fiber. Studies of the polypeptides expressed by the Ad2/Ad5 recombinants have allowed us to map the genes for three additional proteins: penton, the minor core protein V, and 36K protein.

Clockwise from top left: Louise Chow, Ahmad Bukhari and Mike Mathews, Tom Maniatis, Jim McDougall, Denise Galloway, Mike Botchan, Ray Gesteland

Defective adenovirus 2–SV40 hybrid viruses

Two adenovirus 2–SV40 hybrids (Ad2^{+}D1 and Ad2^{+}D2) have been isolated from the Ad2^{++}HEY population. Both hybrids are defective and require helper Ad2 for productive infection of simian cells. The DNA structures of Ad2^{+}D1 and Ad2^{+}D2 have been determined by analysis with restriction endonucleases, hybridization, and electron microscopy. Ad2^{+}D1 contains a 3.2-kb insertion of SV40 DNA which includes the entire "early" region of the SV40 genome between positions 11 and 71. The SV40 insertion replaces a 3.5-kb segment of Ad2 DNA which maps between 0.64 and 0.74 fractional genome length from the left end of the viral DNA molecule and is oriented such that the SV40 *A* gene is transcribed from right to left. Ad2^{+}D2 contains a 4.8-kb insertion of SV40 DNA which replaces a 7.9-kb segment of adenovirus DNA mapping between 0.76 and 0.96 fractional genome length from the left end. The insertion, which includes the entire "late" region of the SV40 genome together with a portion of the early region, lacks only those SV40 sequences between 54 and 62. In contrast to Ad2^{+}D1, the "early" SV40 sequences in Ad2^{+}D2 are transcribed from left to right.

The expression of the SV40 sequences in Ad2^{+}D1 and Ad2^{+}D2 has been determined by hybridization of cytoplasmic RNA extracted at early and late times after infection to the separated strands of defined ^{32}P-labeled fragments of SV40 DNA. RNA complementary to the E strand of fragments encompassing the "early" region of SV40 DNA was detected at both early and late times following infection with Ad2^{+}D1. Cytoplasmic RNA extracted at early times after infection with Ad2^{+}D2 contains no sequences complementary to SV40 DNA. At late times, cytoplasmic RNA complementary to the E strand of a portion of the "early" region of SV40 DNA was readily detected. We suggest that the early Ad2^{+}D1 mRNA complementary to the "early" region of SV40 DNA maps entirely within the SV40 insertion and is subject to the same controls operating in simian cells infected with SV40. In contrast, cells infected with Ad2^{+}D2 contain cytoplasmic transcripts complementary to only part of the SV40 "early" region, which are detected at late times. It seems likely that the 5′ termini of these transcripts map within adenovirus-2 sequences and that their 3′ termini map within SV40 sequences. The expression of this transcript is undoubtedly under adenovirus-2 control.

Proteins antigenically related to SV40 T antigen have been detected in cells infected with Ad2^{+}D1 and Ad2^{+}D2 by immunofluorescence and by immunoprecipitation with sera from hamsters bearing tumors induced by SV40-transformed cells. Ad2^{+}D1 induces the synthesis of a 96,000-dalton-molecular-weight protein which is precipitated with SV40 T-reactive antiserum, is phosphorylated, and comigrates with SV40 T antigen from lytically infected cells through SDS-polyacrylamide gels. By the criteria cited above, the Ad2^{+}D1 96,000-dalton protein appears to be identical to SV40 T antigen.

The major polypeptide species precipitated with SV40 T-reactive antisera from extracts of Ad2^{+}D1-infected HeLa or CV1 cells has an apparent molecular weight of 115,000 daltons, and it too is phosphorylated. The 115,000-dalton protein is probably a fusion polypeptide whose amino terminus is encoded within adenovirus-2 sequences and whose carboxy terminus is specified by the SV40 *A* gene. The 96,000-dalton protein induced by Ad2^{+}D1 is detected by immunofluorescence at both early and late times after infection whereas the 115,000-dalton protein induced by Ad2^{+}D2 is detectable only at late times. These results are consistent with the observation that transcripts corresponding to the "early" region of SV40 DNA are synthesized at early and late times during infection by Ad2^{+}D1 but only at late times after Ad2^{+}D2 infection.

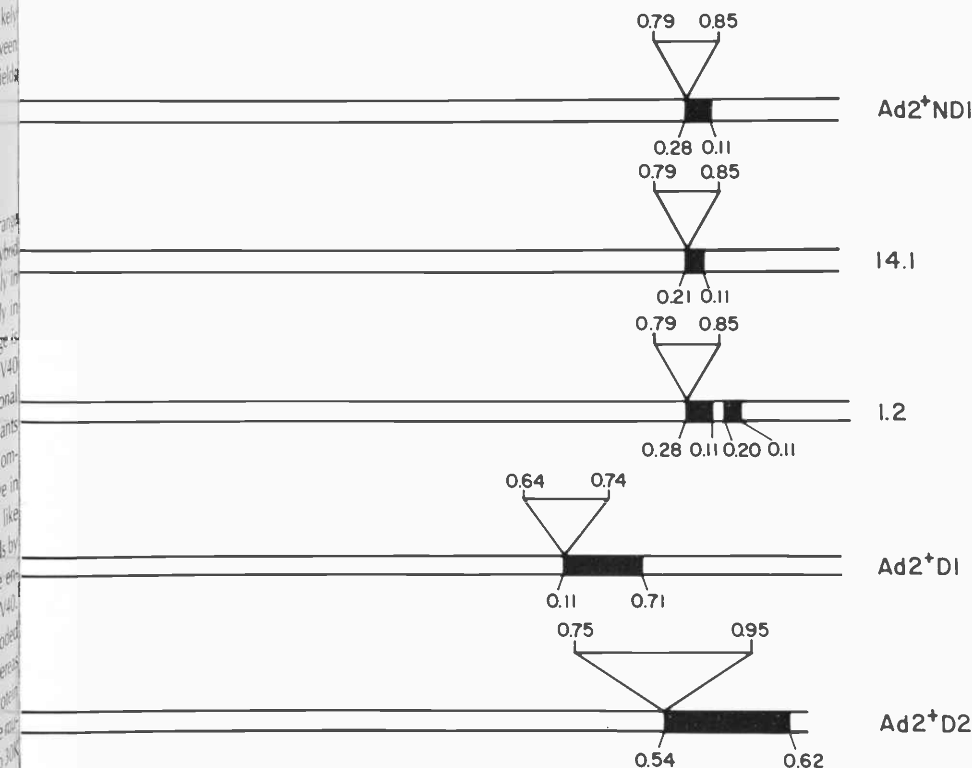
The DNA structures of Ad2^{+}D1 and Ad2^{+}D2 are unrelated to each other or to any of the hybrids in the Ad2^{++}HEY population. It therefore seems unlikely that they have arisen by recombination among the hybrid members of the Ad2^{++}HEY pool or by rearrangement of their genomes. Though the mechanism of formation of Ad2^{+}D1 and Ad2^{+}D2 remains enigmatic, it seems likel that they were generated by recombination betwee free SV40 (Ad2^{++}HEY is genetically unstable and yielc SV40 with high efficiency) and adenovirus 2.

Host-range mutants and revertants of Ad2^{+}ND1

We reported last year the characterization of host-rang mutants of the nondefective adenovirus 2–SV40 hybri virus Ad2^{+}ND1. Unlike Ad2, which grows poorly i monkey cells, Ad2^{+}ND1 grows equally efficiently i human and monkey cells. This extended host range presumably due to the expression of the integrated SV4 sequences which map between 0.28 and 0.11 fraction unit on the SV40 map. The seven host-range mutan which grow well in human cells comprise one com plementation group, grow poorly and are defective i the synthesis of late proteins in monkey cells, and, lik Ad2, are complemented for growth in monkey cells b SV40. Thus they behave as if they have lost the en hancement or "helper" function provided by SV4(Ad2^{+}ND1 expresses a 30,000-MW protein that is code for in part by the SV40 sequences it contains. Wherea four of the host-range mutants synthesize a 30K protei in infected human cells and are probably missense mu tants, three of the host-range mutants synthesize no 30 protein. Instead, mutants *71*, *140*, and *162* direct th synthesis of 10K, 19K, and 19K fragment, respectivel In collaboration with Ray Gesteland and Jim Lewis, w

ave looked at the synthesis of fragments and the 30K ND1 protein directed by mRNA isolated from mutant-nfected human cells, selected by hybridization to SV40 DNA and translated in mammalian cell-free systems upplemented with yeast suppressor tRNA. In the pres-nce of yeast ochre tRNA (but not amber or su⁻ tRNA), nRNA from mutant *71* is translated into 30K protein as vell as the 10K fragment, whereas mutants *140* and *162* ehave as amber nonsense mutants. We hope to use hese mutants to assay for in vivo activity of suppressor RNAs and to look for suppressor cells. On the basis of he size of the fragments produced by the nonsense nutants, and assuming that the carboxy-terminal end of he 30K protein lies near the SV40 *Hin*II,III G-B junction, ve can estimate the approximate positions of the non-ense codons in the mutants. The position of the base hanges in the mutants relative to Ad2⁺ND1 is being xamined by sequence analysis of the DNA and mRNA n the region of interest.

We have also isolated revertants of the nonsense nutants *162* and *71* which have regained the ability to row efficiently on monkey cells. The proteins pro-uced by revertants in vivo and in vitro have been xamined, and their genomes have been analyzed by estriction-enzyme mapping and heteroduplex analysis. evertants of *162* produce a 30K protein, and their genomes appear identical to that of wild-type Ad2⁺ND1. However, several revertants of the ochre mutant *71* appear to contain deletions or genome rearrangements in the region of the SV40 insertion. One of these, the deletion revertant Ad2⁺ND*14-1*, has lost the SV40 sequences which map from position 0.28 to approximately 0.21 (Fig. 1). When *14-1* mRNA selected by hybridization to SV40 DNA is translated in vitro, an 18K protein is produced. This suggests that the deletion has removed the chain-terminating mutation and that only the C-terminal end of the 30K protein actually carried the helper function. Two other revertants, Ad2⁺ND*1-2* (see Fig. 1) and Ad2⁺ND*1-1*, have rearrangements in the region of the SV40 insertion and share common features. In both revertants the parental SV40 insertion is intact; however, to the right of this is a partial duplication comprising the initial sequences of the adjacent adenovirus fiber gene and distal sequences of the original SV40 insertion. Ad2⁺ND*1-2* and *1-1* both produce the 10K *71* fragment and a new protein unique to the revertant. The new protein (23K) produced by *1-2* has been analyzed extensively and shown to contain peptides in common with both adenovirus fiber and SV40 T antigen (see below). The revertant *1-1* contains more fiber sequences in the duplicated region and produces a new polypeptide with a molecular weight of approxi-



gure 1
aps of the genomes of adeno-SV40 hybrid viruses.

mately 55,000. We have isolated two other classes of host-range mutants. The first consists of mutants of the revertant Ad2⁺ND*1-2* which no longer grow on monkey cells. These mutants no longer produce the 23K protein unique to the revertant. The genome structure of these mutants will be analyzed with restriction enzymes to detect possible changes in the mutant DNA. In addition, we have isolated a host-range mutant of Ad2⁺ND4 which has lost the ability to grow on monkey cells but can be complemented by SV40. Ad2⁺ND4 contains SV40 sequences which map between positions 0.59 and 0.11 on the SV40 map and produces polypeptides which can be immunoprecipitated with antibody to T antigen. Human cells infected with the Ad2⁺ND4 host-range mutant do not produce polypeptides which react with anti-T antibody. The genome of this mutant has been analyzed with restriction enzymes and appears identical to the parental Ad2⁺ND4.

Fused proteins from Ad2-SV40 hybrid viruses

HeLa human cells and CV1 monkey cells infected with the new hybrid virus Ad2⁺ND*1-2* contain a new protein of apparent molecular weight 23,000. This protein is not found in cells infected by the parental virus, Ad2⁺ND1 *H71*, a host-range mutant which is unable to grow in monkey cells. Indeed, the 23K protein represents the only major difference between the protein patterns of *H71* and *1-2* and it is therefore a good candidate for a "helper protein." In this respect it is similar to the 30K protein found in Ad2⁺ND1-infected cells. Unlike the 30K protein, which is synthesized both early and late in the infectious cycle and only in moderate amounts, the 23K protein appears late but is made in great quantity—comparable to the 100K and hexon proteins.

Cells infected with *1-2* also contain a new cytoplasmic polyadenylated RNA species which maps at the site of the new insertion of SV40 DNA in the *1-2* genome and contains both adenovirus and SV40 sequences. As described above, these sequences correspond to the N-terminal portion of the Ad2 fiber protein and the C-terminal portion of the SV40 *A*-gene product (T antigen). In collaboration with J. Lewis, we have shown that *1-2* mRNA selected by hybridization to SV40 DNA or to the Ad2 *Eco*RI E fragment directs the synthesis of the 23K protein in a cell-free system. The cell-free product has the same isoelectric point and proteolytic breakdown pattern as the 23K protein isolated from infected cells. This indicates that the 23K protein is translated from the new hybrid mRNA.

From detailed considerations of the genome structure we inferred that the 23K protein should be a fusion product, with its N terminus derived from Ad2 fiber and its C terminus from SV40 T antigen. To test this idea, the lysine-labeled tryptic peptides of the 23K protein were compared with those of Ad2 fiber and SV40 T antigen by high-resolution column chromatography. Of the eight lysine-containing peptides from 23K protein, six corresponded to fiber peptides and two did not. Immunoprecipitated SV40 T antigen appears in two electrophoretically distinct forms, 96K and 88K. Both of these give rise to a large number of lysine-containing peptides, including the two peptides of the 23K protein which are lacking from Ad2 fiber. Thus the 23K protein is a hybrid structure, containing both adenovirus fiber peptides and SV40 T-antigen peptides in a single polypeptide chain. The protein is phosphorylated, and since the fiber protein does not carry this modification whereas T antigen does, the phosphorylation site is possibly contributed by the T-antigen moiety.

The 23K protein and the 96K form of T antigen also share a very prominent proline peptide which is absent from the 88K form. The correspondence between the lysine-labeled peptides of the 96K and 88K T-antigen molecules shows that the two forms are closely related to one another and suggests that the 88K form may be derived from the 96K form. Such processing would be likely to occur from one end of the molecule, and since the SV40 DNA sequences contained in the new insertion in the *1-2* genome can only code for the C-terminal end of the *A*-gene product, we conclude that the 23K protein shares the C-terminal proline-rich tryptic peptide with the 96K form of T antigen. Structural analysis of this peptide, now in progress, will permit assignment of the reading frame for T antigen when the nucleic acid sequence in this region of SV40 DNA becomes available.

We conclude that the C-terminal portion of the 23K protein has helper-function activity. Since this protein is produced in large amounts, it should be useful for biochemical studies of the helper function. The first steps of purification have shown that it is largely located in the cytoplasm, mostly in the postribosomal supernatant. From there it can be greatly enriched by phase partition with polyethylene glycol and dextran.

Characterization and purification of SV40 T antigen

Early after infection by SV40, permissive monkey cells produce a large protein which is coded by the *A* gene of the virus. Recent work done in this laboratory as well as in several other laboratories [Rundell et al., *J. Virol* 21:636 (1976)] has shown that the *A*-gene product, T antigen, is a phosphorylated protein that migrates in SDS gels as a 96K species. Although this protein is preferentially immunoprecipitated by antibody raised against SV40 T antigen, sometimes two additional phosphorylated proteins of 92K and 88K coprecipitate. However, if a rapid isolation procedure is used and care is taken to avoid in vitro proteolytic degradation during extraction, the resulting immunoprecipitate contains very little, if any, of the smaller proteins. These data and recent tryptic peptide analysis of the 92K protein strongly suggest that these smaller species are indeed

specific degradation products of the large 96K T antigen. We have also observed that in vitro proteolytic degradation is most prominent during immunoprecipitation of T-Aq from monkey cells. In contrast, T-Aq from human cells lytically infected with a Ad2-SV40 hybrid (D1) or from human and rat cells transformed by SV40 displays a greatly reduced tendency to break down to the smaller proteolytic products [Ahmad-Zadeh et al., *Proc. Natl. Acad. Sci. USA* 73:1097 (1976); Tegtmeyer et al., *J. Virol.* 21:647 (1977)].

In an attempt to characterize further T-Aq and perhaps elucidate its functional role, we have purified T-Aq in its native form. Thus far, T-Aq from either lytically infected cells or transformed cells has been purified some 500-fold. The isolation procedure employs a phase-partitioning step during the initial extraction of the nuclear lysate, followed by standard chromatographic techniques, including gel filtration, DEAE Sephadex chromatography, phosphocellulose chromatography, and DNA cellulose chromatography. The T-Aq was isolated both from radiolabeled extracts (^{35}S and ^{32}P) and from unlabeled extracts. The T-Aq was assayed at every stage of purification by immunoprecipitation and SDS gel analysis as well as by complement fixation. The final product is usually 30–50% pure as judged by SDS gel analysis and contains the 96K protein as well as varying amounts of the 92K and 88K proteolytic breakdown products. The purified T-Aq remains highly antigenic, is phosphorylated, and retains the ability to bind DNA.

Work is in progress to characterize further the ability of the purified T-Aq to bind to specific sites on the SV40 DNA. To determine the specific binding of T-Aq to DNA, we shall employ a combination of two standard techniques: (1) UV cross-linking of proteins to BrdU-substituted DNA and (2) antibody affinity chromatography.

Finally, we have begun to test the biological activity of the purified T-Aq in in vitro nuclear DNA replication and RNA transcription systems.

Small adenovirus-coded RNAs

The studies on virus-associated (VA) RNA have continued. This RNA accounts for a large proportion of the viral transcripts synthesized late in infection, but its function remains tantalizingly unknown. As a result of recent work it has been established that there are two species of VA RNA, a major one called VA RNA$_I$ and a minor one, VA RNA$_{II}$. There is little sequence homology between the two VA RNAs, and they exhibit different kinetics of synthesis. Both are made early as well as late in the infectious cycle, but the synthesis of the major species accelerates dramatically after the onset of viral DNA replication, whereas production of the minor species holds steady. The synthesis of both species can be initiated in vitro, and both start with a pppGp residue. This indicates that RNA polymerase initiation sites are associated with the structural genes for both VA RNA species and suggests that a determination of the sequence of the DNA in these regions should be informative.

The locations of the genes for the two RNA species have been determined with considerable accuracy. The gene for VA RNA$_I$ is bisected by a *Bam* restriction-enzyme cleavage site at position 30 on the physical map of the viral genome. The gene for VA RNA$_{II}$ lies downstream from that of the major species (relative to the direction of transcription) and is separated from it by a short spacer region. The spacer is only about 80 base pairs long and must contain signals for completion of VA RNA$_I$ synthesis as well as for commencement of VA RNA$_{II}$ synthesis. Sequence analysis in and around the gene for the major RNA species is largely completed, and the complementary studies in the vicinity of the minor species are under way.

In the course of experiments designed to screen for additional species of VA RNA—a search which proved unsuccessful—an RNA species of substantially greater length was detected. This RNA is 550 bases long, sediments at 9S, and contains the poly(A) tract characteristic of messenger RNAs. Its map position has been found to lie at position 10 on the viral genome, a considerable distance from the location of the VA RNA genes. In a cell-free system for protein synthesis, the 9S RNA is translated into a polypeptide known as protein IX. It is a structural component of the adenovirus particle and is only synthesized late in the infectious cycle. The DNA sequences coding for it are transcribed both early and late, however, so there must be a mechanism to prevent the premature translation of this protein. One possibility under investigation is that in the early RNA the 9S mRNA sequences occur in a modified form, such that the signals for the initiation of polypeptide IX synthesis are incomplete or hidden.

Structure of naturally occurring strains of adenovirus

Several field strains of adenovirus, isolated over several years from a number of locations, were obtained from Dr. J. Hierholzer at the Center for Disease Control, Atlanta, Georgia. By standard serological tests, which recognize principally the viral fiber protein, these strains had been classified as normal adenovirus types 1, 2, or 5. We have isolated the DNA from such natural strains and analyzed it by digestion with several restriction enzymes. Maps of the cleavage sites show that the DNA of each field strain differs markedly from the DNA of the standard serotype with which it had been identified. We have also analyzed the proteins made in cells infected by the natural isolates. Again, the pattern of polypeptides is not identical to that elicited by the standard serotypes. On the basis of the DNA and protein

studies, it would appear that the characteristics of the standard strains occur in the natural isolates in reassorted combinations. One possibility is that the natural strains originated by recombination between two standard serotypes, a process which occurs readily under laboratory conditions. In order to identify the crossover points for different recombinations, regions of homology between the DNAs of the standard and field strains are being examined by heteroduplex mapping.

Isolation of viable SV40 mutants

The restriction enzymes *Taq* and *BglI* each make a single cut in the SV40 genome, at map positions 0.57 and 0.67, respectively. When circular form-I SV40 DNA is cleaved with both of these enzymes and the resulting mixture of fragments is used to infect monkey cells, virus is produced. The progeny of individual plaques were grown in monkey cells and their genomes were analyzed by digestion with restriction enzymes. Although no virus was found whose DNA was resistant to cleavage by *BglI*, 5% of the virus clones contained DNA which was resistant to *Taq* digestion. These *Taq*-resistant mutants have suffered deletions of up to 4% around the *Taq* cleavage site. Nevertheless, these mutants appear to grow normally and to produce normal T antigen. Their ability to transform rat cells is currently being tested. Mutants with deletions in the same region have been isolated by Shenk et al. [*J. Virol.* 18:664 (1976)]. Infection of monkey cells with DNA altered at the *BglI* site (by *BglI* cleavage followed by digestion with exonuclease or single-strand-specific endonuclease to remove single-stranded tails) also failed to produce a *BglI*-resistant mutant among approximately 200 plaque isolates tested. Since the *BglI* cleavage site lies at or close to the origin of DNA replication, it is possible that the DNA sequence in this area cannot be altered without loss of viability.

Analysis of the structure of integrated viral DNA in transformed cells and revertants

Over the past year significant progress has been made in our analysis of the arrangement of viral sequences in transformed cells. To a large extent, we have brought to a conclusion hybridization experiments which were initiated several years ago, through the application of E. Southern's "transfer technique" and the development of efficient hybridization probes. The experiments follow the general design described in the 1974 Annual Report; however, they differ in certain particulars of procedure. High-molecular-weight DNA, extracted from transformed cells, is cleaved by a sequence-specific restriction endonuclease. The resulting DNA fragments are fractionated by electrophoresis through an agarose gel, denatured in situ, transferred to a nitrocellulose filter as described by Southern [*J. Mol. Biol.* 98:503 (1975)], and hybridized to viral DNA that has been labeled in vitro to high specific activity by the "nick-translation" reaction [Kelly et al., *J. Biol. Chem* 245:39 (1970)]. The distribution of viral sequences among the different-sized fragments of transformed-cel DNA is then determined by autoradiography. The important feature of this method of hybridization is that the labeled viral DNA used as a probe is present in vas excess and provides the driving force for the reaction From the results of these experiments we have drawn the following conclusions concerning SV40 integration

1. After transformation, selection, and subsequen cloning, the chromosomal location for the SV4 genes within any cell line is stable. For example, hamster transformed cell line (The-2) was injecte into a hamster, and the solid tumor that was eventu ally isolated from this animal contained SV40 DN integrated in its cell chromosomes in the same man ner as in the tumor-inducing cell line.
2. There can be multiple copies of intact or partial vira genomes integrated at different chromosomal loca tions. We have now examined the distribution o restriction sites surrounding integrated SV40 DNA i 16 independently transformed Fisher rat cell line No indication of a specific chromosomal insertio site was found.
3. The junction points between viral DNA and cellula DNA contain viral sequences which map at differen positions on the viral genome (see Fig. 2). In thre cell lines the viral DNA appears to have been incor porated into the chromosome by a simple recombi nation event. That is, the integrated SV40 DNA ha the same break points on both sides of the insertion In these cases, then, we can say that the viral genom probably does not have a special sequence (unless i is small and reiterated) for integration. In a fourth cel line (rat cl9) the integrated viral DNA is arranged i the form of a tandem duplication. The stretch of vira DNA is equivalent in length to about 1.5 copies o the SV40 genome.

In our rat and mouse cells transformed by SV40, fr circular viral DNA has not been detected. However, t situation seems much more complex in human ce transformed by SV40. In collaboration with Ji McDougall, we have been analyzing the viral D present in human fibroblast cells transformed by SV4 Preliminary results from experiments addressed to t specificity of integration of SV40 DNA in these cells a parallel to those in rat and mouse cells. However, fr molecules of closed-circular SV40 viral DNA can al be extracted from all of these cell lines. Some cell lin produce on the average of 1000 molecules of free SV per cell. By in situ hybridization techniques, it appe that only a small fraction of the cells contain lar amounts of free viral DNA at any given time. The nu ber of these cells is equivalent to the number

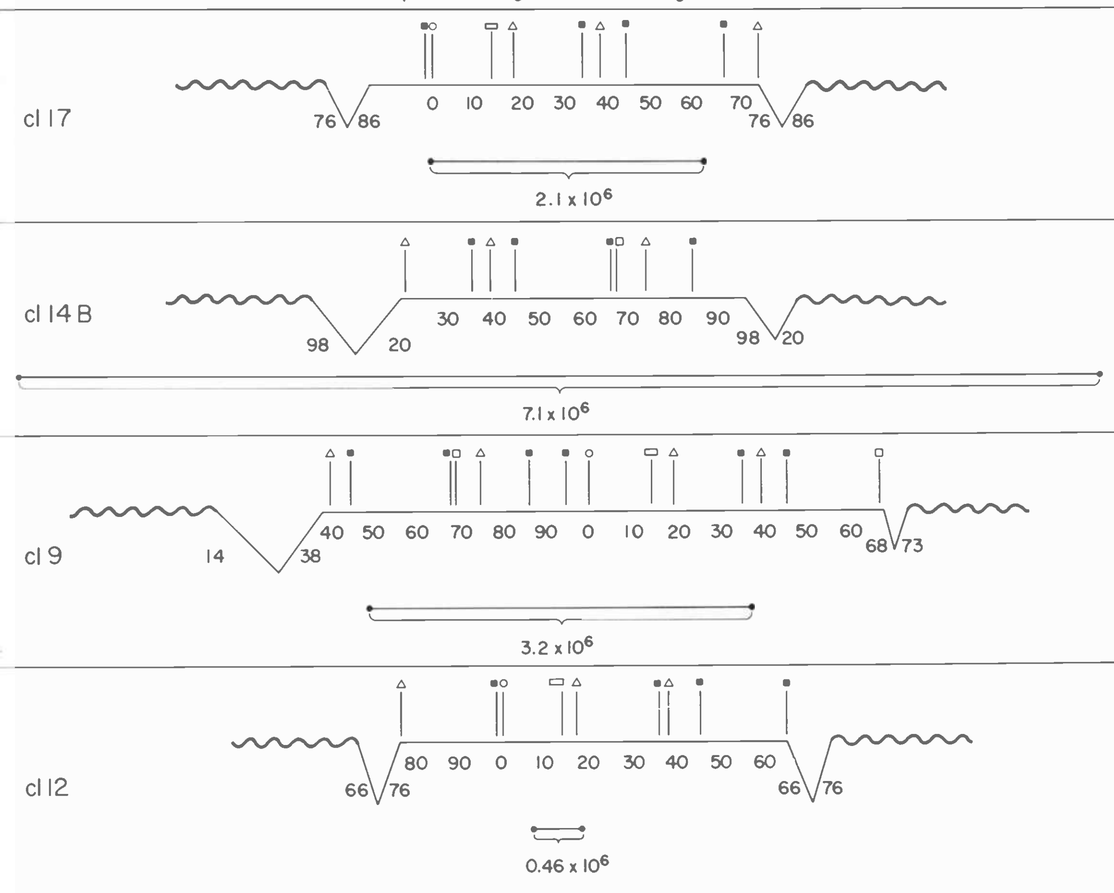


Figure 2
Maps of viral DNA sequences integrated into the genomes of four lines of SV40-transformed rat cells. The maps were constructed as described in detail in the text. The wavy lines represent flanking cellular DNA sequences; the solid lines represent integrated SV40 DNA. V indicates the regions of the SV40 map which contain the junctions between cellular and viral DNA sequences. The sites of cleavage within the integrated viral sequences were identified as described in the text. The symbols represent the various restriction enzymes used: (■) HindIII; (△) HpaI; (○) EcoRI; (□) BglI; (▭) BamHI. The lines marked •–• represent the length of the BalI segment of cellular DNA into which the SV40 DNA is inserted. The molecular weights of these segments, calculated as described in the text, are given in daltons.

V-antigen-positive cells in the culture. Thus SV40 replication seems to be induced in a certain constant fraction of cells from any given cloned line. The induction of SV40 DNA replication in these cells brings into prominence the problem of SV40 excision from chromosomes in general. We now know that SV40 DNA can be rescued from nonpermissive transformed cells that have only single copies of SV40 DNA (e.g., rat clones 17 and 12) by fusion of these cells with permissive monkey cells. Quite remarkably, very early in this rescue process (in some cases 12 hours after fusion) perfect SV40 circles are formed within the heterokaryons. How a stably integrated piece of viral DNA which is joined to cell DNA at different sites can be excised from the chromosome remains a mystery. Current models which are being investigated involve the activation of the viral origin for DNA synthesis while the viral DNA is still integrated.

Human transformed cell lines have also been selected after exposure to specific segments of SV40, obtained by the use of restriction endonucleases, that lack substantial portions of the late region of the viral genome. These lines, if they are similar to whole virus transformants, may provide a source of cloned defective SV40 DNA molecules that have been propagated in the absence of infectious helper virus and are suitable for use as vectors in recombinant DNA research. By propagating the recombinant molecules in transformed cells, it should be possible to carry large segments of foreign DNA and circumvent many of the problems

imposed by the packing of DNA into virus particles.

The state of the integrated viral DNA has also been studied in several lines of rat-embryo cells transformed by adenovirus 2. Data from recent experiments utilizing Southern's technique complement the results of our earlier renaturation kinetic analyses. Thus, in DNA from cell lines such as F17, F18, and 2T4, the pattern of fragments observed on autoradiograms is consistent with the presence of only the leftmost 14% of the adenoviral genome in these cells. By renaturation kinetic analysis, F18 has three copies and 2T4 has seven copies of this 14% of the adenoviral genome. Surprisingly, only one adenovirus DNA insertion is seen in F18, and only two in 2T4. This finding suggests that the number of initial integration events in any given cell is small, and that the multiple copies of viral genes found in the stable transformants may be due to aneuploidy or to amplification of the chromosomal region that contains integrated viral sequences.

DNA fragment transformation

Experiments were conducted during the past year to determine the amount of viral genetic information necessary for transformation of nonpermissive cells by SV40. We have used the calcium phosphate technique of Graham and van der Eb [*Virology* 54:536 (1973)] for DNA infection of an established rat fibroblast line, measuring transformation by the number of dense foci in 5% serum. Our results show that only restriction fragments that contain DNA corresponding to the entire "early" region of SV40 give a measurable frequency of transformation. Defining ΔA as the amount (in map units) of "late" DNA attached at the 5′ end of the "early" region and ΔB as the amount of "late" DNA present at the 3′ end, we find that the efficiency of transformation is directly proportional to the *product* $\Delta A \Delta B$. The purely statistical nature of this dependence argues against the existence of one or a few specific integration sites within the virus and suggests that integration (by whatever mechanism) of SV40 into rat cells is not site-specific. We also find that the region of SV40 capable of complementing TsA (early) mutants of SV40 in a lytic infection is almost exactly coextensive with the transforming region, and that the efficiency of this complementation shows the same dependence on $\Delta A \Delta B$ as does transformation. The only exception is the restriction fragment generated by cleavage with restriction endonucleases *Bam*HI and *Bgl*I, which will complement TsA but which will not transform. It is unknown whether the block to transformation by this fragment lies in the integration or in the expression of the DNA.

Revertants of SV40-transformed cells

Revertant cell lines selected from SV40-transformed cells have in general been T-antigen-positive; on the other hand, a direct role for the viral genome in the maintenance of transformation was indicated from the temperature-sensitive growth properties of cells transformed by the TsA mutant of SV40. Many reasons have been proposed to explain these paradoxical findings, including the following: (1) The SV40 genome is not directly involved in maintaining the phenotype for which reversion is selected. (2) There are so many cellular genes involved in maintaining transformation that finding a revertant with an altered viral genome would be unlikely. (3) The cell lines used had multiple copies of an active dominant viral gene (T antigen), so that a cellular variant would necessarily be selected.

In collaboration with Betty Steinberg and Bob Pollack at Stony Brook, we have been reexamining this question.

The transformed rat cell line 14B contains one copy of SV40 DNA per cell [see Fig. 2 and Botchan et al., *Cell* 9:269 (1976)]. Line 14B contains nuclear T antigen as determined by indirect immunofluorescence and immunoprecipitation. It grows to a very high density, grows in low serum and in methocel, and is highly tumorigenic in "nude" mice. Revertant cells which have been isolated from 14B by FdU selection exhibit the T-antigen-minus phenotype. These T-antigen-negative revertant cell lines can be divided into three classes: those in which the integrated SV40 DNA is retained with no detectable changes, others in which the integrated SV40 DNA is retained but has acquired a small deletion in the T-antigen gene, and those in which the integrated viral DNA is completely lost. All three groups are T-antigen-negative under both sparse and confluent culture conditions and after passage at 32°C. All three groups are density-inhibited and do not grow in methocel. Finally, some of the revertants can be retransformed with SV40 DNA.

These findings reinforce the earlier conclusion that some of the growth properties of transformed cells are the direct consequence of the continued expression of the integrated SV40 *A* gene.

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PROTEIN SYNTHESIS

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Suppression

Last year, we showed that the yeast ochre and amber nonsense suppressors could function in a cell-free system derived from eukaryotes [R. F. Gesteland et al., *Cell* 7:381 (1976)]. It was shown that this suppression is due to the tRNA fraction from suppressor-carrying cells. Perhaps this provides a foot in the door toward the development of a suppressing system for mammalian cells.

Purification of the suppressing tRNAs

The recessive lethal amber suppressor RL1, isolated by M. C. Brandress et al. [*Genetics* 79:551 (1975)], inserts serine in response to an amber codon and can be assayed by suppression of an amber mutation in the $Q\beta$ RNA phage synthetase gene. Using this assay we have partially purified the suppressing species on a benzolated DEAE (BD) cellulose column where the serine-accepting activity comes off in a late and rather broad peak and the suppressor activity is on the leading edge. The active fractions are then chromatographed on Sepharose 4B, where at least one of the serine-accepting species separates from the suppressing activity, which again comes off late, giving a good purification. At least 50% of the tRNA in the preparation accepts serine, and the preparation has a high specific activity for suppression. With Peter Piper, who was here for a visit this summer, we showed that this partially purified fraction has a mobility on two-dimensional gels that corresponds to the mobility of a component that has a UCG-decoding tRNA sequence. Furthermore, he has shown that this component has a major and a minor UCG-decoding tRNA, which differ by a few nucleotides, and that it is the minor species which is mutated to a sequence that will read the amber codon in the amber suppressor. We also showed that if the crude tRNA from the amber-suppressing strain is run on a two-dimen sional RNA gel, the suppressing activity can be recov ered in the spot predicted by Piper's results. We do no yet know whether our column-fractionation schem discriminates between major and minor UCG-decodin species; if it does not, we will make an effort to fin some method that will discriminate between these.

We are at the same time purifying the suppressin tRNA from a strain which carries an ochre allele of th amber suppressor. The ochre suppressing species is as sayed by read-through of the normal terminator UAA a the end of the $Q\beta$ synthetase gene. In this latter case w would like to look at the sequence of the suppressin tRNA to ask why the yeast ochre suppressors are specifi for ochre, whereas the *coli* ochre suppressors read bot ochre and amber.

Suppression in mammalian systems

Our original cell-free suppression experiments wer done in mixed systems having a yeast S-100 fraction an other components from a mammalian source. We hav now found conditions under which we can get hig levels of suppression in the standard mammalian systen that we use [M. Schreier and T. Staehelin, *J. Mol. Biol.* 73:329 (1973)] by adding just the yeast tRNA. Lowerin the temperature of incubation to 25°C seems to be th major factor in attaining higher suppression levels, an purification of the tRNA species allows inclusion o more suppressing tRNA without concomitant additio of other species that tend to inhibit protein synthesis which also results in higher levels of suppression. Ver recently we have found that we can also get high level of suppression in the very simple reticulocyte lysate system described by Pelham and Jackson, from whic the endogenous messenger RNA activity is removed b treatment with micrococcal nuclease. This provides

very simple, active, and clean system for doing these experiments.

Together with Terri Grodzicker we are using the cell-free suppression system to ask whether any of the host-range mutants of Ad2$^+$ND1 that she has isolated and characterized are amber or ochre mutants. We have good evidence that a number of these mutants make, at least in vitro, fragments rather than the normal gene product; also, we have preliminary evidence that one of the mutants, which makes a 19,000-molecular-weight fragment, can be suppressed by an amber suppressor in vitro to give back some of the 30,000-MW wild-type product. Hence we conclude that this particular mutant is an amber mutant, and we hope this can be used to help develop suppressing cell lines. These host-range mutants are defective in the SV40 function that allows adenovirus to grow on monkey cells. The purpose of one of our current experiments is to see whether we can suppress these mutations in vivo by getting suppressing tRNA into mutant-infected monkey cells and then see whether or not virus is produced. We are trying this by using red blood cells preloaded with purified tRNA species with the infected monkey cells. To make a suppression system useful it will obviously be necessary to find mutant monkey cell lines that are capable of suppressing this amber mutant.

Cloning

We are trying to clone the suppressor genes from yeast, first to study their structure and second to develop vectors carrying these genes. T. Petes, D. Botstein, and P. Wensink have constructed a set of 2500 clones, each with a randomly generated piece of yeast DNA integrated into the *coli* PMB9 plasmid. We have screened these with ^{32}P-labeled leucine and serine tRNAs and have identified a number of clones that have these sequences. The serine-tRNA-containing plasmids are being investigated further by restriction-nuclease digestion and Southern-type blotting experiments to identify those fragments specifically carrying the tRNA sequences. These fragments will then be made very radioactive by nick translation and used as probes for hybridization to restriction digests of DNA from the suppressor-carrying cells to identify those DNA fragments that are of interest. These can then be cloned by conventional ligation into PMB9.

β-Galactosidase synthesis in *E. coli*

Jim Manley has finished his thesis work on the synthesis of abnormal proteins from the β-galactosidase gene of *E. coli*. He has been able to identify fragments of β-galactosidase that result from premature termination of translation of the messenger RNA, both in vitro and in vivo. Such fragments had been observed in other cases among in vitro products, and it had been assumed that this was an artifact of cell-free systems. However, it is now clear that the same fragments are produced in vivo, in surprisingly large amounts. The calculation in Manley's experiments says that one out of three ribosomes that start translating the β-galactosidase gene in vivo does not make it to the end but falls off prematurely. He has ruled out that this is due to proteolytic degradation. He has also shown that the internal restarts (initiation by ribosomes at internal sites in the message rather than at the normal start site) can be demonstrated in vitro, and that the resulting fragments are stable in the cell-free system whereas in vivo they are usually rapidly degraded. The restart fragments made in vitro have a funny solubility problem; at 37°C they are in some large complex that sediments very rapidly but when made at 25°C they are in the soluble fraction and can be readily detected. He has also made the surprising observation that if the Zubay-type cell-free system is run at a lower than normal temperature one sees suppression of amber mutants under otherwise nonsuppressing conditions.

Yeast *his* genes

Ray Bigelis here, in collaboration with Gerry Fink at Cornell, has been looking at the expression of the *hisA*, *B*, and *C* genes in yeast. He has obtained very good antibody against the purified histidinol dehydrogenase protein and has been able to show that the three genes produce one large protein of MW 90,000 which can be cleaved to give some separation of the activities. He is now looking at various fragments produced by nonsense mutations within the cluster and trying in vitro synthesis of the gene product to identify the primary translation product.

Yeast galactose genes

Jim Broach is initiating work to clone the *gal* genes in yeast. This is an interesting system in that three structural genes of the operon are controlled together by two controlling genes that map at other locations in the genome. He is currently screening the Petes collection of clones for those that contain the *gal* gene sequences by using ^{32}P-labeled or iodinated RNA from induced and uninduced cells. Since the *gal* messages should be among the relatively abundant species, it should be possible to pick up these clones by the difference in hybridization of these RNA preparations. Broach is also fractionating mRNA so that partially purified probes can be used for screening if that becomes necessary. The long-term hope here is to understand the organization of this cluster and to get at the function of the two controlling genes.

Abortive infection of monkey cells by Ad2

A new variant of Ad2 has been isolated that multiplies efficiently on monkey cells without the aid of SV40. The

variant was selected after mutagenesis by sequential passage on monkey cells. The kinetics of its growth, its burst size, and the efficiency of plaque formation are similar on human and monkey cells. Electron-microscope, restriction-endonuclease, and hybridization analyses have failed to detect any differences between the genomes of the variant and its parent. The pattern of protein synthesis of the variant is the same in human cells and in monkey cells, with the full complement of viral protein being synthesized in normal amount. This is in marked contrast to the much reduced synthesis of late viral proteins, particularly fiber, during Ad2 infection of monkey cells. The mutation appears to act in *trans*, since it enhances the synthesis of Ad5 fiber protein in coinfections of monkey cells. Together with John Hassell we are mapping the mutation responsible for the increased host range by modified marker rescue techniques.

5′ Ends of fiber and 100K mRNAs

Our earlier studies on the block to Ad2 multiplication in monkey cells indicated that although the concentration of fiber mRNA was approximately ten times lower in abortive than in productively infected monkey cells, the synthesis of fiber protein was below the level of detection. Recently, with the more sensitive technique of immunoprecipitation, fiber was found to be synthesized at only 0.3% of the level observed in productively infected monkey cells. This large difference in fiber synthesis between the two types of infection, compared with the more modest difference in fiber mRNA levels, suggested that the mRNA should be examined. We have looked specifically at the 5′ ends and have found that in both types of infection the fiber mRNA and also the 100K mRNA are capped. Unexpectedly, the fiber and 100K mRNAs both contain similar (if not identical) T_1 oligonucleotides at their 5′ ends. These oligonucleotides have a structure and nucleotide composition consistent with that found by R. Gelinas and R. Roberts for the major cap-containing oligonucleotides found in mRNAs late in Ad2 infection of human cells. Mild nuclease digestion of the RNA in the DNA-RNA hybrid state removes the oligonucleotide from these two messengers, which suggests that this common oligonucleotide may not be encoded at the beginning of each gene. To investigate this we are doing fine-structure mapping of the sequence corresponding to the 5′ end of fiber mRNA in the Ad2 genome, and together with R. Roberts' group, we hope to compare the DNA sequence of this region with the sequence of the common 5′ T_1 oligonucleotide.

Mapping of adenovirus genome

This year efforts have continued to identify and characterize the protein products of the Ad2 genes, particularly the early genes. As we reported last year, cell-free translation of early mRNA selected by hybridization to specific fragments of Ad2 DNA identified five Ad2 early mRNAs. Experiments this year, using mRNA prepared from cells infected at much higher multiplicity (200 pfu/cell compared to 10–25 pfu/cell used previously) have identified additional polypeptides as products of cell-free translation of Ad2 early mRNA. Current results are summarized in Table 1.

The existence of multiple polypeptide products from each of the two right (75.9–83.4, 89.4–98.5) Ad2 early mRNAs raises the question of whether there is more than one mRNA coded for by either or both of these regions of the genome, or whether either of these mRNAs might be polycistronic. To investigate this question further, these mRNAs were given to L. Chow, R. Roberts, and T. Broker for R-loop analysis in the electron microscope. The mRNAs complementary to the region 75.9–83.4 were found to be heterogeneous in size, but the rightmost region coded for only one very homogeneous population of molecules, which covered map positions 91.5–96.8, that is, an estimated coding capacity of 68,000 daltons of protein. The five polypeptides resulting from cell-free translation of this mRNA total 92,000 MW. Some of these polypeptides may thus be overlapping in sequence. This possibility is currently being investigated by peptide mapping. If at least some of these polypeptides are unique in sequence, then this mRNA is a particularly attractive candidate to study as an example of a eukaryotic polycistronic mRNA.

Of particular interest are those early proteins that are products of the transforming gene(s) of Ad2. To this end we have been especially interested in characterizing the 41K–57K and 15K proteins, since the genes for at least the 41K–57K and possibly also the 15K map within the transforming region of Ad2. The 41K–57K protein as synthesized in vitro is heterogeneous, in that a number of bands in the range 41,000–57,000 apparent molecular weight are found in different experiments. R-loop analysis of this mRNA by our Electron Microscopy Section has shown that this mRNA maps between 1.3 and 4.0 map units and thus can code for only 36,000 daltons

Table 1
Identification of polypeptide products of cell-free translation

Portions of DNA within which sequences complementary to mRNA map (map units)	*Polypeptide products of cell-free translation (K)*
0 – 4.4	41 – 57
4.4 – 17.0	15
58.5 – 70.7	72
75.9 – 83.4	15.5, 14,* 13*
89.4 – 98.5	24,* 21,* 19, 17,* 11

*New polypeptides not seen with RNAs prepared at lower multiplicities of infection.

of protein. Thus the heterogeneity of this polypeptide as synthesized in vitro must be due to some variable degree of posttranslational modification, which also presumably confers upon the polypeptide an anomalously slow mobility on SDS-polyacrylamide gels. Indeed, comparison of the peptides produced by partial digestion of these polypeptides with protease has shown that two prominent in vitro products of 43,000 and 46,000 MW have very closely related sequences.

This year we have also shown that proteins of 15K, 42K, and 50K can be specifically labeled early after infection of cells with Ad2; that is, they are not found in uninfected cells. These proteins are best resolved from host-cell proteins of similar molecular weight by two-dimensional isoelectric focusing–SDS-polyacrylamide gel electrophoresis. Preliminary studies comparing peptides produced by partial protease digestion indicate that the 42K and 50K in vivo proteins are very similar to each other and to the 43K and 46K in vitro polypeptides. Similarly, the 15K protein synthesized in vivo gives a partial digestion peptide pattern very similar to that of the in-vitro-synthesized 15K. Thus the two polypeptides (41K–57K, 15K) shown by our in vitro studies to be the products of the genes associated with the transforming activity of Ad2 can now be correlated with particular proteins found in infected cells. This result opens up the possibility of purifying these proteins in their native form from infected or transformed cells by using two-dimensional gels as an assay.

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MAMMALIAN CELL GENETICS

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This year has seen not only the transfer of much of our work from the Department of Cancer Studies at Birmingham University Medical School, England, but also the beginning of very promising collaborations with other established sections at Cold Spring Harbor. Our main research interests are (1) the biological characteristics of cells transformed to a neoplastic state by a variety of agents (e.g., viruses and chemicals), (2) the extent to which the in vivo behavior of such cells is a reflection of their in vitro properties, (3) the cytogenetics of tumor cells, with particular reference to specific translocations and deletions in experimentally induced animal tumors and in human tumors, and (4) the cytogenetic changes induced in human cells after virus infection in relation to the induction and localization of human genes.

From this it can be seen that our intent is to deal with the same question previously posed for this section by Bob Pollack: What allows cancer cells to grow where normal cells are inhibited? Our method of study is to search for the comparisons which can be made in the properties of tumor cells induced in the same species by different oncogenic agents. We already know that not all cells which exhibit the properties of cancer cells in vitro are tumorigenic in vivo; this phenomenon provides us with a basis for studying those characteristics which correlate with cancer induction but are absent from both normal cells and cells only partly along the pathway to becoming malignant.

We have enjoyed a valued collaboration over the past three years with Frank Ruddle's group at Yale and with Raju Kucherlapati at Princeton, pursuing our joint interests in somatic-cell hybrids. One of the many benefits of being at Cold Spring Harbor is that these mutual interests are continuing without the problems of transatlantic experiments.

Transformation of human cells by SV40

We have initiated a series of experiments designed to study the integration of SV40 into the chromosomes of human cells. Human cells are semipermissive for the replication of SV40 virus, and transformed cells tend to have multiple copies of virus DNA. In order to examine the association of viral DNA with particular chromosomes, we fuse the parental SV40-transformed cloned cell lines with mouse cells, using polyethylene glycol to promote fusion. The resultant hybrid cells, which initially contain a full complement of chromosomes from each species, lose most of the human chromosomes during subsequent divisions of the hybrid. The loss of human chromosomes, with some exceptions, is random, and thus a panel of cloned hybrid cell lines is developed in which different complements of human chromosomes are represented. By examining the hybrid cell lines for virus-transformed phenotypes (e.g., T antigen) and for viral DNA, it is possible to assign viral integration sites to particular chromosomes. The initial results of Croce et al. [*Proc. Natl. Acad. Sci. USA* 72: 1397 (1975)] suggested a specific integration site in only one human autosome, C7, but subsequent studies in this and other laboratories argue for a more complex situation, with integration of virus DNA into more than one chromosome. In parallel with our experiments in somatic-cell hybrids, the SV40-transformed cells are being examined by Michael Botchan and Joe Sambrook for both integrated and free viral DNA. Apart from the integration studies, one of the properties amenable to study in these somatic-cell hybrids is replication of free viral DNA in the established cell lines. We are examining the hybrids for both integrated SV40 and continued extrachromosomal viral DNA synthesis in order to establish which human chromosomes carry genes which regulate SV40 DNA replication.

In collaboration with Raju Kucherlapati at Princeton, a large suite of hybrids formed from the fusion of long-established SV40-transformed human cells and mouse cells is being examined for viral markers. These hybrid cell lines will extend our ability to determine associations between human genetic markers and integrated SV40 virus.

Transformation by adenoviruses

Ad2 transformation of rodent cells, a semipermissive system, results in cell lines which share many characteristics while differing in others. Epithelioid morphology, growth to high density, increased protease production, and virus-specific T antigen are all common phenotypes, and the integration of a minimum segment of the left end of the virus genome [Sambrook et al., *Cold Spring Harbor Symp. Quant. Biol.* 39:615 (1974)] is consistently observed. Further studies on these cell lines and an Ad5-transformed human cell line [Graham et al., *Cold Spring Harbor Symp. Quant. Biol.* 39: 637 (1974)] carried out by Phil Gallimore in Birmingham and in this laboratory have identified other phenotypes that are not uniformly expressed: serum-independent growth, tumorigenicity in syngenic rats or "nude" mice, and the synthesis of large, external, transformation-sensitive (LETS) protein. Anchorage-independent growth, as measured by the ability to form colonies in methylcellulose, did not give a good correlation with tumorigenicity in adult athymic nude mice, as two of the seven lines tested plated well in methylcellulose but were nontumorigenic in adult nude mice. This is in contrast with the results reported for SV40-transformed cells [Shin et al., *Proc. Natl. Acad. Sci. USA* 72: 4435 (1975)], where growth in methylcellulose and tumorigenicity appear to be well correlated.

The distribution of the cell-surface LETS protein on these cell lines does, however, correlate well with tumor induction. These experiments were carried out in collaboration with Lan Bo Chen and are reported fully in the Cell Biology section. The only other factor identified as having an association with oncogenicity of these cell lines is the initial virus input used to transform the cells. Adenovirus-2-transformed cell lines derived from dishes infected at a virus/cell ratio of 50 plaque-forming units (pfu):1 can produce tumors in syngenic animals without immunosuppression [Harwood and Gallimore, *Int. J. Cancer* 16: 498 (1975)]. Cell lines initiated from dishes infected with 1–40 pfu/cell may only produce tumors in immunosuppressed syngenic animals, and those initiated with less than 1 pfu/cell are, with one exception, nontumorigenic. There is a significant difference between the behavior of another serotype (Ad12) and Ad2 in this respect: Ad12-transformed cell lines are all highly oncogenic and have most or all of the viral genome incorporated.

Exposure of cells to higher virus doses (Ad2) or integration of more of the viral DNA molecule (Ad12 and possibly Ad2 at high m.o.i.) may result in an increased probability of integration into a specific site(s) in the host genome. Our recent results from studies with somatic-cell hybrids suggest that at the host chromosome level there is no indication that Ad2 sequences integrate at specific loci. As most virus-transformed cell lines are derived from primary embryo cell cultures, there may be transformation of cell types at varying stages of differentiation, and a particular differentiated state could exhibit increased growth potential both in vitro and in vivo. The probability of transformation of particular "target" cells is increased at higher virus m.o.i. It may be that transformation results from the random integration of fragments of viral DNA which include the transforming genes but that further conversion to the tumorigenic state requires a genetic change in the host such as randomly induced mutation following virus infection, or even a stable epigenetic change.

One interpretation of all these facets of Ad2-transformed cells is that oncogenicity is the result of at least a two-stage progression. Viral transformation in vitro can be achieved by infecting cultures with 0.25 pfu/cell of Ad2 or by transfection with DNA of molecular weight 1.6×10^6 daltons, and the cell lines will have the virus-associated transformation phenotype yet be unable to establish tumors. The increased oncogenicity of cells transformed at higher virus m.o.i. may reflect other effects on the cell genome, e.g., increased structural damage to cell DNA, which can in fact be seen at the cytogenetic level. Induction of chromosome damage by adenoviruses, and other DNA viruses, is dose-dependent. Although the majority of cells with chromosome aberrations do not survive, error-prone repair of damage may be one of the most important factors in oncogenesis.

A series of hybrid cell lines resulting from the fusion of Ad2-transformed rat cell lines F4, F17, and F18 with mouse cells has been made. The rat/mouse hybrids segregate rat chromosomes, and established cell lines contain only a limited number of rat chromosomes. None of the chromosomes consistently segregates with adenovirus T antigen, although in Ad2/F18, for example, a cell line containing an estimated 2.9 copies of the 14% left end of the viral DNA molecule [Sambrook et al., *Cold Spring Harbor Symp. Quant. Biol.* 39: 615 (1974)], T antigen is associated with retention of rat chromosome no. 14. The absence of this chromosome from some T-antigen-positive hybrids made from Ad2/F17 or Ad2/F4 argues strongly for random integration sites in the host.

Transformation by herpesvirus

Herpes simplex virus type 2 (HSV-2) has been associated with carcinoma of the human cervix by seroepidemiological criteria, reported recovery of

virions, and detection of virus-specific antigens in cervical carcinoma cells. The oncogenic potential of the virus has been investigated by transforming rodent cells in vitro. We have taken one line of Syrian hamster cells transformed by UV-inactivated HSV-2 and, after cloning, analyzed the cloned lines cytogenetically for tumorigenicity and for virus mRNA by in situ hybridization. The original cell line [333-8-9; Duff and Rapp, *Nat. New Biol.* 233: 48 (1971)] is hyperdiploid with a mode of 49 chromosomes and contains a spectrum of cytogenetic marker chromosomes present in varying frequencies. The clones demonstrate a segregation of these marker chromosomes and other abnormal chromosomes. Cloned lines have distinct stem-cell types, with the characteristic abnormal chromosomes present in a high percentage of cells. The marker chromosomes have been identified by Giemsa banding techniques. All the clones have distinctive, persistent in vitro morphologies, ranging from purely epithelial to purely fibroblastic. They differ from the line 333-8-9 and among themselves in tumorigenicity, varying from the highly tumorigenic to the entirely nontumorigenic. Herpesvirus-2-specific sequences have been detected in the original cell line and in all but two of the clones. There does not appear to be any obvious correlation between the three parameters of detection of HSV-2 information, marker-chromosome occurrence, and tumorigenicity. As with the Ad2-transformed rat cell lines described above, one characteristic which showed a clear correlation with oncogenicity of the clones was the distribution of LETS surface protein.

We are using fragments of HSV-2 DNA to transform rodent cells in two ways. LMTK$^-$, mouse cells deficient in thymidine kinase, have been converted to TK$^+$ using either restriction-endonuclease (*Eco*RI and *Hin*dIII) fragments or sheared viral DNA. Cytosol extracts of the cloned cell lines possess TK activity identical to the enzyme synthesized de novo in HSV-2-infected cells, as determined by thermolability, mobility on polyacrylamide gel electrophoresis, and substrate specificity. The *Eco* RI and *Hin*dIII fragments which convert TK$^-$ cells of TK$^+$ overlap on the map of HSV-2 and therefore allow a suggested assignment of the TK gene to the long unique region of the viral genome between map units 53.2 and 64.6. Whether in fact the HSV-2 DNA fragments are integrated into cellular DNA remains to be determined, but the TK activity has remained stable through eight passages in nonselective medium. These experiments demonstrate that defined fragments of HSV-2 DNA can transform cells in culture. We have also transformed primary rat liver cells using sheared HSV-2 DNA; the cloned lines have cytogenetic abnormalities and are positive for HSV-specific antigens by immunofluorescence. We will be testing the oncogenic potential of these cell lines and of others being derived by transformation of cells with defined fragments of HSV DNA.

Cytogenetics of tumor cells

In experimental systems it is clear that some chromosomes are more frequently involved in rearrangement of the karyotype than are others. We have observed that rat cells transformed by adenoviruses, SV40, and herpesviruses all show similar marked chromosomes, and that two of the autosomes regularly involved in rearrangements in adenovirus-transformed cells are also preferentially involved in marker formation in chemical transformants. In hamster cells transformed by adenovirus, herpesvirus, polyoma virus, or carcinogenic chemicals, identical chromosome rearrangements have been described; some of these were cataloged during our studies of the 333-8-9 cell line. This could suggest involvement of specific loci on the autosomes which carry genes important in the regulation of cell growth and therefore of malignancy.

A survey of 287 human neoplasms carried out by Mittelman and Levan [*Hereditas* 82: 167 (1967)], and since extended by these workers to include more cases, has identified a limited number (nos. 1, 7, 8, 9, 14, 17, 21, and 22) of chromosomes regularly involved in structural aberrations. We are studying interactions between viruses and human chromosomes, and it is perhaps of more than passing interest that six of the seven human chromosomes (nos. 1, 7, 9, 14, 17, and 22) which we and others have reported as specifically affected by adenoviruses, herpesviruses, and SV40 fall into the same group. Although no virus etiology is implied, the nonrandom involvement of a chromosome in human neoplasms [Rowley, *J. Natl. Cancer Inst.* 52: 315 (1974)] and in cellular responses to virus infection may point out important regulatory pathways. In collaboration with Janet Rowley in Chicago and Felix Mittelman in Lund, Sweden, we are examining these phenomena in detail.

Human gene mapping

Our previous work in this area has used adenovirus-induced uncoiler regions on human chromosomes nos. 1 and 17 to facilitate the localization of the gene coding for thymidine kinase, galactose kinase, lactate dehydrogenase-A, and esterase-A4 and the major locus for 5S DNA on chromosome 1. We are currently investigating in somatic-cell hybrids three further uncoiler regions induced by adenovirus 12 on chromosome 1 in order to determine whether there is an association between these regions and the gene loci for adenylate kinase-2, guanylate kinase-1, and uridine monophosphate kinase, all of which are known to map on chromosome 1. Recent results obtained in Frank Rud-

dle's laboratory at Yale show that collagen-4 can be assigned to chromosome 17; we are currently collaborating in examining virus-infected somatic-cell hybrids to investigate the extent of linkage between TK, Gal-K, and this gene.

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MOLECULAR GENETICS

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Protein degradation

We have known for many years that *Escherichia coli* is able to selectively degrade aberrant proteins. For example, proteins loaded with an amino acid analog, the polypeptide termination and reinitiation fragments produced by nonsense mutants, and the small fragments produced by premature termination after treatment with puromycin are rapidly and selectively degraded, whereas their normal counterpart proteins are completely stable. Our laboratory has been studying both the genetic and the biochemical bases of this phenomenon. In the past we have isolated mutants in the degradation system itself, and, in collaboration with Susan Gottesman of the National Institutes of Health, we have continued our mapping of these mutants. So far, all deg^- mutations mapped have been shown to be in the same location as the *lon* gene of *E. coli*, as was shown for the original deg^- mutation several years ago by Barry Shineberg.

So far in the study of the substrates of the *deg* system only proteins with relatively large aberrations, such as those described above, have been used. In order to simplify our study we decided to look at proteins which were altered in only one amino acid—that is, missense mutations—to see if they too were substrates for the *deg* system of *E. coli*.

Over the past decade our laboratory has isolated a large number of Z^- mutants, many of which have been tentatively classified as missense mutants. During the course of this year we confirmed that many of these were missense mutants—a somewhat complex job—and then tested them to see if any were degraded. We found that of 32 independently isolated missense mutants mapping at 17 different sites, seven were unstable and the rest were stable. These seven mapped at only three sites, five at a single site and the other two at separate sites. Thus we conclude that degrading missense mutants are a minor class of all missense mutants in β-galactosidase. This suggests that the system which recognizes aberrant proteins needs some prominent alteration in the protein to distinguish it from the native configuration. We hope that further study of what is wrong with these missense mutants will give us some clue as to what this distinguishing feature of aberrant proteins is.

An obvious question to ask is "What is the effect of the *deg* mutations on the degradation of the degradable missense mutants?" This was tested in a straightforward way which showed that all *deg* mutations slowed the degradation of the degradable missense mutants to a considerable degree, implying that the degradation of missense mutants is carried out by the same system used to degrade nonsense fragments. Presumably the only reason that nonsense mutations are more likely to lead to unstable proteins is the much greater alteration they produce.

From January through August, Professor Charles Miller from Case Western Reserve University was working full-time in our laboratory. Dr. Miller has isolated mutations in a large number of genes known to specify protease or peptidase mutants in bacteria. He was interested in finding out if any of these mutants, which lack a known protease or peptidase, is in any way deficient in degrading aberrant proteins. He studied the two major proteases of bacteria, proteases I and II, and about five peptidases. An extremely careful and extensive study showed that there was no difference in the degradation of a variety of nonsense mutants in the absence of any or all of these protein degradation functions. In addition, he tested for the presence of known proteases and peptidases in the deg^- strains. There was no alteration whatsoever in the pattern of these enzymes, including several proteases for which no mutants yet exist. Thus, whatever the function lacking in deg^- mutant, it is not among the known proteolytic enzymes of the cell.

Clearly, one way to get a handle on which enzymes are doing the degradation would be to have the degradation take place in vitro, and thus have an assay for purification of the system. Although this is relatively trivial with most protein-degradative enzymes, in the case of the *deg* system there are certain problems. First of all, the *deg* system is known to require energy for degradation. Also, it does not function—at least it does not function nearly as actively—in vitro as in vivo. For example, in the case of the long nonsense fragment X90, degradation is completely blocked by cyanide in vivo, completely blocked by the addition of toluene to the cells, and completely blocked by opening of the cells in any manner we have yet employed. Stymied in using a long nonsense fragment, we decided to use the smallest possible piece of β-galactosidase we could detect, and chose the auto-α fragment, which is a peptide of about 7500 molecular weight that comes from the N-terminal end of β-galactosidase and can be detected in a complementation assay. We were able to show that extracts led to the disappearance of the complementing activity of auto-α. This disappearance has a nonlinear kinetics, going on for a short period and then stopping. It is very difficult to get all of the auto-α activity to disappear, but the 75–80% that does disappear does so quite rapidly. A variety of other features of this reaction suggest that it is of considerable interest. For example, it does not involve any of the known peptidases or proteases of *E. coli*. Thus, at the very least, it may represent the action of a new proteolytic enzyme. However, we have not shown that the auto-α activity is disappearing because it is degrading. It could conceivably be disappearing for other reasons. In order to distinguish between these possibilities, we engaged in making pure labeled auto-α. With this material, disappearance due to binding or some form of chemical addition could be distinguished from disappearance of complementing activity due to actual cleavage of the peptide.

The λ attachment site

Another project in which we engaged was an attempt to define the minimum physical structure of the DNA of λ which is required for attachment to the host chromosome. In pursuit of this, a fine-structure map of the attachment site and the surrounding DNA region has been constructed with the use of 39 restriction enzymes. It contains 227 sites, 180 of which are in a 5000-base-pair *Eco*RI fragment containing the λ*att* site. We hope that this detailed mapping will enable us eventually to place limits on the sequence of DNA used for integration. One of the problems in such a study is to devise a biological assay for the attachment site. An approach that is now showing some signs of success is to cut out an *att*-site-containing piece of DNA with restriction enzymes and clone it on a plasmid. A piece about 420 nucleotides long was chosen, which was certain to contain the attachment site. This piece was then inserted, by ligation, into plasmid PBR313 at a *Bam Hin*dIII site. Since the plasmid can be opened with these two restriction enzymes, which are the same as those used to define the attachment-site DNA piece, simple annealing and ligation served to close the plasmid. The plasmid was then grown up in the appropriate bacteria. Subsequent restriction-enzyme analysis indicated that the desired piece had been cloned. Now, how do we know that this *Bam Hin*dIII piece contains a functioning attachment site? We are testing a variety of approaches to answer this question. One that seems to show considerable promise is infecting the plasmid-containing strain with wild-type λ. When this is done, it is found that the number of λ-immune survivors from a cloned attachment-site strain is ten times fewer than from the control. This result was expected, but unfortunately there are a rather large variety of explanations. All, however, involve the presence of a λ*att* site on the plasmid. For example, the plasmid could be competing with the bacterial chromosome for λ. Once the λ integrates into the plasmid, the now greatly enlarged plasmid is at such a disadvantage in replication that it will soon be replaced by the much smaller non-λ-containing plasmids. This is an effective way of preventing the development of the stable lysogen. Another possibility is that the integration system of λ integrates plasmids carrying attachment sites into the host chromosome; this is known to lead to the death of the host chromosome because of the extra replication origin in the plasmid. This would also lower the number of surviving immune cells. We appear to have the beginning of a biological assay for the λ attachment site, but considerably more work is needed before this is completely clarified.

Phage Mu

Another area in which our laboratory is working is the somewhat enigmatic bacteriophage Mu. This phage integrates at random into the bacterial chromosome. Its integration appears to be necessary for its replication, and indeed the phage is always associated with bacterial DNA, even in the virion state, having a little piece of bacterial DNA at one end and a large piece at the other end. One of the more bizarre features of Mu is the G loop. This is a region of DNA which can invert, apparently because of the presence of short inverted DNA repetitions at each of its ends. The inversion, however, occurs only upon induction of a lysogen, when the phage particles produced have an approximately equal mixture of G loops in each possible orientation. When the phage is grown lytically, the G loops all point in one particular orientation, called the G^+ orientation. By a somewhat fortuitous means, we have been able to iso-

late mutants that do not rotate their G loops; that is, mutations seem to have occurred in a function, which we call Gin$^-$, which is responsible for G-loop rotation. The function maps in the β region of Mu, and the mutation producing the effect is a deletion which does not overlap the G loop. All the Gin$^-$ phage mutants initially obtained have their G loops pointing in the G$^+$ direction and grow lytically, form lysogens, and insert randomly into the chromosome in a manner very like that of wild-type Mu. If a lysogen of a Gin$^-$ G$^+$ Mu is induced, the progeny phage particles all have their G loops pointing in the G$^+$ direction. The G loop can, however, be rotated if a wild-type Mu phage is introduced into the cell, for instance on an episome. The episome, with its wild-type Mu, can be removed by curing, and among the cured progeny are cells which have apparently had the G loop rotated. When these are induced, the cells lyse and liberate phage particles which can be purified and visualized in cesium chloride gradients but which themselves do not produce any plaques. Analysis of the DNA from these noninfectious particles shows them to have G loops all pointing in the opposite direction, that is, the G$^-$ direction.

The repressor of Mu has been mapped at the extreme left end of the phage by genetic means. In order to define its exact physical position, we decided to clone a *Hind*III fragment, about 1000 base pairs long, which contains the left end of Mu including the attached bacterial DNA. Since the bacterial DNA end of this fragment is random in sequence, it was not possible to clone it by ligation, and so terminal transferase was used instead to add poly(A) to the fragment and poly(T) to an *Eco*RI-restricted PMB9 plasmid. From this, a series of PMB9 plasmids containing the left end of Mu were isolated. Strains carrying these plasmids could be divided into two major classes, one immune to Mu and the other sensitive. Preliminary restriction-enzyme analysis indicates that the ones that are immune contain a reasonably large insertion into the PMB9 plasmid, consistent with the hypothesis that most of the 1000-base-pair *Hind*III fragment has been cloned, whereas some of those that are sensitive appear to contain less cloned DNA.

Further analysis of those that were immune showed that they seem to overproduce the repressor tremendously. This is not unexpected since there are perhaps 20 copies of the PMB9 plasmid in the cell whereas there is generally only one copy of Mu in a normal lysogen. Overproduction was demonstrated by the observation that even virulent Mu did not form plaques on the repressor plasmid-containing strains. This indicates that there is no feedback control of Mu repressor on these plasmids. The 20-fold excess of repressor should greatly facilitate identification of its protein, which has so far not been physically detected. Our initial attempts to find it on two-dimensional gels are still in progress and have not yielded a positive result. Another strategy which we are in the process of trying is to allow the plasmid to segregate into minicells. These have a very simple pattern of plasmid proteins and should greatly facilitate the identification of the Mu repressor protein.

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INSERTION ELEMENTS AND PLASMIDS

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For the last few years we have been trying to develop a genetic and biochemical framework for analyzing the insertion system of the temperate bacterial virus Mu. This bacteriophage presents an extraordinary model for studying integrative recombination. It inserts its DNA at randomly distributed sites on the genome of its host bacterium *E. coli* and has many properties strikingly different from those of other temperate phages. Work done by us during the past year has indicated that Mu deviates from the classic Campbell model for λ integration. This work has led to the proposal that the form of DNA active in integrative recombination is generated by the replication of Mu DNA. We think this type of mechanism may be shared by transposable elements in general. Thus Mu might represent a class of elements, different from most temperate phages, in which replication of the inserting molecule is a necessary step in the process of its integration.

Structure and packaging of the Mu genome

Experiments initiated during the previous year on the structure of Mu DNA have now been completed and have provided direct proof that both ends of mature Mu DNA contain heterogeneous host sequences. The two end fragments generated by endonucleases from Mu DNA specifically hybridize to *E. coli* DNA. All parts of the *E. coli* chromosome are represented at the ends of Mu DNA. The left end, called the *c* end, contains about 100 host base pairs, whereas the *S* end contains from 500 to 3200 host base pairs (mean length: 1500 base pairs). The heterogeneous host sequences arise because of headful packaging of DNA from maturation precursors in which Mu DNA is flanked by host sequences. The packaging reaction apparently starts from the *c* end and terminates at the *S* end when a headful is reached. In collaboration with Louise Chow, we have shown that insertion of Tn9 (a 2800-base-pair transposon carrying a gene for chloramphenicol resistance) results in a loss of host sequences as well as part of Mu DNA at the *S* end. The insertions, however, do not affect the presence of host sequences covalently linked to the *c* end. The packaging proteins thus recognize a specific Mu sequence at the *c* end, but the DNA is cut to the left of the recognition sequence.

Genetic studies on the Mu life cycle

Mode of heterogeneous circle formation

We have shown that when an Hfr *E. coli* strain containing a temperature-inducible Mu prophage is heat-induced, F′ episomes are generated. These F′ episomes carry markers adjacent to the integrated F factor and are formed even if the Mu prophage is located away from the F factor in the chromosome of the Hfr strain. Almost all of these F′ episomes have at least one copy of Mu. The F′ episomes apparently represent the circular DNA molecules of varying sizes that can be detected during the lytic cycle. Since each of the F′ episomes generated after Mu induction appears to carry a Mu prophage, it can be inferred that integration of Mu is a necessary step in the formation of heterogeneous circles.

Excision of the X mutants

We have previously described prophage Mu mutants that can be excised from the host chromosome at a low frequency (10^{-5} to 10^{-7} per cell). Precise excision of the prophage DNA can restore the activity of the gene into which Mu was inserted. However, prophage excision generally does not lead to restoration of the gene activity. Experiments with newly isolated Mu *X* mutants have shown that for every precise excision, 10 to 100 imprecise excision events can be detected. Imprecise excision frequently results in a deletion covering both sides of the prophage. Some deletions are several hundred base pairs long, but most are exceedingly small, covering less

than 100 base pairs. Precise excision cannot be detected in $recA^-$ host cells. Imprecise excision can be seen in $recA^-$ cells but it occurs at a lower frequency (by a factor of 2 to 10). Our working hypothesis is that excision of the Mu *X* mutants involves two separate steps: precise cutting of Mu DNA from the host chromosome and repair of the host chromosome. Mu-specific functions are involved in recognition of the Mu-host junction, and various host functions, including the *recA* function, might be needed for repair of the chromosome. Deletions would occur because of degradation of host DNA before it is fully resealed.

Biochemical studies on Mu integration

An important step in understanding the process of Mu integration would be the elucidation of the form of Mu DNA which inserts itself into the host chromosome. We define this form as the integrative precursor. To examine the Mu integrative precursor, we have studied the fate of Mu DNA after infection of cells and the behavior of prophage Mu DNA upon induction.

Inability of Mu DNA to become circular

We infected Mu-sensitive and Mu-immune (lysogenic) cells with ^{32}P-labeled phages and examined the intracellular forms of the labeled DNA at different times after infection. No circular form of Mu DNA was detected by sucrose gradient centrifugation, CsCl–ethidium bromide density centrifugation, or restriction-endonuclease cleavage analysis of the DNA. We have reached the conclusion that Mu lacks an efficient mechanism for fusing its ends to form circles. Most of the infecting DNA remains in a linear form. A form of Mu DNA moving twice as fast as linear DNA in neutral sucrose gradients is seen upon infection of immune cells. This form apparently represents a protein-DNA complex which protects Mu DNA from degradation.

Failure of infecting DNA to be integrated

Not only does the infecting parental DNA not become circular, it is not even integrated into host DNA. We have separated Mu DNA from host DNA by electrophoresis in 0.3% agarose gels. About 90% of the ^{32}P-label remains in the phage DNA band as late as 40 minutes after infection. This is a highly intriguing result since we know that integration of Mu DNA is a necessary step in the growth cycle of Mu. This result implies that Mu DNA first replicates and then the replication products are used for integration into host DNA.

State of prophage DNA upon induction

We have examined the fate of prophage Mu DNA in situ, at different sites on the host chromosome, after induction. The prophage DNA was located with a technique in which DNA of the lysogenic cells is cleaved with a restriction endonuclease and fractionated on agarose gels. The DNA fragments are denatured on gels, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled mature phage DNA. The fragments containing prophage DNA become visible after autoradiography. In experiments with λ lysogens, we could readily monitor excision of λ DNA. The phage-host junction fragments disappeared after induction and the fragments containing the λ*att* site appeared. No such excision could be seen with prophage Mu. The Mu-host junction fragments remained intact well into the lytic cycle, when Mu DNA had undergone many rounds of replication and many copies of Mu DNA had integrated at different sites on the host chromosome. (As pointed out above, Mu DNA is continuously integrated into the host during the lytic cycle.) Figure 1 shows the results of results of a similar experiment in which a λ lysogen and a Mu lysogen were induced for a long time in the presence of nalidixic acid, an inhibitor of DNA synthesis. This figure emphasizes the difference between the λ system and the Mu system—whereas λ is excised promptly after induction, no change in prophage Mu DNA can be seen. These experiments thus show that Mu DNA is not excised but replicates in situ after induction.

It should be noted that although a mechanism for the excision of Mu DNA exists (i.e., excision of the *X* mutants), induction of a prophage with subsequent integration of Mu DNA at different sites apparently does not involve excision of prophage DNA. In the transposable element systems also, the present evidence suggests that transposition does not require excision of the element. The transposable element can be excised, but the element is, in general, lost upon excision. Perhaps both Mu and transposable elements undergo replication, giving rise to copies which are integrated into host DNA. It is in view of this mechanistic similarity that we have proposed that Mu is a representative of transposable elements.

The G segment of Mu

The G segment is an invertible 3-kilobase sequence of Mu DNA. Mu particles grown by induction of a lysogen have both G orientations (about 50% have the "flip" orientation; the other 50% have the "flop" orientation). Mu particles grown by infection almost always have the "flip" orientation. The flip-flop process of G is an interesting model for an on-and-off switch for gene expression. In collaboration with Louise Chow, we have found that the G segment of Mu is homologous to the invertible segment found in bacteriophage P1. We have extensively analyzed homology between P1 and Mu by DNA-DNA hybridization techniques. These experiments have shown that endonuclease-generated fragments from the G segment of Mu hybridize to the fragments from the P1 invertible segments. These results raise the possibility that P1 and Mu have acquired the G sequence by transposition from another source. The

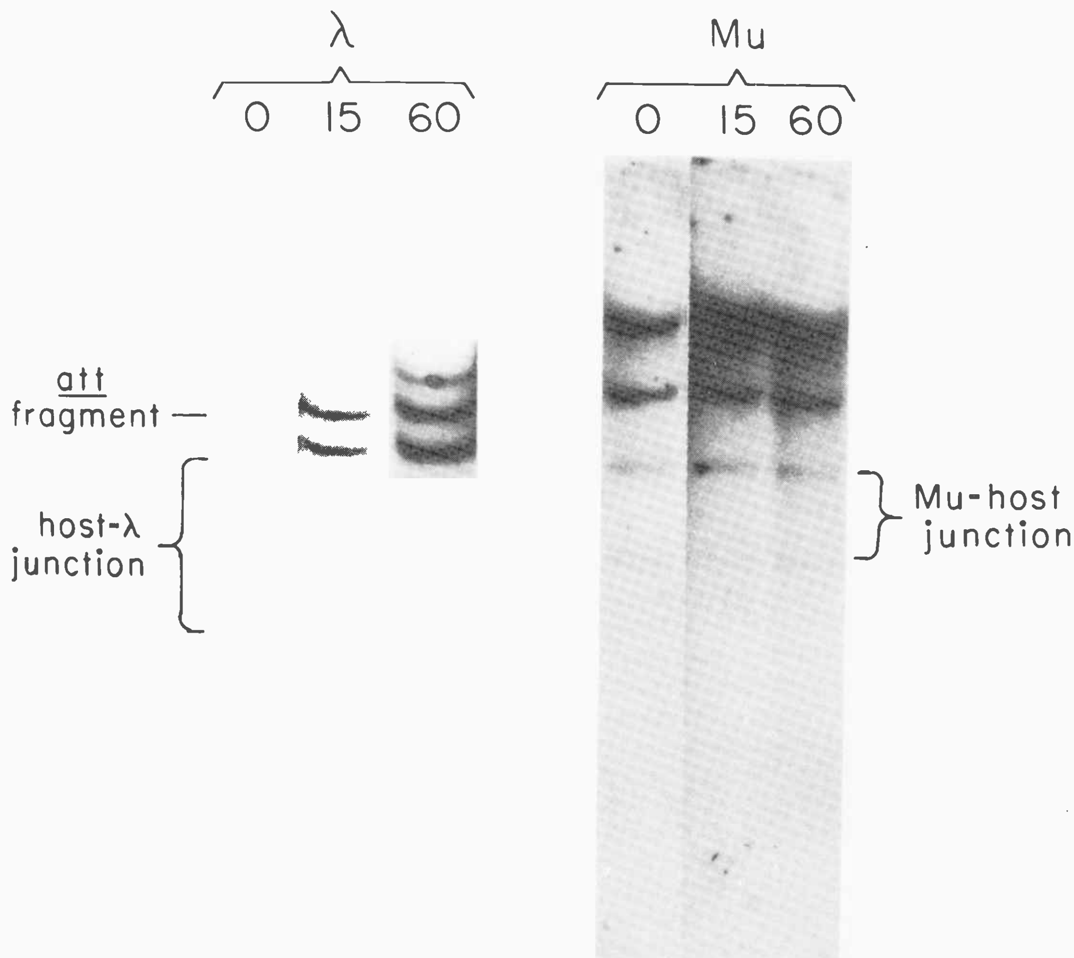

Figure 1
Comparison of λ and Mu systems. A λ lysogen and a Mu lysogen, carrying temperature-inducible prophages, were heat-induced in the presence of nalidixic acid. Samples were taken at different times (indicated in minutes) after induction and the DNA was extracted. DNA from the λ lysogen was digested with the exonuclease BglII *and DNA from the Mu lysogen was digested with* BalI. *The fragments were fractionated by electrophoresis on agarose gels, "blotted" onto nitrocellulose paper, and hybridized to* ^{32}*P-labeled λ or Mu DNA. In λ, the fragment containing the* att *site appears with time, while the host-phage junction fragments disappear. In Mu, the host-phage junction fragments persist (Ljungquist and Bukhari 1977a).*

idea that G in Mu or in P1 may be a transposable element (G in P1, as compared to G in Mu, has longer inverted repetitious sequences at its ends) is being tested.

Transposition elements

IS1 and Tn9

The *X* mutations in Mu are generally caused by the insertion of IS*1*, an 800-base-pair transposition element found in the chromosomes and plasmids of many prokaryotic organisms. Mu *X* mutants can also be generated by the insertion of Tn9, a transposon carrying a gene for chloramphenicol resistance. These insertions occur near the *c* end of Mu DNA and cause the Mu prophage to become defective. The Mu *X* mutants with IS*1* insertions and Tn9 insertions have also been studied by electron microscopy in Dr. Chow's laboratory. Both IS*1* and Tn9 have the same insertion sites in Mu DNA, and it has now been shown that Tn9 contains one copy of IS*1* at each end in direct repeats.

We have been able to study the Tn9 transposon both genetically and biochemically by using the Mu *X*::Tn9 mutants. The Tn9 transposon can be precisely excised from Mu DNA. However, the Tn9 insertions in Mu fall into two categories with respect to excision. One class of Mu *X*::Tn9 mutants loses the Tn9 at a frequency of 10^{-3} to 10^{-5}, whereas the other class reverts to Mu wild

type at a frequency of about 10^{-7}. Structural organization of the Tn9 transposon in these mutants is being examined. The Tn9 segment encoding chloramphenicol resistance can be purified by cleaving the DNA molecules carrying Tn9 with the restriction endonuclease *Bal*I (*Bal*I cleaves the *IS1* element once). Furthermore, *Eco*RI cleaves Tn9 approximately in the middle. Thus Tn9 can be used, in vivo or in vitro, to introduce an *Eco*RI cleavage site at a given position in a DNA molecule.

IS1 in phage P1

We have discovered by DNA-DNA hybridization techniques that bacteriophage P1 has two copies of IS*1*. This finding has prompted us to explore further the structural relationship between the genomes of Mu, P1, and R plasmids.

DNA insertions meeting

The sudden emergence of insertion sequences as genetic elements of fundamental interest was reflected at the meeting on DNA insertions organized by the Cold Spring Harbor Laboratory in May, 1976. It was the first such meeting devoted specifically to a discussion of insertion phenomena, and it brought together biologists working in many different areas. The complete program of this exciting meeting appears elsewhere in this annual report. The Cold Spring Harbor Laboratory is publishing a comprehensive monograph based on the papers presented at the meeting.

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ELECTRON MICROSCOPY

T. R. BROKER, L. T. CHOW, J. M. Scott, J. N. Roberts

This has been a particularly active and productive year for electron-microscopic analyses of the organization of chromosomes from adenovirus 2, *E. coli,* and bacteriophages, Mu, lambda, P1, and ϕ29. Descriptions of those projects follow.

Adenovirus transcription map

Together with Jim Roberts, an undergraduate visiting our lab for the summer, we used a recently developed method, RNA-loop mapping, to refine the transcription map of adenovirus 2. The map had previously been determined by other techniques, including genetics, restriction-endonuclease cleavage of the DNA, saturation hybridization of RNA to DNA, and in vitro translation of RNA. Duplex adeno DNA, or restriction fragments of it, was mixed with an equivalent amount of RNA extracted from the cytoplasm of human KB cells at either early or late times after infection. (The DNA was provided by R.J. Roberts and the RNA was isolated by J.B. Lewis.) The DNA-RNA mixture was adjusted to 70% formamide and incubated at a temperature at which the duplex DNA was largely denatured. Under these conditions, RNA is still able to form stable hybrid duplexes with complementary DNA sequences. When the solution was chilled, diluted, and prepared for electron-microscopic examination, the partially melted DNA paired again, except where RNA transcripts had hybridized. There, the homologous DNA strand remained displaced as loops (Fig. 1). Thus the loops in each DNA molecule indicated the precise coordinates of the transcripts. About 1000 loops were analyzed in order that we might determine the adenovirus transcription map to 0.5% (200-base-pair) accuracy. Our results are summarized in Figure 2.

Additional conclusions:

(1) Some early (e.g., 19K–11K) and some late (IVa_2–IX, hexon, and fiber) messages have very discrete sizes, whereas others (e.g., in the penton region, the 100K region, and the E15.5 region) have a limited heterogeneity in length at either or both the 5′ and 3′ ends. We believe this reflects variability in endonucleolytic processing of primary nuclear transcripts.

(2) Switch points where transcription changes from one DNA strand to the other could be visualized. Loops with RNA hybridized to the left portion of one limb of the loop and the right portion of the other limb were observed. The junction between RNA:DNA hybrid and single-stranded DNA toward the middle of both limbs revealed the position of the strand switch.

(3) The message for the fiber protein uniquely forms a reproducible branch near its 3′ end. This may result from secondary structure within the RNA which restricts it from pairing with the DNA.

Our attention in the future will be directed toward those regions of the adenovirus chromosome with heterogeneous transcripts.

Visualization of covalent protein-DNA complexes

Covalent association of proteins with nucleic acids has been reported in a diverse group of viral and plasmid chromosomes. The most probable linkages are between 5′-terminal phosphoryl groups of the nucleic acid and lysine or serine residues of the protein. Potential roles of the proteins in DNA metabolism include protection of termini from exonucleases and ligases, initiation of strand displacement during replication, interaction with the cell membrane during replication, and orientation of the DNA during conjugation or during packaging into the virion.

The proteins range in size from 7000 daltons to 70,000 daltons on various chromosomes. Direct visualization of such small proteins by electron microscopy is at best tedious and difficult and may not be certain unless the presence and location of the proteins can be

Figure 1
Adenovirus-2 DNA containing RNA loops formed by transcripts from the hexon, 100K protein, and fiber regions. The branch at the 3′ end of the fiber message is reproducible. Collapsed molecules in the background are unhybridized RNA.

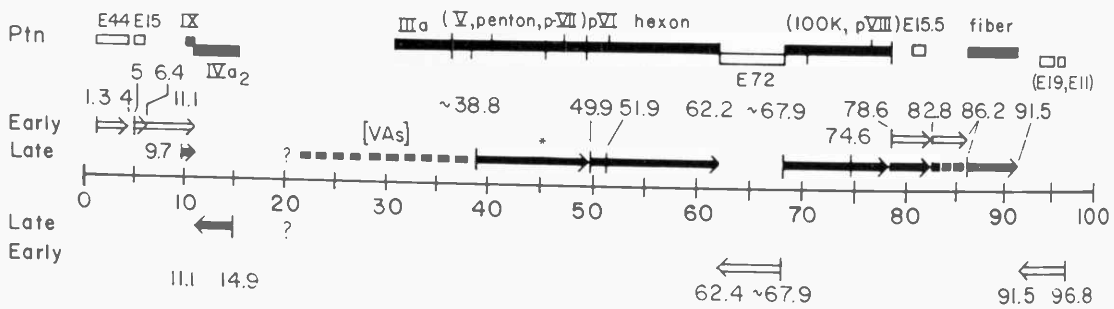
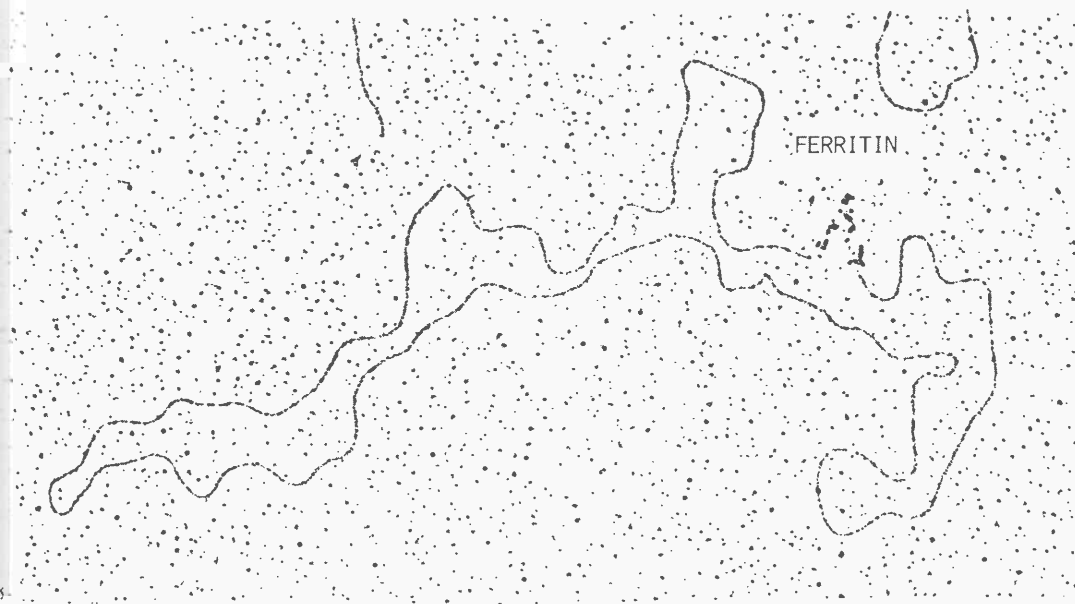


Figure 2
Adenovirus transcript coordinates determined by electron microscopy of RNA loops on adenovirus-2 DNA. Arrows indicate the direction of transcription, which was determined by Sharp et al. [Cold Spring Harbor Symp. Quant. Biol. 39:457 (1974)] and Philipson et al. [Cold Spring Harbor Symp. Quant. Biol. 39:447 (1974)]. Thin arrows show the positions of early transcripts and thick arrows show the positions of late transcripts. Dashed lines indicate the positions of infrequently observed R loops. Vertical bars on arrows indicate preferential 5′ termini. The cleavage sites of EcoRI and SalI endonucleases are also represented.

stablished in advance by other techniques. T. Broker nd L. Chow have recently developed a relatively easy, apid, efficient, and reliable method to detect and study rotein–nucleic acid complexes. It uses the standard asic protein film technique rather than direct adsorpon methods. The proteins are visualized with ferritin bels. The basis of our labeling technique is the exaordinary affinity of the egg-white protein avidin for itamin H (biotin) characterized by a K_{diss} of 10^{-16} M. his binding is a million times stronger than that of a ood antibody–antigen complex. Avidin can be oupled to ferritin (an electron-dense iron-storage proin from spleen) in a variety of ways. Biotin is coupled amino groups of macromolecules via the reactive *N*-ydroxysuccinimide ester of biotin. Thus ferritin can be ttached to macromolecules through an avidin-biotin nkage, a labeling scheme also devised independently y H. Heitzmann and F.M. Richards [*Proc. Natl. Acad. Sci. USA* 71:3537 (1974)]. Proteins bound covalently to ucleic acids were derivatized with biotin and were hen localized with $(\text{ferritin-biotin}_{20}\text{:avidin})_{n,1\text{-}20}$ olymeric labels. We have refined our chemical devatization and labeling protocols to allow greater than 0% efficiency in attaching ferritin to the proteins at the ermini of adenovirus-2 (Fig. 3) and *B. subtilis* phage 29 DNAs. We have confirmed that proteins are stably nked to both ends of these chromosomes. Furthernore, these terminal proteins are present in and preumably are responsible for the cyclized and concatemerized structures observed by electron microscopy of isolated complexes. In control experiments, the labels do not bind to single-stranded DNA or to deproteinized double-stranded DNA. We shall use our labeling techniques to identify other protein–nucleic acid conjugates and to study in detail the formation and structures of protein–DNA conjugates during DNA replication.

Chromosome structures of viable Mu mutants

A Mu lysogen established by Dietmar Kamp was found by Chow to contain an unknown insertion of 2.6 kilobases (kb) in the Mu beta region. The insertion is sufficiently long to force Mu chromosome deletions in nonessential regions in order to package Mu DNA that will be viable in the next round of infection. Indeed, Regine Kahmann and Dietmar Kamp have isolated seven independent, nondefective Mu mutant phages from this lysogen. The sequence arrangements of the mutant chromosomes were characterized by Chow using EM heteroduplex methods. The DNA of each mutant contains either a deletion or a substitution in the G and/or beta regions. The regions deleted are thus nonessential. The lengths of the bacterial DNA on the *S* (or right) end of these mutants varied according to the amount of DNA inserted or deleted from the Mu genome. Therefore, the model of headful encapsidation of Mu DNA from the *c* (or left) end proposed by A.I. Bukhari and A.L. Taylor [*Proc. Natl. Acad. Sci. USA*



gure 3
denovirus DNA dimer. The proteins at both termini and at the internal concatemer junction are labeled with otin:avidin-ferritin conjugates.

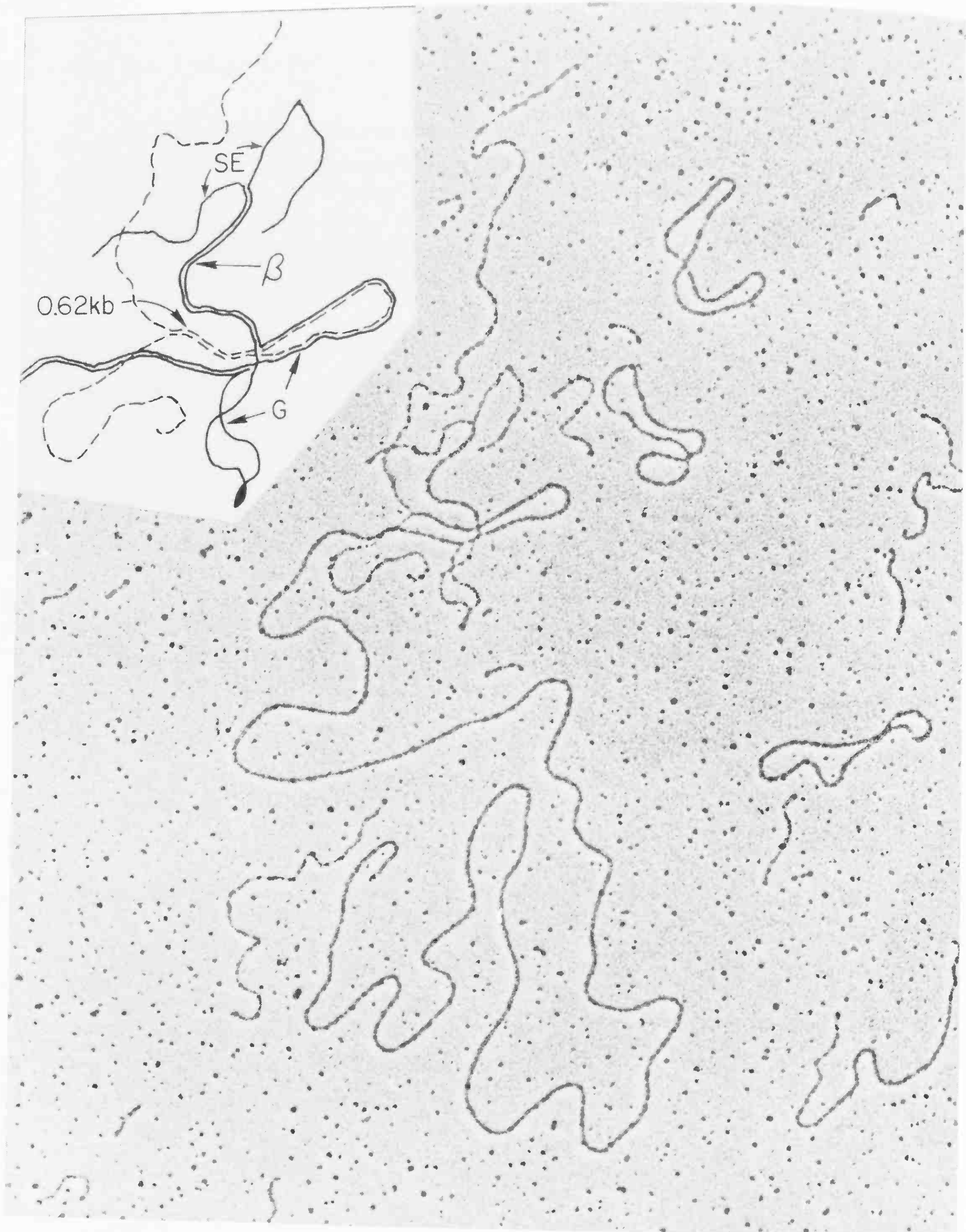


Figure 4
Heteroduplex between the G loop of renatured Mu DNA and the G segment of a single-strand restriction fragment of P1 DNA. The beta region and the split ends (SE) of Mu and the 0.62-kilobase stem formed from the inverted duplications flanking the P1 G segment are also indicated in the schematic representation.

2:4399 (1975)] was strongly supported. We have also localized at least part of the Mu G inversion system (*gin*) to the left portion of the beta region, for deletions there prevent inversion. All mutants have their G sequence in the lytic orientation regardless of whether or not the G segment is intact. No deletions have been observed within the left 2000 bases of the G sequence in the lytic orientation. Thus essential functions may be encoded there. Finally, our preliminary results indicate that Mu phages with the G loop fixed in the nonlytic orientation are not viable.

Homology between bacteriophage P1 and Mu DNAs

Chow has shown, by electron-microscopic studies, that the G sequence of phage Mu DNA, an invertible region 3 kb in length near the *S* end, is identical to the invertible region found in phage P1 DNA. The first indication of their relatedness came with the observation that they have the same pattern of secondary structure. Their homology was confirmed by the formation of perfect heteroduplex pairing between the P1 and Mu G sequences (Fig. 4). Since the P1 and Mu are quite different phages in most respects, and no other sequence homology has been found between their DNAs, the G sequence has probably moved from one phage to another by translocation.

Identification of insertion sequence *1* (IS*1*) in Mu·*X* and Mu·*Xcam*

In last year's report, Chow presented a preliminary report on the characterization of DNAs from Mu mutants, Mu·*Xcam*. Briefly, Mu·*Xcam* DNAs have a transposable chloramphenicol resistance element of 2.8-kb pairs near the c end. The observation of the complete loss of their split-end bacterial DNA in the Mu·*Xcam* mutants further supports the headful packaging model of Bukhari and Taylor.

This year Chow has continued to characterize the Mu·*X* and Mu·*Xcam* mutant DNAs. Mu·*X* mutant had been shown by Bukhari and Taylor to have a 0.8-kb insertion near the c end of the Mu genome [*Proc. Natl. Acad. Sci. USA* 72:4399 (1975)]. Chow has found, by electron-microscopic–heteroduplex studies, that the insertion in Mu·*X* is an IS*1* sequence, and that the *cam* insertion in the Mu·*Xcam* consists of the chloramphenicol resistance marker flanked by two IS*1* elements in direct order. This is the same as the structure of the *cam* transposon in phage lambda *cam*, determined by L.A. MacHattie and J.B. Jackowski [in *DNA Insertion Elements, Plasmids, and Episomes* (ed. A.I. Bukhari et al.), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (in press)].

Inverted sequences in the *E. coli* chromosome

Chow has studied the arrangements of insertion sequences in the *E. coli* chromosome. These elements can be the sites of recombination of bacterial chromosomes and episomes such as the fertility factor and drug resistance determinants. Some of the approximately seven different insertion sequences also modulate RNA transcription. Total *E. coli* DNA was denatured, reannealed, and observed by electron microscopy. The single-strand DNA lengths ranged from about 50 to 150 kb. In some molecules, a short duplex region with a single-stranded fork at each end was observed. The duplex lengths were 0.75 kb, 1.30 kb, 5.22 kb, and 5.62 kb, which correspond to those of IS*1* and of IS2, IS3, or IS4 of the ribosomal RNA genes and of the $\gamma\delta$ sequence, respectively. Duplexes of 1.0 kb and 0.5 kb were also found. Most of the duplexes of lengths 0.5 kb, 0.75 kb, 1 kb, and 1.3 kb were observed as intramolecular stem-loop structures and were therefore interpreted to be sequence duplications in inverted order on the same DNA strand. Surprisingly, the separation of the putative inverted insertion sequences was often around 22 and 27.5 ± 1.5 kb. About 18% of the *E. coli* chromosome is estimated to be involved in the sequence arrangements that give rise to stem-loop structures upon denaturation and reannealing. Continuing studies will attempt to locate some of the inverted duplications with respect to bacterial markers and to determine whether the unusual arrangement of large blocks of the bacterial chromosome has a function.

Underwound heteroduplex loops can indicate inverted duplications

Broker and Chow have identified and characterized a new heteroduplex structure. DNA that contains inverted duplications of at least 20 base pairs separated by noninverted sequences of at least 600 base pairs can form characteristic "underwound loops" when it is denatured and reannealed. An underwound loop is a partially duplex, partially denatured circle between the inverted duplications and is produced as follows: During the early stages of the reannealing, intrastrand stem-loop structures form with first-order kinetics when the inverted duplications pair. In a slower, second-order reaction, complementary strands (each with a stem loop) pair. The stem-loop structures may be retained to produce a cruciform. The cruciform can isomerize to a linear duplex by double-strand exchange with coordinated axial rotation of the four duplex arms. In this process, the loops rotate in opposite directions with respect to one another. But if the loops start to pair with one another, axial rotation is prevented and the stem-loop structures are trapped. However, there can be no net interwinding of the strands of separate, closed rings. As a result, the loops observed by electron microscopy have variable patterns of single-stranded denaturation bubbles and duplex segments with both right-handed and left-handed winding, and they may also have a few tertiary twists.

We have used underwound loops to identify a short inverted duplication flanking the $\gamma\delta$ recombination se-

quence of *E. coli* F factor (isolated on $\phi 80$ d_3 ilv^+ transducing phage by Larry Soll) and to study the G segments of DNA from phages Mu and P1, which are flanked by inverted duplications. When deproteinized adenovirus-2 DNA is denatured and reannealed, some underwound circles, the length of the entire chromosome, are observed by EM. These result from the restricted interaction of complementary single-strand rings generated when pairing of the short inverted terminal duplications closes the ends of single strands. Another type of underwound loop is seen in heteroduplexes containing complementary insertion loops located at different positions in the hybridized strands, such as occur with P1 *cam* DNAs. All these underwound structures are similar in appearance to the hybrids formed when topologically separate, complementary single-strand circles of Colicin E_1 DNA are allowed to anneal.

Recombination of the bacterial $\gamma\delta$ sequence

On the basis of our discovery that the $\gamma\delta$ sequence is flanked by inverted duplications, Broker has developed a recombination model to explain their function. Unlike the Mu and P1 G loops, which also have flanking inverted duplications, inversion of the $\gamma\delta$ sequence has not been observed. Recombination generally requires two complete copies of $\gamma\delta$. Despite the presence of the inverted sequences, recombination can occur with only one orientation. Synapse must therefore include pairing of the internal $\gamma\delta$ sequences. The model postulates that crossover initiates at a single-strand nick at one end of the $\gamma\delta$ sequence, continues with double-strand exchange through the entire $\gamma\delta$ segment, and terminates 5700 base pairs away at a nick in the flanking duplication at the other end.

Modifications of the Philips 201 electron microscope

An audible alarm system was designed and installed by John Scott to minimize the possibility that the specimen holder of a Philips 201 or Philips 301 electron microscope may be left accidentally in the airlock prepump position. The modification is simple, inexpensive, and readily performed by laboratory electronics personnel. It can prevent contamination of the microscope column with oil vapor backstreaming from the mechanical vacuum pump.

Scott and Broker adapted a simple closed-circuit television (CCTV) system for use with a Philips EM 201 transmission electron microscope. A similar video system was previously developed at Cold Spring Harbor by G. Albrecht-Buehler and B. DeTroy for light microscopy. The system can be assembled at a modest cost of approximately $1200. The television display has been particularly valuable for the summer courses using electron microscopy and for other demonstrations to large groups.

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NUCLEIC ACID CHEMISTRY

R. J. ROBERTS, J. Bonventre, B. Doretsky, S. A. Endow, R. E. Gelinas, T. R. Gingeras, M. Moschitta, P. A. Myers, M. Zabeau, S. Zain

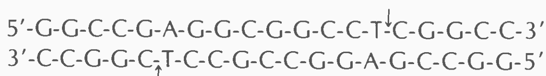
New restriction endonucleases

The search for new restriction endonucleases continues, and nearly 100 such enzymes are now known, showing more than 50 different specificities. Among the endonucleases isolated recently, *Xba*I (from *Xanthomonas badrii*) seems useful for mapping as it makes relatively few cuts on most DNAs examined. It has proved particularly useful during the mapping of herpesvirus DNA. The endonucleases *Bby*I (from *Bacillus brevis*), *Pfa*I (from *Pseudomonas facilis*), *Hin*1056I (from *Haemophilus influenzae* 1056), and *Sac*III (from *Streptomyces achromogenes*) make many cuts on most DNAs and should prove useful for DNA sequence analysis. Recognition sequences have been deduced for *Bgl*II (A↓GATCT) from *Bacillus globiggi*, *Xma*I (C↓CCGGG) from *Xanthomonas malvacearum*, and *Mbo*I (GATC) from *Moraxella bovis*.

Mapping

The *Bal*I, *Xba*I, and *Bgl*II maps of the adenovirus-2 genome are now complete and have been related to maps previously derived for six other restriction endonucleases.

Mapping of SV40 has also been continued, and the sites for the single-hit enzymes *Kpn*I, *Taq*I, *Uba*I, and *Bgl*I have been located. In addition, maps for *Pst*I (two cuts) and *Hph*I (five cuts) have been obtained. The site at which *Bgl*I cleaves the SV40 genome is of particular interest. It cleaves the sequence



5′-G-G-C-C-G-A-G-G-C-G-G-C-C-T-↓C-G-G-C-C-3′
3′-C-C-G-G-C-↑T-C-C-G-C-C-G-G-A-G-C-C-G-G-5′

at the positions indicated by the arrows to generate a linear molecule containing 9-nucleotide 3′ single-stranded extensions. The center of this 9-nucleotide stretch is also the center of a 27-nucleotide palindrome which has been suggested as the origin of replication in SV40. The mode of cleavage by *Bgl*I is unusual, and the precise sequence recognized by this enzyme cannot be deduced from this single site.

Integration site sequenced

The nondefective hybrid virus Ad2⁺ND1 is a recombinant between adenovirus 2 and SV40 in which a section of the SV40 genome replaces a nonessential section of the Ad2 genome. A restriction-endonuclease fragment containing one of the junctions between Ad2 and SV40 sequences had been identified earlier and another fragment containing the corresponding sequences from the parental Ad2 genome had also been identified. The sequences of both these fragments have now been determined and compared with the corresponding sequences from the parental SV40 DNA, determined by Zain and Weissman. No obvious homology exists between the parental SV40 and Ad2 DNAs within 30 nucleotides of the site at which the recombination event took place. This finding suggests (a) that recombination in these viral systems does not depend on the presence of homologous sequences within the parent molecules, and (b) that the selection of the recombination sites on both SV40 and Ad2 was determined by some feature other than DNA sequence homology. The Ad2 DNA sequence immediately adjacent to this recombination site should prove of great interest. The 5′ end of the messenger RNA for fiber, one of the major structural proteins of the Ad2 capsid, appears to map within this region of the Ad2 genome. From companion studies on the 5′ ends of Ad2 mRNAs, the sequence present at the 5′ end of the fiber mRNA should soon be available, and it will then be possible to locate it within the DNA sequence adjacent to this integration site.

\denovirus-2 mRNA

\ new and general method has been developed for the solation of oligonucleotides containing the 5′-terminal equences of eukaryotic mRNA. The method depends ipon the presence of a 7-methylguanosine residue at-ached by a 5′-5′-triphosphate linkage to the 5′-ter-inal nucleotide of the messenger RNA (the "capped" ructure). Since this nucleotide is linked through a ′-phosphate group, it contains a 2′-3′-*cis*-diol struc-ire, and oligonucleotides containing such a structure an be purified using an affinity column of dihydroxy-oryl cellulose. When the method is applied to RNase oligonucleotides derived from Ad2 mRNAs iso-ed late after infection, a single oligonucleotide has een isolated. This oligonucleotide, m^{7}G$^{5'}$ppp$^{5'}$ AmCmU[C_4U_3]Gp, appears to be the only "capped" ligonucleotide found at the 5′ end of all late Ad2 RNAs. It is present on different size classes of RNA and also present on RNA selected by hybridization to ifferent fragments of the Ad2 genome. Furthermore, it different from the 5′-terminal RNase T_1 oligonucleo-de obtained from Ad2 mRNA isolated early during ifection. Experiments are in progress (a) to determine if e homology among the various mRNAs extends be-ond these first 10 nucleotides, (b) to determine at what oints on the adenovirus-2 genome this structure is oded, and (c) to assess the significance of this particular ructure (e.g., is it part of some signal for the control of te transcription?).

rminal structure of the adenovirus-2 genome

details of the structure and the nucleic acid sequence the ends of the Ad2 genome become clearer, their nction remains enigmatic. Direct confirmation of the spected covalent linkage between the terminal pro-in and the ends of the DNA has now been obtained by e isolation and characterization of covalently linked igonucleotide-oligopeptides. The nature of the bond etween the terminal protein and the 5′ ends of the NA has not yet been elucidated, and the significance f this covalent linkage is unknown.

Another interesting feature of the termini is that upon eatment with exonuclease III, the resulting 5′ strand at ach end is capable of secondary-structure formation. his has been observed by electron microscopy and volves nucleotides within the inverted terminal repeti-on; however, the exact nature of the secondary struc-ire is unclear. Much of the DNA sequence of this egion is now available, and several possible secondary structures can be drawn. Further experiments are in progress to clarify the remaining doubts in the sequence and hence allow the derivation of the most stable secondary structure.

A model for the initiation of DNA replication has been proposed which invokes this capacity for secondary-structure formation and suggests a mechanism by which the terminal protein becomes covalently bound to the DNA. Several predictions of this model are now being tested experimentally.

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EUKARYOTIC GENE REGULATION

T. MANIATIS, D. Goldberg, L. Villa Komaroff, E. Lacy, G. K. Sim

Molecular cloning of eukaryotic genes

This year we have continued efforts to develop an approach for isolating eukaryotic structural genes and the DNA sequences which flank them. The goal of this work, which is being carried out in collaboration with Argiris Efstratiadis and Fotis Kafatos at Harvard University, is to understand the structure of eukaryotic genes and the mechanism by which their expression is controlled.

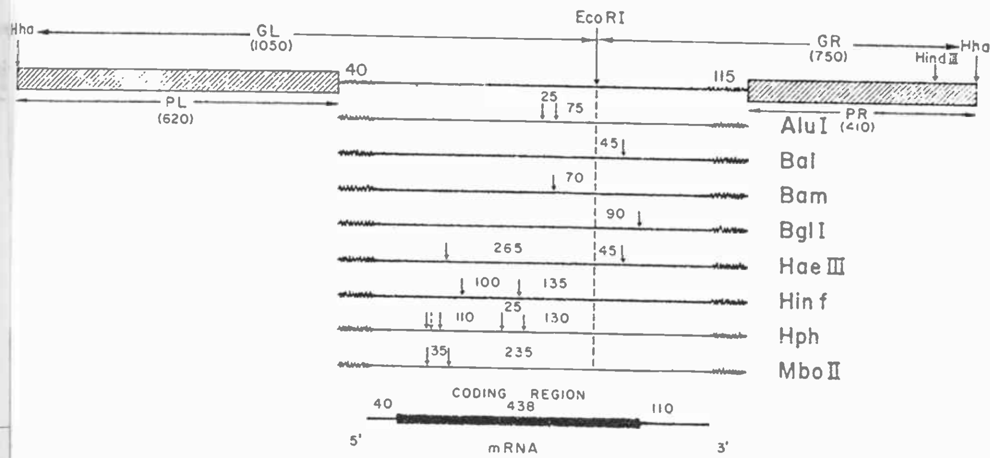
The approach we have chosen involves the in vitro synthesis of full-length, double-stranded DNA copies of mRNA using the sequential enzymatic activities of AMV reverse transcriptase, DNA polymerase I, and S1 nuclease. The synthetic duplex DNA is then purified by polyacrylamide gel electrophoresis, joined to a bacterial plasmid, and amplified using molecular cloning techniques. Thus it is possible to obtain large amounts of eukaryotic gene sequences which can be studied directly or used as specific hybridization probes for identifying and isolating flanking sequences in chromosomal DNA.

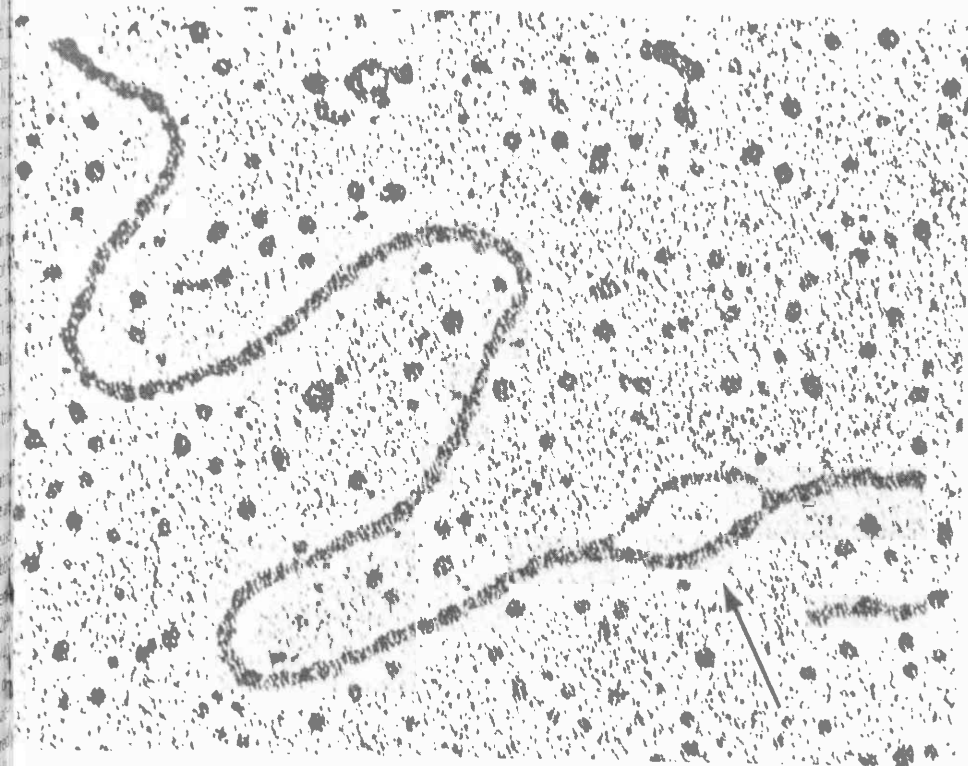
One chimeric plasmid (pβG-1) constructed in this manner carries a synthetic DNA copy of rabbit β-globin mRNA and has been characterized in detail (see Fig. 1). Last year an in-depth analysis of the globin insertion in pβG-1 was undertaken to show that the sequence is a faithful copy of the mRNA and that no sequence rearrangements occurred during the cloning or propagation of the chimeric plasmid DNA in *E. coli*. This year we have extended this characterization in order to address the question of the validity of using recombinant DNA techniques to determine the primary structure of eukaryotic mRNA. Taking advantage of the detailed restriction map of the insertion derived by Gek Kee Sim and using the rapid DNA sequencing method of Maxam and Gilbert, the nucleotide sequence of the entire β-globin insertion in pβG-1 was derived (see Fig. 2). The sequence is in complete agreement with previously reported partial mRNA sequencing data and with the predictions from the primary structure of the protein. Moreover, the globin DNA insertion is missing only 13 nucleotides corresponding to the 5′-terminal sequence of the mRNA. The rabbit β-globin mRNA consists of a coding region of 438 nucleotides, flanked by a 5′ noncoding region of 57 nucleotides (including the initiation codon AUG but excluding the 7-methylguanine of the "cap structure") and by a 3′ noncoding region of 95 nucleotides (including a UGA termination codon). The faithful representation of β-globin mRNA in pβG-1 establishes the validity of using cloned DNA, initially derived from double-stranded DNA transcripts of mRNA, for studying the structure of eukaryotic structural genes.

To guide the development of procedures for isolating DNA sequences which flank the β-globin gene, we are mapping the position of restriction-endonuclease cleavage sites located at either side of the gene in rabbit chromosomal DNA. We are using the procedure employed by Botchan, Topp, and Sambrook [*Cell* 9:269 (1976)] to study the structure of integrated SV40 genomes. In this procedure, chromosomal DNA is digested with a restriction enzyme, the products are fractionated by agarose gel electrophoresis and transferred to nitrocellulose paper by the method of Southern [*J. Mol. Biol.* 98:503(1976)], and the filters are hybridized to in-vitro-labeled DNA. By constructing plasmids which carry sequences from either the 5′ end or the 3′ end of globin mRNA, it is possible to locate restriction sites at either end of the gene.

Chorion genes

Gek Kee Sim and Lydia Villa Komaroff are studying the organization of developmentally regulated genes in the silk moth, *Antherea polyphemus*. During the terminal





ure 1

p) Restriction map of the β-globin DNA insertion of pβG-1. The wavy lines indicate the dA-dT sequences at each end the globin DNA insertion. The hatched bar represents plasmid pMB-9 DNA. PL and PR: pMB-9 restriction fragments nerated by the combined digestion with the enzymes EcoRI and Hha. GL and GR: pβG-1 DNA restriction fragments responding to PL and PR, respectively. The orientation of the globin insertion and the distribution of the coding and ncoding globin sequences are also shown. The numbers of base pairs between restriction sites are indicated. printed, with permission, from Maniatis et al., Cell 8:163 (1976).]

Bottom) Localization of the globin insertion in pβG-1 using the R-loop displacement method. The plasmid pβG-1 was earized by digestion with the restriction enzyme HindIII and mixed with globin mRNA under partially denaturing nditions which result in the formation of an mRNA-DNA duplex and the displacement of the complementary DNA and. The arrow indicates the position of the double-stranded globin RNA-DNA duplex. The electron micrograph was en by Tom Broker and Louise Chow of Cold Spring Harbor Laboratory. Magnification: 160,000×.

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A C A C U U G C U U U U G A C A C A A C U G U G U U U A C U U G C A A U C C C C C A A A
-56        -50              -40                -30                -20
                             Val | His | Leu | Ser | Ser | Glu | Glu | Lys | Ser | Ala | Val
                            G U X|C A Y|C U X|U C X|A G Y|G A R|G A R|A A R|U C X|G C X|C U X
A C A G A C A G A [A U G] G U G|C A U|C U G|U C C|A G U|G A G|G A G|A A G|U C U|G C G|G U C
 -10                -1    1                 10                20                30

 Thr | Ala | Leu | Trp | Gly | Lys | Val | Asn | Val | Glu | Glu | Val | Gly | Gly | Glu
A C X|G C X|C U X|U G G|G G X|A A R|G U X|A A Y|G U X|G A R|G A R|G U X|G G X|G G X|G A R
A C U|G C C|C U G|U G G|G G C|A A G|G U G|A A U|G U G|G A A|G A A|G U U|G G U|G G U|G A G
       40                    50   Hph            60 MboII           70    Hph

 Ala | Leu | Gly | Arg | Leu | Leu | Val | Val | Tyr | Pro | Trp | Thr | Gln | Arg | Phe
G C X|C U X|G G X|A G R|C U X|C U X|G U X|G U X|U A Y|C C X|U G G|A C X|C A R|A G R|U U Y
G C C|C U G|G G C|A G G|C U G|C U G|G U U|G U C|U A C|C C A|U G G|A C C|C A G|A G G|U U C
Hae III            90                100                110                120

 Phe | Glu | Ser | Phe | Gly | Asp | Leu | Ser | Ser | Ala | Asn | Ala | Val | Met | Asn
U U Y|G A R|U C X|U U Y|G G X|G A Y|C U X|U C X|U C X|G C X|A A Y|G C X|G U X|A U G|A A Y
U U C|G A G|U C C|U U U|G G G|G A C|C U G|U C C|U C U|G C A|A A U|G C U|G U U|A U G|A A C
Mbo II Hinf                     140                150                160

 Asn | Pro | Lys | Val | Lys | Ala | His | Gly | Lys | Lys | Val | Leu | Ala | Ala | Phe
A A Y|C C X|A A R|G U X|A A R|G C X|C A Y|G G X|A A R|A A R|G U X|C U X|G C X|G C X|U U Y
A A U|C C U|A A G|G U G|A A G|G C U|C A U|G G C|A A G|A A G|G U G|C U G|G C U|G C C|U U C
170              Hph 180               190                200                210

 Ser | Glu | Gly | Leu | Ser | His | Leu | Asp | Asn | Leu | Lys | Gly | Thr | Phe | Ala
A G Y|G A R|G G X|C U X|A G Y|C A Y|C U X|G A Y|A A Y|C U X|A A R|G G X|A C X|U U Y|G C X
A G U|G A G|G G U|C U G|A G U|C A C|C U G|G A C|A A C|C U C|A A A|G G C|A C C|U U U|G C U
        220        Hinf  Hph                     240                250

 Lys | Leu | Ser | Glu | Leu | His | Cys | Asp | Lys | Leu | His | Val | Asp | Pro | Glu
A A R|C U X|A G Y|G A R|C U X|C A Y|U G Y|G A Y|A A R|C U X|C A Y|G U X|G A Y|C C X|G A R
A A G|C U G|A G U|G A A|C U G|C A C|U G U|G A C|A A G|C U G|C A C|G U G|G A U|C C U|G A G
260  Alu           270                280    Alu         290          Bam      300

 Asn | Phe | Arg | Leu | Leu | Gly | Asn | Val | Leu | Val | Ile | Val | Leu | Ser | His
A A Y|U U Y|A G R|C U X|C U X|G G X|A A Y|G U X|C U X|G U X|A U Y|G U X|C U X|U C X|C A Y
A A C|U U C|A G G|C U C|C U G|G G C|A A C|G U G|C U G|G U U|A U U|G U G|C U G|U C U|C A U
        310                320                330                340

 His | Phe | Gly | Lys | Glu | Phe | Thr | Pro | Gln | Val | Gln | Ala | Ala | Tyr | Gln
C A Y|U U Y|G G X|A A R|G A R|U U Y|A C X|C C X|C A R|G U X|C A R|G C X|G C X|U A Y|C A R
C A U|U U U|G G C|A A A|G A A|U U C|A C U|C C U|C A G|G U G|C A G|G C U|G C C|U A U|C A G
350              360       RI      370                380                390

 Lys | Val | Val | Ala | Gly | Val | Ala | Asn | Ala | Leu | Ala | His | Lys | Tyr | His
A A R|G U X|G U X|G C X|G G X|G U X|G C X|A A Y|G C X|C U X|G C X|C A Y|A A R|U A Y|C A Y
A A G|G U G|G U G|G C U|G G U|G U G|G C C|A A U|G C C|C U G|G C U|C A C|A A A|U A C|C A C
        400                  HaeIII           420                430
                              Bal

[U G A] G A U C U U U U C C C U C U G C C A A A A A U U A U G G G G A C A U C [A U G] A A G
440    BglII    450                460                470                480

C C C C U U G A G C A U C U G A C U U C U G G C [U A A][U A A] A G G A A A U U U A U U U U C
        490                500                510                520

A U U G C-poly A          X = A, U, G or C
530    533                R = Purine
                          Y = Pyrimidine
```

Figure 2

The primary structure of β-globin mRNA. The numbers below the sequence indicate the positions of the nucleotides, numbered by reference to the first nucleotide in the codon for the N-terminal valine (position 1). The amino acid sequence of β-globin and the RNA sequence predicted by the genetic code are listed above the derived sequence. Restriction-endonuclease recognition sites are underlined; also shown are the 5′ and 3′ untranslated regions, with the AUG and UGA initiation and termination codons enclosed by solid lines. Within the 3′ untranslated region, a single potential initiation AUG and two contiguous potential UAA terminators, all in phase with the globin UGA terminator, are enclosed in dashed lines.

stages of oogenesis in this animal, an egg shell (chorion) consisting of approximately 50 structural proteins is synthesized and assembled. This process occurs in distinct, identifiable stages (see Fig. 3), and the large number and size of the eggs in each female provide the opportunity for biochemical analysis of each stage. In particular, it is possible to isolate chorion mRNAs from each stage. In order to study the temporal expression of specific chorion genes, we have synthesized and cloned double-stranded DNA copies of the mRNA for use as hybridization probes. On the basis of restriction-endonuclease cleavage analysis, heteroduplex analysis, and nucleic acid hybridization experiments, approximately 20 different chorion gene plasmids have been identified. We are presently trying to classify each of these gene sequences on the basis of its ability to hybridize developmental-stage-specific chorion mRNA. In addition, sheared chromosomal DNA has been cloned, and the colonies are being screened for chorion sequences using in-vitro-labeled chorion mRNA. Chimeric plasmids containing chorion sequences can then be assigned to a specific stage in development by hybridization to the previously staged cDNA clones. We anticipate that a detailed analysis of these chorion-specific chromosomal insertions will reveal interesting features of the sequences that flank coordinately expressed genes.

SV40 chromatin

As a means of establishing conditions for the in vitro transcription of eukaryotic genes, Liz Lacy and Walter Keller have been studying the in vitro transcription of SV40 minichromosomes by the endogeneous RNA polymerase activities associated with isolated SV40 chromatin. In addition, the transcripts made by exogenous RNA polymerases isolated from *E. coli* or from human cells is being studied. Although specific transcripts have not been observed using exogenous polymerases, specific "late" SV40 transcripts have been observed with nuclear extracts or purified minichromosomes isolated from SV40-infected cells late in infection. We are presently using these extracts in an attempt to establish conditions for the specific in vitro initiation of SV40 transcription.

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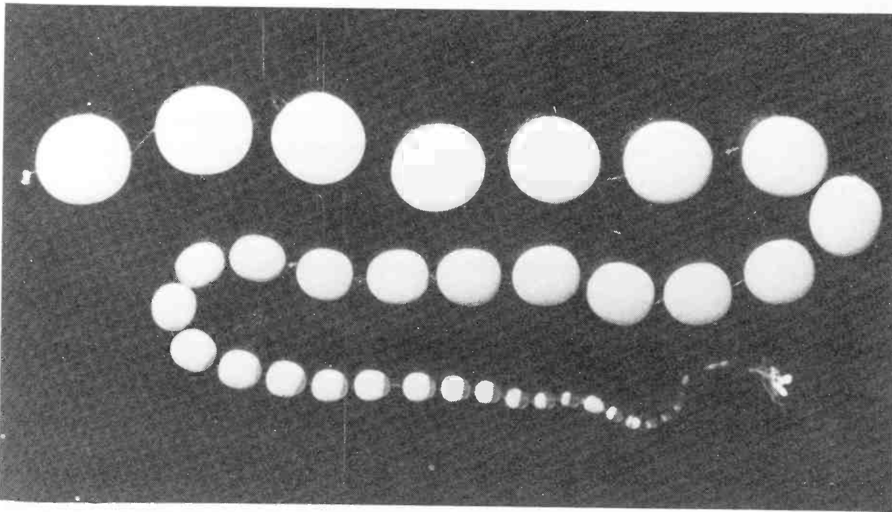


Figure 3
A silk moth ovariole. One of the eight strings of eggs carried by each female silk moth is shown. The anterior (youngest) end is at the lower right and the posterior (oldest) end is at the upper left. A cellular cord connects the developing eggs (follicles) in a linear array. The choriogenic follicles in the upper row are separated in time by approximately 3–4 hours. Ninety percent of all protein synthesis in these cells is devoted to the production of the chorion.

CELL BIOLOGY

G. ALBRECHT-BUEHLER, K. Burridge, A. Bushnell, S. Chait, L. B. Chen, W. Gordon, R. Gudor, L. Jordan, R. Lancaster

Since the majority of the scientists at the Cold Spring Harbor Laboratory work on tumor viruses and tumor-virus-related topics, the Cell Biology section was established about a year ago to provide the cell-biological basis for and to interact with the tumor-related research at the Laboratory. Our predominant research topics are the primary functions of animal cells, such as cell motility, structure and composition of the cell surface, and means of cell-cell communication. Not only does the understanding of such fundamental aspects of animal cells seem mandatory from a general biological point of view, it would seem in particular to be a prerequisite for the understanding of tumor formation and metastasis in animals and humans.

Last year's annual report of our group concentrated on two aspects of animal cells. One aspect was the motility of the surface protrusions and their biological function. Since the time of that report, we have found a convenient way of quantitating the motile actions of surface protrusions in large numbers of cells and established some of the basic parametric dependencies of these actions. The method immediately suggested a way also to quantitate cellular locomotion. One of the first applications of this method suggested a rather fundamental relationship between daughter cells in an established cell line—in contrast with the tacit assumption of biologists that those daughter cells are identical twins, they seem to be mirror images of each other.

Another aspect of last year's report was the localization by indirect immunofluorescence of structural proteins of muscle in nonmuscle cells. Elias Lazarides, the protagonist of the application of indirect immunofluorescence to nonmuscle cells at Cold Spring Harbor, left for Boulder, Colorado, in the fall of 1975 to work as a postdoctoral fellow in Keith Porter's laboratory. Bill Gordon succeeded him in position and interest; he has added various new aspects to the localization studies and is now working on promising ways to localize cellular antigens on the electron-microscopic level.

During the past year, Keith Burridge developed a valuable research tool both for our group and for others. Using this method, it is now possible to further analyze and identify cellular glycoproteins as to their antigenic character and sugar composition after they have already been separated on gels. This method promises to allow rapid determination of changes in the glycoprotein composition of cells following transformation and differentiation.

With the arrival of Lan Bo Chen from the Massachusetts Institute of Technology, a new and very exciting aspect of cell-surface structure was introduced into our group's efforts to understand primary functions of animal cells. Lan Bo Chen's major object of research is the so-called large, external, transformation-sensitive (LETS) protein. Its intriguing nonhomogeneous distribution on cell surfaces and the fact that it eventually forms three-dimensional networks connecting all cells of a normal fibroblast culture may lead to a new understanding of cell surfaces. During the last year, Lan Bo found that this protein originates from cell-cell contact areas and that its distribution changes drastically during myogenesis. Furthermore, in collaboration with James McDougall of the Mammalian Cytogenetics Section, he found an inverse correlation between tumorigenicity and the expression of this protein on the cell surface.

The past year was a period of consolidation of the group and exploration of the potential of the methods which we developed. We are now confident that our approaches, the studies of cell movements, of intracellular contractile elements, and of the structure and function of the major surface glycoproteins, form a promising basis for our future joint efforts toward a more fundamental understanding of normal and transformed animal cell behavior.

A quantitative description of the extension and retraction of surface protrusions in spreading 3T3 mouse fibroblasts

Small particles placed on a cell substrate can be picked up by cells and transported to the perinuclear region. We found that gold particles 0.2–0.4 μm in diameter coat a glass coverslip very densely and evenly. If suspended cells are plated on such a coated substrate, the particles are removed around 80–90% of the cells within 30–60 minutes. Easily identifiable rings can be seen which have twice the diameter of the cell bodies, with most of the cells still spherical. Live-cell studies confirmed the conclusion suggested by this observation: The cells extend projections which reach as far as the perimeter of the rings, attach to the loose particles, and transport them to the cell body either by a centripetal flow mechanism or by retraction as a whole together with the particle.

Making use of this phenomenon under standardized and experimentally controlled conditions, one can readily quantitate the capacity of cell projections to perform extensions followed by retraction by determining the percentage of cells that produce particle-free rings.

Studying 3T3 cells with this assay, we found that the presence of Na^+, K^+, Cl^-, and Mg^{++} or Ca^{++} in a neutral or slightly alkaline phosphate or bicarbonate buffered solution is sufficient to support the optimal particle removal by the cells for at least 50 minutes.

Two metabolic inhibitors, 2,4-dinitrophenol and Na-azide, inhibit the particle removal. If D-glucose is added along with the inhibitors, particle removal can be restored, whereas the addition of three glucose analogs which are generally believed to be nonmetabolizable cannot restore the activity.

Serum is not required for the mechanism(s) of the motile actions of surface protrusions in spreading 3T3 cells. However, it contains components which can neutralize the inhibitory actions of bovine serum albumin and several amino acids, particularly L-cystine or L-cystein and L-methionine. Furthermore, serum codetermines which of the major surface extensions, filopodia, lamellipodia, or lobopodia, is predominantly active.

A quantitative description of cellular displacements

So far, time-lapse cinematography, followed by a very time-consuming frame-by-frame analysis, and the migration of cells from a confluent sheet into a "wound" have been the only ways to study quantitatively cellular locomotion in culture. The use of gold-particle-coated substrates offers a new and more convenient way to study and quantitate the total displacements of single cells.

As mentioned above, if freshly suspended cells are plated on top of a gold-particle-coated coverslip, they produce various surface protrusions and remove the particles within a ring around each cell during the first hour. Within the following 10 hours, the cells begin to locomote while cleaning more particles out of their way. The particles become partly internalized; partly the cells accumulate them on their backs in big clumps which are pinched off once in a while and can be found floating in the medium.

In order to distinguish locomotion on plain surfaces from this combination of phagocytosis and cellular displacement, we suggest the use of the term "phagokinetics." The particle-free tracks (phagokinetic tracks) can be visualized conveniently in dark-field illumination as black lines (Fig. 1).

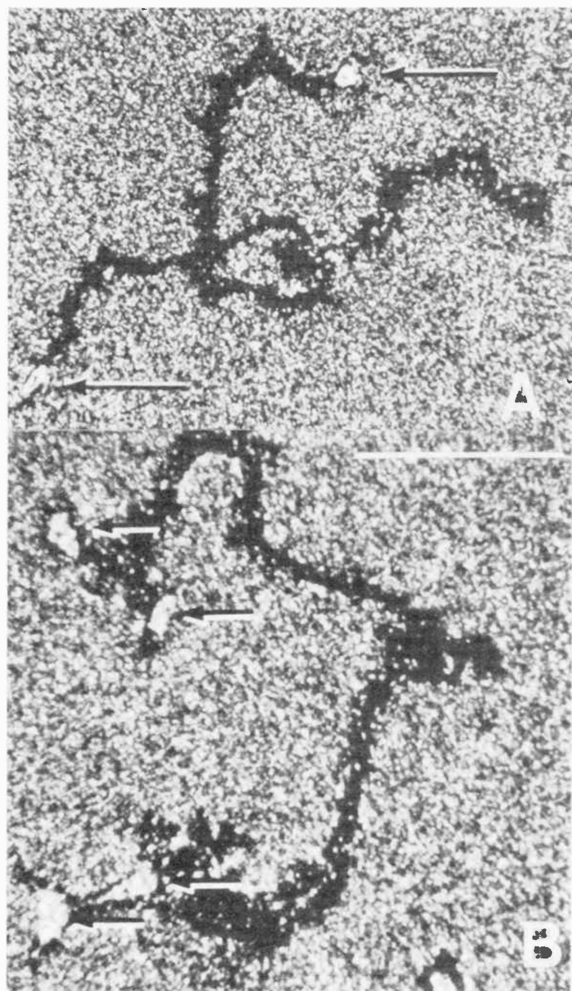


Figure 1
Phagokinetic tracks of mitotic 3T3 cells (48–72 hr after plating in culture medium with 10% or 20% calf serum) suggesting mirror-symmetric directional changes of the cells even after spatial separation. Locations of the cells are indicated by arrows. Second-generation cells are shown in panel B.

We examined various different cell lines in this assay and found quite different track patterns. The tracks can be used for rapid quantitative screening of the extracellular parameters which are required for cellular displacements. At first, however, we concentrated on a rather startling aspect of animal cell structure and movement which was an immediate result of the study of phagokinetic tracks.

The mirror symmetry of daughter 3T3 cells

Branching isolated tracks with the cells located at the ends of the branches indicate mitosis and subsequent

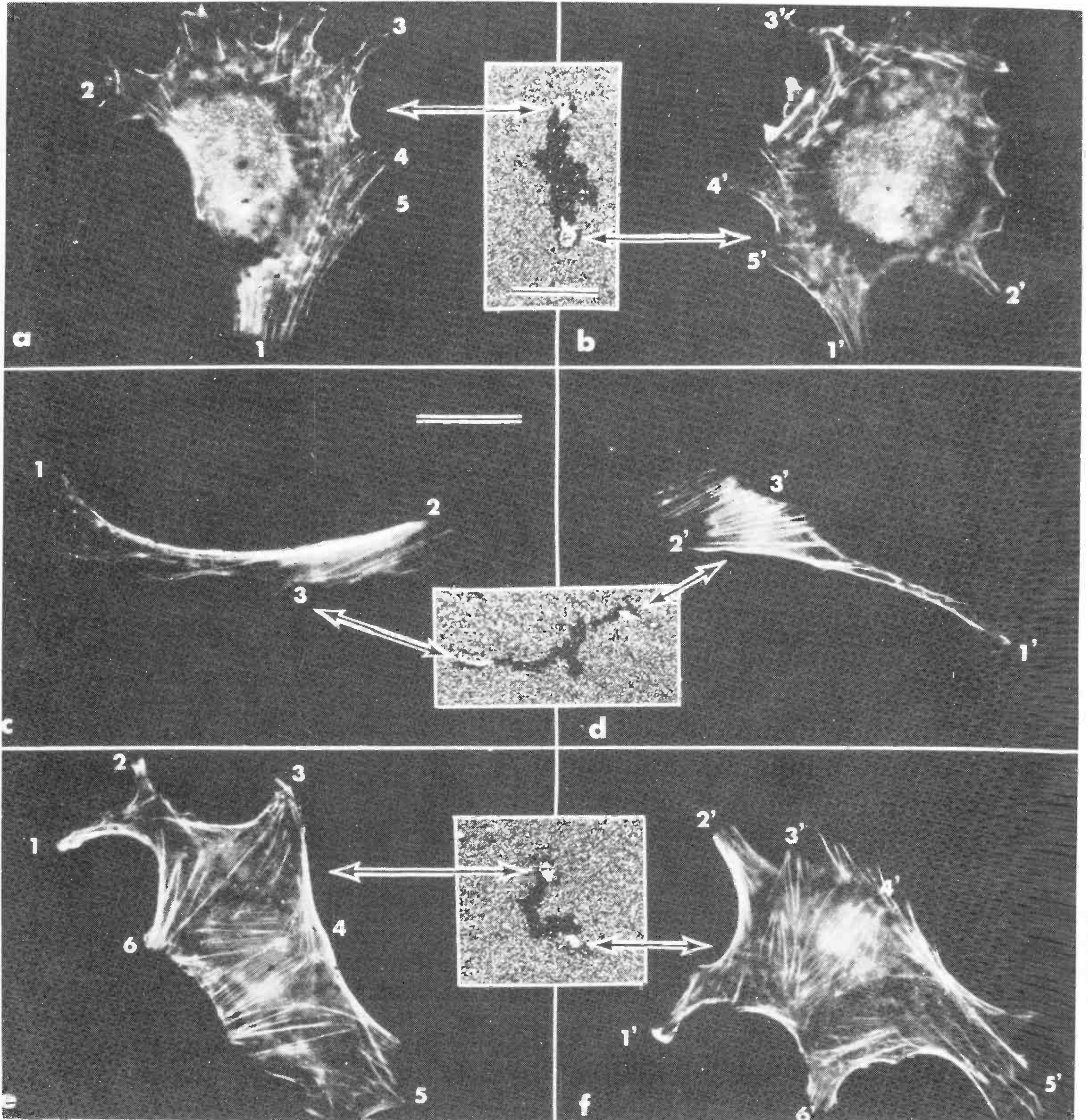


Figure 2

Actin patterns of daughter 3T3 cells in phagokinetic tracks (symmetry relationships between daughter cells after spatial separation). The insets show the tracks formed by the same cells, which are shown in indirect immunofluorescence. Bar in inset a-b *indicates 250 μm; bar in panel* c *indicates 20 μm. With the exception of the cell in panel* b*, which was rotated by 180°, the cells in the fluorescence micrographs are shown in the same orientation as in the insets. The numbers around the cells' peripheries indicate shape characteristics which we suggest correlate in the daughter cells. They are also to help the reader to follow corresponding fluorescent bundles inside the cells. Our interpretation as to the symmetry is as follows.* (a,b)*: symmetrical patterns;* (c,d)*: identical patterns in elongated cell;* (e,f)*: identical patterns.*

separate movement of both daughter cells (Fig. 1). At more than 48 hours after plating of the cells, we found branching tracks with further branching at each end Fig. 1B), showing the four descendents of the cell which tarted the track. The observed simultaneous occurrence of all four second-generation cells suggests that in ells which do not collide with or cross the tracks of thers, the length of the cell cycle is the same in both laughter cells.

More startling, however, was that 40% of such racks showed mirror symmetry in the sense that one daughter cell made directional changes opposite to the other at approximately the same positions of their respective tracks. Fourteen percent of the tracks were dentical in the sense that both daughter cells made the ne directional changes relative to their direction of ovement (Fig. 1). The remaining 47% could not be etermined because the branches either showed no irectional change or were overly distorted.

As to the split angle between the branches, we found he two ranges 80°–120° and 80°–180°. About two-hirds of the observed angles were between 80° and 120°.

The branching tracks of mitotic cells allow an easy dentification of daughter cells, even after spatial separation. Therefore, we examined the actin patterns of laughter pairs in indirect immunofluorescence after hey had formed branching phagokinetic tracks. We ound a strong resemblance in the complex bundle patterns and shape characteristics of the daughter cells, as shown in Figure 2. This finding suggests that these patterns are likely to be predetermined by the parental cell.

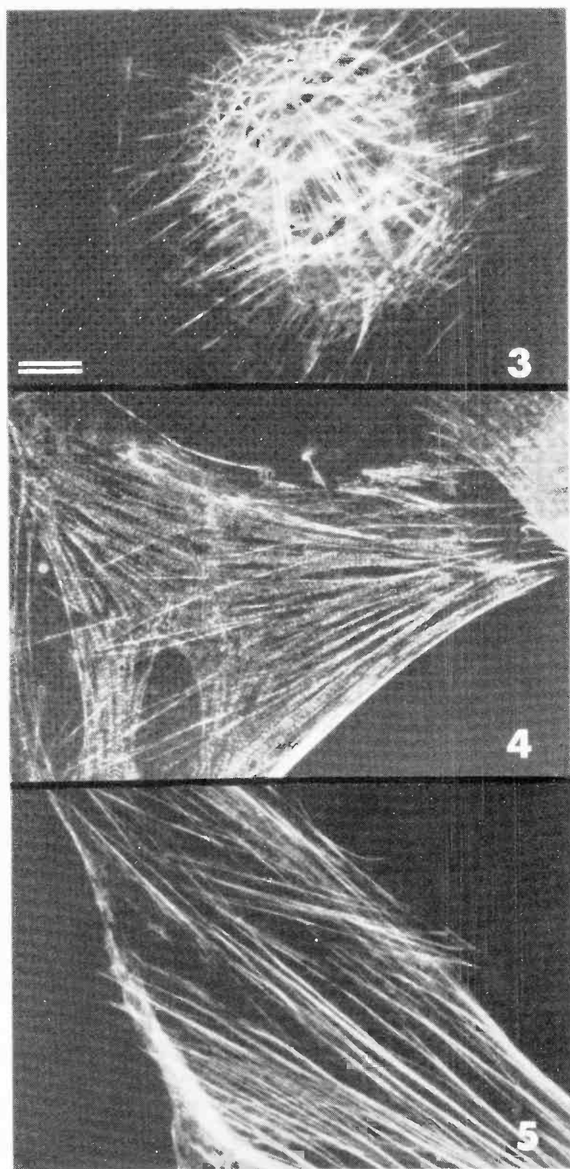
As to the symmetry relationships between the patterns, out of 42 daughter-cell pairs examined we found 34% symmetrical (Fig. 2a, b), 24% identical (Fig. 2c, d, e, f), and 42% unrelated actin-bundle patterns.

We consistently found about twice as many cases of nirror symmetry as of identity among the examined patterns and tracks. Therefore if any of the two relationships between daughter cells holds in general, it is more ikely the symmetry. The additional occurrence of identity and unrelatedness is not surprising, considering the possibility that a daughter cell can be rotated to various degrees during cleavage.

It remains to be seen whether the symmetry between he actin patterns and the directional changes of two laughter 3T3 cells reveals a fundamental consequence of the spindle formation during mitosis of animal cells, nd whether it presents the cellular basis for the universally found bilateral symmetry of organisms. As to the ccurrence of split angles around both 90° and 180°, one may suspect that mitotic cells are programmed to nove one of the daughter cells vertically out of a cell ayer or a string of cells.

Localization of contractile elements in nonmuscle cells

Studies described in last year's annual report on actin, myosin, and α-actinin localization in a variety of cell types have continued. Antibodies to these three proteins have been made and rigorously characterized by immunochemical tests. Myosin has been the main interest



Figures 3–5
Indirect immunofluorescent localization of myosin in cells fixed normally or pretreated with contraction or rigor buffers containing Triton X-100 for various times prior to fixation. Bar = 10 µm.
Fig. 3: *Rat-embryo fibroblast, myosin.*
Fig. 4: *Human skin fibroblast, rigor, 2 min, actin.*
Fig. 5: *Human skin fibroblast, contraction, 30 sec, myosin.*

because it carries the enzymatic activity of the actomyosin system and was the least well described protein in previous studies. The observations of Weber and Groschel-Stewart [*Proc. Natl. Acad. Sci. USA* 71:4561 (1974)] have been reconfirmed in our finding that myosin is localized periodically along stress fibers every 1.5 μm. However, we have shown that myosin is also localized between stress fibers in "sheets" of periodicity. This arrangement is particularly common at the ends of stress fibers, where they appear to splay out into smaller units. Myosin has also been localized in the polygonal network pattern of rat-embryo cells (see Fig. 3). It is like tropomyosin in that it is absent from the vertices, occurring only between them.

The localization studies have given a static image of the distribution, with only a few hints of the dynamics that must occur in vivo with force production. "Contractile models" of muscle fibers, that is, fibers that have been completely permeabilized by glycerol or detergent treatment, provided a major step forward in the study of muscle physiology [for review, see N.I. Arronet, *Motile Muscle and Cell Models* (Consultants' Bureau, New York, 1973)] in that they allowed the use of defined buffers to determine the exact ionic conditions for contraction, relaxation, and rigor. Hoffmann-Berling [*Naturwissenschaften* 50:256 (1963)] pioneered the use of contractile models in the study of nonmuscle cells. Following up the ideas of Hoffmann-Berling, we have recently begun studying contractile models of human fibroblasts. The localizations of actin, myosin, and α-actinin have been determined in cells permeabilized by detergent (Triton X-100) made up in contraction (10^{-5} M calcium, 1 mM ATP), relaxation (10^{-7} M calcium, 1 mM ATP), and rigor (10^{-7} M calcium, no ATP) buffers. Cells grown on glass coverslips were removed from the culture medium and placed directly into contraction, relaxation, or rigor buffer containing Triton X-100. At various times after this treatment, the cells were fixed and stained for immunofluorescence. In rigor buffer, the patterns of actin, myosin, and α-actinin are stabilized, and no significant change occurs for hours while the cells are kept in rigor buffer (see Fig. 4). Contraction buffer at 21°C produces a dynamic retraction and complete rounding up of the previously well-spread cells within 2 minutes. If the retraction process is stopped after 30 seconds by fixation and staining, considerable changes can be observed in the actin and myosin distributions. The most striking change is in the myosin pattern, which loses all signs of periodicity, appearing in a solid staining of stress fibers with no remaining "sheets" of periodicity (see Fig. 5). Interruptions appear in the actin staining pattern, though they are not regular enough to be called periodic. The α-actinin pattern remains periodic, though somewhat altered. From these results it appears that the normal distribution of actin, myosin, and α-actinin in well-spread fibroblasts represents a rigor configuration. Certain ionic conditions which appear to be the addition of ATP and calcium can cause redistribution of the force-generating components actin and myosin.

In an attempt to gain a higher level of resolution than immunofluorescence provides, transmission electron microscopy was applied to the contractile models of human fibroblasts as well as to normally fixed, unpermeabilized cells. A new technique of specimen preparation devised by Buckley and Porter [*J. Microsc. (Oxf.)* 104:107 (1975)] was used for these studies. Cells were grown on Formvar-coated glass coverslips. After being treated with detergent in contraction, relaxation, or rigor buffer, or without any treatment at all, the cells were fixed by standard procedures (using glutaraldehyde and osmium tetroxide), the Formvar was removed from the coverslips and mounted on electron microscope grids, and the cells were dehydrated and finally critical-point-dried. The advantage of preparing cells in this way is that they can be viewed directly, i.e., unsectioned, in the 80-kV Philips 201 electron microscope. The image is that of the internal components of the entire cell. If the specimen stage is tilted and pairs of micrographs are taken at various angles of tilt, a stereo viewer can be utilized to give a three-dimensional view of the internal components. This 3-D image provides much more information than the usual two-dimensional thin-section image of resin-embedded cells. Studies already completed with this "whole-mount" technique have shown that dynamic ultrastructural changes occur in the contractile models. Work is in progress to determine the correlations between immunofluorescent images and the 3-D whole-mount images.

LETS protein and oncogenic transformed cells

There are several adenovirus-transformed cell lines that are nontumorigenic. For instance, cell lines Ad2/F17 and Ad2/F18, which are rat-embryo cells transformed by adenovirus type 2, are T-antigen-positive and able to grow in low serum and to high saturation density, yet they are nontumorigenic in normal syngenic rats, immunosuppressed newborn syngenic rats [P.H. Gallimore, *J. Gen. Virol.* 16:99 (1972)], and "nude" mice. Ad2/F19, on the other hand, is tumorigenic in nude mice but not in rats, whether immunosuppressed or not. In comparison with the other Ad2 lines studied, Ad2/F19 showed a lower level of tumor induction in nude mice. Ad2/F19 tumors had a longer latency period than the other lines (e.g., T2C4—7.5 days, REM—8.5 days, F4—19 days, F19—30 days), and whereas T2C4, REM, and F4 showed invasion of local mouse tissues, this was not the case with Ad2/F19, which classified histologically as a benign tumor (P.H. Gallimore, manuscript in preparation). Ad2/F4 and Ad2/REM are tumorigenic in immunosuppressed syngenic rats, and, at the other ex-

treme, some lines (e.g., T2C4) are tumorigenic in syngenic rats without immunosuppression. This series of cell lines thus provides a spectrum of oncogenicity within a single species.

We decided to examine the surface characteristics of these cells as a starting point toward understanding the gradation of oncogenicity of this series of adenovirus-transformed cells. We used indirect immunofluorescence techniques to study the distribution of the cell-surface LETS protein on normal, transformed, and tumorigenic cells (Fig. 6). The results of these experiments have been published in detail elsewhere [Chen, Gallimore, and McDougall, *Proc. Natl. Acad. Sci. USA* 73:3570 (1976)]. In brief, a correlation has been established between the expression of fibril-like LETS protein and the oncogenic capabilities of a series of adenovirus-transformed cell lines. In cells expressing a transformed phenotype in vitro, LETS protein is only detected in cell-cell contact areas, whereas in "untransformed" cells, LETS is distributed over the cell surface. Transformed cells capable of inducing invasive tumors and the cells of established tumor lines have low or undetectable levels of LETS, as measured by this method. The results indicate that LETS protein has a role in cell-cell adhesion, and that reduced expression of this protein at the cell surface is related to the oncogenic phenotype. This relationship has been established for both experimentally induced and spontaneous tumors.

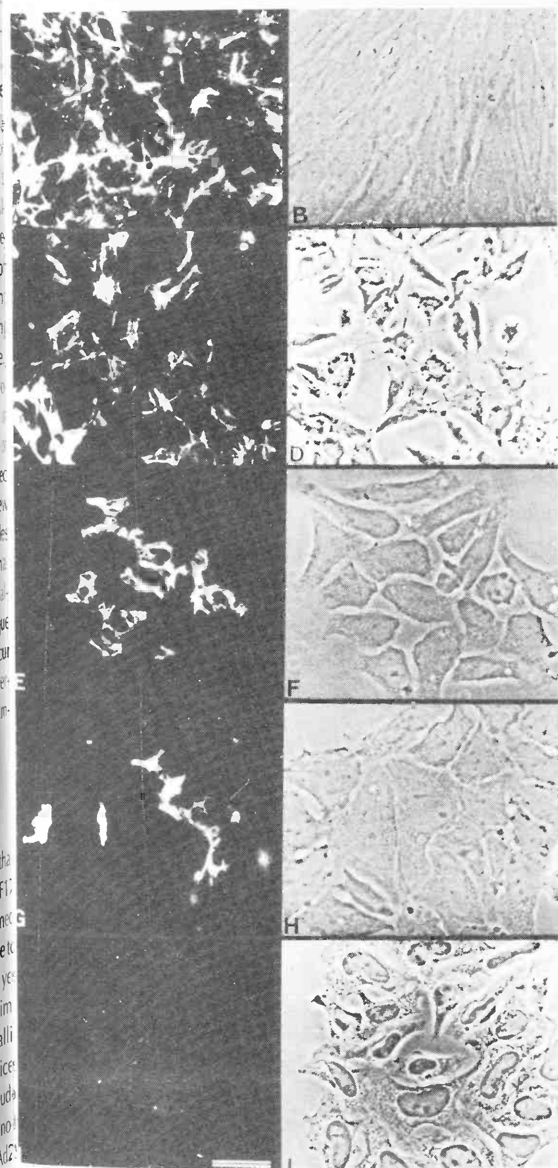


Figure 6
Indirect immunofluorescent stains of normal and adenovirus-2-transformed rat-embryo fibroblasts with anti-LETS antiserum. Photographs A, C, E, G, and I are fluorescent photomicrographs of normal rat cells and cell lines F18, F17, B1, and T8 stained with anti-LETS antiserum, respectively; B, D, F, H, *and* J *are the corresponding phase-contrast photomicrographs of the same cell lines. The bar represents 20 μm.*

At the moment, we do not know why there should be a correlation between the loss of LETS protein and tumor induction. The simplest explanation may be that LETS protein is directly involved in growth control, and that the loss of LETS protein is responsible for unrestricted growth during tumor formation. Our previous finding [Teng and Chen, *Proc. Natl. Acad. Sci. USA* 72:413 (1975)] argues against this possibility. In view of the distribution of LETS protein in adenovirus-transformed cells reported here and the findings of Yamada et al. [*Proc. Natl. Acad. Sci. USA* 73:1217 (1976)], it is possible that LETS protein may be the "glue" involved in the extracellular matrix system. Classical embryology tells us that the extracellular matrix plays an important role in differentiation and organogenesis. Perhaps the maintenance of a normal pattern of cell growth in vivo depends on a proper intercellular matrix system. The integrity of such a matrix may be impaired by the loss of one (LETS protein) or two (LETS protein and, for example, collagen) of its elements.

LETS protein and myogenesis

Recently we have shown that the replacement of ^{125}iodide by ^{131}iodide and the use of gradient polyacrylamide gels can greatly increase both the number of detectable cell-surface proteins and the resolution of protein bands in SDS polyacrylamide gel electrophoresis [Teng and Chen, *Nature* 259:578 (1976)]. When such procedures were applied to compare prefused, fused, and postfused stages of Yaffe's myogenic cell line L8, twice as many bands were detected as with ^{125}iodide, but, in agreement with Hynes [*Biochim. Biophys. Acta* 458:73 (1976)], no significant changes were found.

As a continuation of these studies, cell-surface alterations during myogenesis have been investigated in Yaffe's myogenic cell line L8 using indirect immunofluorescence with an antibody against the LETS protein [Chen, *Cell* (1977, in press)]. Whereas lactoperoxidase-catalyzed iodination shows no changes during myogenesis in the composition of cell-surface components, including LETS protein, the immunofluorescent technique reveals a substantial alteration in the distribution of this surface antigen. With the prefused myoblasts, LETS protein is dispersed all over the cell surface and is particularly prominent in the regions of cell-cell contact and cell-substrate contact. However, following myoblast fusion, this pattern is markedly changed. All of the fibril-like surface LETS protein disappears, and in some myotubes discrete clusters of LETS protein become conspicuous.

Whether LETS-protein clusters play a role in muscle development is currently being studied in collaboration with Dr. Eric Frank of Harvard Medical School. In particular, we will explore whether LETS-protein clusters are involved in the interaction of motor neuron and muscle fiber.

Identification of antigens and specific glycoproteins in SDS gels

Last year two of us (K.B. and W.G.) were involved in the localization of structural proteins in nonmuscle cells using the immunofluorescent-antibody technique. Whenever antibodies are used, it is important to know their specificity. While we were testing the specificity of our antisera by conventional immunological methods, it occurred to us that some (perhaps many) proteins might preserve their antigenicity during SDS gel electrophoresis. It seemed that if this were so, a useful way to test the specificity of antisera would be to "stain" SDS gels directly with antisera and then to reveal the antigenic gel bands by using a second antiserum which was labeled in some way (e.g., with a radioactive, enzymatic, or fluorescent tag). Accordingly, we decided to test this idea and tried to "stain" gels with a specific antiserum and then follow with a second, radioactively labeled antibody directed against the first antiserum. The radioactivity was detected by autoradiography. Somewhat to our surprise, the technique has worked successfully for more than a dozen different antisera tested. So far only an antiserum against collagen has failed to react with its antigen on SDS gels.

While developing this technique we realized that besides antibodies recognizing antigens, other types of interaction might also be possible in SDS gels. For example, it seemed probable that SDS gel electrophoresis would not affect the carbohydrate portion of glycoproteins and these might still be able to bind plant lectins. This prediction was soon confirmed by applying radioiodinated lectins directly to gels. Moreover, by using lectins with different carbohydrate specificities, different sets of glycoproteins could be visualized. The technique seemed to offer a new means of analyzing glycoproteins according to both their apparent molecular weight and their carbohydrate specificity. Shortly before we presented this technique at the 1976 Cold Spring Harbor Symposium, it was a disappointment to find out that essentially the same technique had already been developed independently by two other laboratories [Robinson et al., *FEBS Lett.* 58:330 (1975); Tanner and Anstee, *Biochem. J.* 153:265 (1976)]. Nevertheless, the techniques should have many applications. So far we have concentrated on analyzing the glycoproteins of normal, transformed, and tumorigenic cells (Burridge 1976b). Now we are also using the two techniques to analyze glycoproteins in a number of developmental systems. Certainly, in the area of developmental biology, both the antibody and the lectin techniques may be useful, since even in differentiation the expression of new surface antigens, many of which will also be glycoproteins, is critical.

The glycoproteins of 3T3 cells and various virus-transformed 3T3 cells have been analyzed by the direct application to SDS gels of four iodinated lectins with different specificities (Con A, ricin, WGA, and PHA) (Burridge 1976b). With all four lectins differences are seen between the normal and the transformed cell types. On the gel of 3T3 cells, for example, WGA strongly stains a doublet with a molecular weight of about 190,000 and another band at about 205,000, but WGA does not stain equivalent bands in the transformed cells or stains them only very weakly. Glycoprotein differences are also seen among the various transformants. For example, a band at about 250,000 daltons, the region to which the LETS protein moves, is labeled in all four cell types by Con A and PHA, but the band is labeled strongly with WGA and ricin only in the gels of the SV101 and Py3T3 cells. Since glycoprotein differences are seen between the two lines of SV40-transformed 3T3 cells (SV513 and SV101), this suggests that at least some of the differences arise from clonal variations unrelated to viral transformation.

The glycoproteins of normal and RSV-transformed chick cells have also been analyzed. Here the most prominent glycoprotein differences involve the LETS protein. In the normal chick cells, this band is stained strongly by all four lectins, and it would seem, therefore, that LETS not only contains considerable carbohydrate but exposes several different terminal specificities. In the RSV-transformed chick cells, the staining of this band is reduced, and other glycoprotein bands appear or become more prominent.

Recently, Chen, Gallimore, and McDougall at Cold Spring Harbor have found a correlation between the tumorigenicity of many transformed cell lines and th

reduction of LETS protein expressed on these cells' surfaces. With the lectin technique, we have begun to examine the glycoproteins of the series of adenovirus-2-transformed rat fibroblasts which show a range of tumorigenicity and which Chen et al. [*Proc. Natl. Acad. Sci. USA* 73:3570 (1976)] have correlated with reduction in LETS. So far we have examined F18 and T8 cells. F18 is nontumorigenic and expresses LETS on its surface, whereas T8 is highly tumorigenic and does not express LETS. A prominent band is stained in the region of the LETS protein by both ricin and PHA in gels of F18 but not in gels of T8. Similarly, a band just below this is stained by PHA in the F18 gel, whereas in the T8 gel an additional band, with an approximate molecular weight of 40,000, is stained by Con A.

We have used anti-LETS antiserum to identify this antigen in SDS gels of the various normal, transformed, and tumorigenic cell types mentioned above. The antibody stained a high-molecular-weight band found in the gel of each cell type, but the intensity of this staining varied considerably; the lowest level was seen in the tumorigenic, adenovirus-transformed rat cell line T8. The intensity of this band was also much reduced in the RSV-transformed chick cells compared with that in the normal chick fibroblasts. An unexpected result was that the gels of two of the 3T3 cell transformants (SV101 and Py3T3) showed increased intensity of staining with the LETS antibody.

The staining of gels with the anti-LETS protein antibody is complementary to the examination of intact cells by immunofluorescent microscopy. This latter method looks at the LETS expressed on the surface, whereas the antibody applied to gels of whole cells can reveal the presence of LETS within these cells even though it may not be expressed on the surface. This is the case with the RSV-transformed chick cells. Both the antibody staining and the lectin staining on gels of these cells confirm the presence of LETS within them, though it is absent from their surfaces. These lectin results are interesting since they indicate that at whatever stage LETS is being degraded or lost from these chick cells, it occurs subsequent to the glycosylation process. With the SV40- and polyoma-transformed 3T3 cells, a different situation exists. These cells do express some surface LETS, particularly when highly confluent, but in reduced amounts when compared with confluent 3T3 cells. However, the intensity of the antibody staining on these gels indicates that the transformants have at least as much LETS as the normal cells. This would suggest an accumulation of LETS within these cells, perhaps related to the incomplete expression on the surface. In this respect, it is interesting that the LETS band of the Py3T3 and SV101 cells binds significantly greater amounts of both ricin and WGA, indicating either an increased level or an altered pattern of glycosylation.

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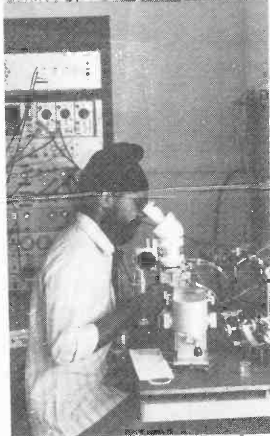
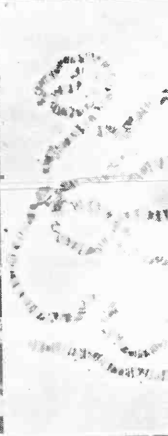
POSTGRADUATE TRAINING PROGRAMS





SUMMER 1976

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

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

ANIMAL CELL CULTURE, June 10 – June 30

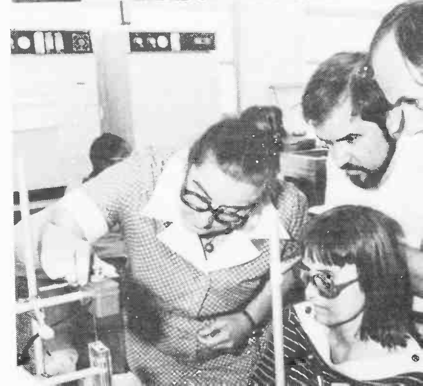
INSTRUCTORS

Coon, Hayden, Ph.D., National Cancer Institute, Bethesda, Maryland
Sato, Gordon, Ph.D., University of California, San Diego, California

ASSISTANTS

Yarkin, Rhoda, B.A., National Cancer Institute, Bethesda, Maryland
Ambesi, Saverio, M.D., National Institutes of Health, Bethesda, Maryland
Izumi, Hayashi, B.A., University of California, San Diego, California

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



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Wolfe, Jason, W., Ph.D., Wesleyan University, Middletown, Connecticut

SEMINARS

Pollack, R., State University of New York, Stony Brook. *A strategy for the in vitro analysis of the oncogenic process.*
Blobel, H., Rockefeller Institute. *Processing of secretory proteins.*
Hsie, A., Oak Ridge National Laboratory. *Quantitative mutagenesis in vitro.*
Kates, J., State University of New York, Stony Brook. *Reconstruction of cells from cytoplasts and karyoplasts.*
Green, H., Massachusetts Institute of Technology. *Life of the epidermal keratinocyte—A lesson learned from the study of teratomas.*
Anderson, W.F., Arthritis Institute, NIH. *Use of mouse erythroleukemia cells in the study of globin gene expression.*
Meiss, H., New York University Medical School. *Temperature-sensitive mutants.*
Eisenstadt, J., Yale University. *Behavior of cytoplasmic genes in mammalian cells.*
Rechsteiner, M., University of Utah. *Studies on human-mouse hybrid cell formation and chromosome segregation.*
Scharff, M., Albert Einstein Medical School. *Mutants of mouse myeloma cells.*
Sambrook, J., Cold Spring Harbor Laboratory. *Nucleic acid studies in transformed cells.*
Shin, S., Albert Einstein Medical School. *Use of "nude" mice in cancer research.*

THE NERVOUS SYSTEM OF THE LEECH, June 10–June 30

INSTRUCTORS

Nicholls, John, Ph.D., M.D., Stanford University Medical School, Stanford, California
Jansen, Jan, Ph.D., M.D., University of Oslo, Oslo, Norway
Muller, Kenneth J., Ph.D., Carnegie Institution of Washington, Washington, D.C.
Sawyer, Roy, Ph.D., University of California, Berkeley, California

The aim of this workshop was to provide students with an intensive lab and seminar course that would enable them to pursue independent work on the leech. To this end, we hoped to provide the students with techniques for recording from leech cells, now considered straightforward and relatively easy, which took much time and effort to be refined. With this knowledge, they might avoid many of the trivial technical difficulties that bedevil anyone starting on the nervous system of the leech or other animals.

The initial work was devoted mainly to recognizing the individual cells, learning how to record from them with intracellular and extracellular electrodes, getting familiar with the equipment, and performing dissections. The students then progressed to more difficult experiments, such as recording synaptic potentials while changing the fluid bathing the preparation or injecting individual cells with marker substances in order to study their geometry.

The final phase of the course consisted of devising and performing original experiments, some of which proved to be of sufficient interest to be pursued in greater detail. For example, the nervous system of a hitherto unknown South American leech was explored in detail. This animal may provide a uniquely useful preparation for examining the organization and development of a simple brain.

PARTICIPANTS

Buckley, Kathleen M., B.A., Harvard Medical School, Boston, Massachusetts
Ferster, David, B.S., Harvard Medical School, Boston, Massachusetts
Hogan, Patrick, B.A., Harvard Medical School, Boston, Massachusetts
Kavinsky, Clifford J., B.A., University of Chicago, Chicago, Illinois
Ready, Donald, B.A., California Institute of Technology, Pasadena, California
Singh, Satpal, M.S., Tata Institute, Bombay, India
Victor, Jonathan, B.A., Rockefeller University, New York, New York
Weeks, Janis, B.S., University of California, San Diego, California
Weisblat, David, B.A., California Institute of Technology, Pasadena, California

SEMINARS

Adal, M., Stanford University School of Medicine. *Fine structure of cultured, regenerating CNS.*
Stent, G., and W. Kristan, University of California. *Locomotion and heartbeat (I, II, III).*
Cohen, L., Yale Medical School. *Recording without electrodes.*
Sawyer, R., University of California, Berkeley. *Biology of leeches.*
Fernandez, J., University of California, Berkeley. *Development of leech CNS.*

TECHNIQUES FOR STUDYING THE VERTEBRATE CENTRAL NERVOUS SYSTEM, June 10–July 5

INSTRUCTORS

Kirkwood, Peter, Ph.D., Institute of Neurology, London, England
Shatz, Carla, Ph.D., Harvard Medical School, Boston, Massachusetts
Stryker, Michael, Ph.D., Harvard Medical School, Boston, Massachusetts

ASSISTANT

Mates, Sharon, B.A., Harvard Medical School, Boston, Massachusetts

One aim of the course was to provide students with the basic technical competence necessary for conducting independent experimental work. Another was to provide an introduction to the functional organization of several different areas in the vertebrate central nervous system. The course consisted of four different laboratory exercises. In all labs students learned the appropriate surgical techniques, the preparation of microelectrodes, and simple histological procedures for reconstructing electrode tracks. In one lab the reflex activity of motoneurons in the cat's spinal cord was studied using both intracellular and extracellular recording techniques. Two other labs focused on the visual system. In one, extracellular recordings were made from the cat's visual cortex, and the response properties of neurons to visual stimuli were examined. In the other, stereotaxic techniques were used to place electrodes within deeper structures (lateral geniculate nucleus and superior colliculus) for recordings. The retrograde transport of horseradish peroxidase injected into these structures was used to trace afferent connections. In the fourth lab, the regions of the cortex and superior colliculus receiving somatosensory input from the whiskers of the rat were studied. The relationship between these two structures was examined using both anatomical and extracellular recording techniques. Additional anatomical methods, such as silver stains selective for degenerating fibers, were demonstrated.

PARTICIPANTS

Bixby, John, B.A., California Institute of Technology, Pasadena, California
Botticelli, Lawrence J., M.S., Harvard School of Public Health, Boston, Massachusetts
Demian, Jeff, M.S., State University of New York, Stony Brook.
Grobstein, Carolyn Smith, B.A., University of Chicago, Chicago, Illinois
Jarvis, Charlene D., Ph.D., National Institutes of Health, Bethesda, Maryland
Nass, Menasche, Ph.D., Brown University, Providence, Rhode Island
Norris, Charles, Ph.D., Tulane Medical School, New Orleans, Louisiana
Reichardt, Louis, Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Bizzi, E., Massachusetts Institute of Technology. *Central and peripheral contributions to the coordination of eye and head movements studied in alert animals.*
Burke, R., National Institutes of Health. *Physiological and histochemical characterization of a spinal motoneuron pool.*
Freeman, J.A., Vanderbilt University Medical School. *Organization of the frog optic tectum studied using current source density analysis and electrical models of tectal cells.*
Hubel, D.H., Harvard Medical School. *Functional organization of the monkey's visual cortex.*
Kiang, N., Massachusetts Eye and Ear Infirmary and Massachusetts Institute of Technology. *Functional organization of primary relay sites in the auditory system.*
Kuno, M., University of North Carolina at Chapel Hill. *Trophic interactions between motoneurons and muscle.*
Schiller, P.H., Massachusetts Institute of Technology. *Role of the superior colliculus in eye movement and vision.*

MOLECULAR CYTOGENETICS, June 13 – July 3

INSTRUCTORS

Pardue, Mary Lou, Ph.D., Massachusetts Institute of Technology, Cambridge
Gall, Joseph, Ph.D., Yale University, New Haven, Connecticut

ASSISTANT

Erba, Harry, Yale University, New Haven, Connecticut

The molecular cytogenetics course emphasized the integration of classical and molecular techniques for the analysis of chromosome structure and function. Current problems of chromosome structure were approached by chromosome banding techniques, DNA renaturation kinetics, the isolation of chromatin subunits, in situ hybridization, visualization of transcription, restriction-enzyme digestion of DNA, nucleic acid sequencing, and heteroduplex mapping. A new technique introduced this year was the localization of proteins in cytological preparations by fluorescent antibodies. As always we benefited from the many contributions of Barbara McClintock.

Students came from Denmark, England, Germany, and Israel as well as various parts of the U.S., and most had primarily biochemical training. Previous research experience of the students ranged from *Drosophila* embryo culture to analysis of chloroplast DNA, so participants contributed a wide range of expertise.

PARTICIPANTS

Bohr, Vilhelm A., Ph.D., Panum Institute, Copenhagen, Denmark
Carlson, Jonathan, Ph.D., University of Colorado, Denver
Donady, James J., Ph.D., Wesleyan University, Middletown, Connecticut
Gershoni, Jonathan, B.S., Hebrew University, Jerusalem, Israel
Goldberg, Sheldon, B.S., New York University Medical Center, New York, New York
Jamrich, Milan, M.S., Institut Molekulare Genetik, Heidelberg, Federal Republic of Germany
Lockwood, Arthur H., Ph.D., New York University, New York, New York
McKnight, Steven L., B.A., University of Virginia, Charlottesville.
Morgan, Garry T., B.S., University of Leicester, Leicester, England
Silver, Louise S., B.A., New York University, New York, New York

SEMINARS

Hsu, T.C., M.D. Anderson Hospital and Tumor Institute. *Cytochemical analysis of mammalian chromosomes.*
Cech, T., Massachusetts Institute of Technology. *Psoralen cross-linking as a probe for in vivo chromatin structure.*
Judd, B., University of Texas. *Gene-chromomere relationships in* Drosophila.
Gall, J., Yale University. *Gene amplification in* Tetrahymena
Pardue, M.L., Massachusetts Institute of Technology. *The "heat shock" response in cultured* Drosophila *cells.*
Elgin, S., Harvard University. *Studies on* Drosophila *chromosomal proteins.*
Davis, R., Stanford University. *Expression and mapping of eukaryotic genes in* Escherichia coli.
Roberts, R., Cold Spring Harbor Laboratory. *Restriction-type enzymes.*
———. *Nucleic acid sequencing.*
Noll, M., Universität Basel. *Chromatin structure in eukaryotes.*
Bakken, A., University of Washington. *Visualization of transcription in mouse oocytes.*

ADVANCED BACTERIAL GENETICS, July 6 – July 26

INSTRUCTORS

Miller, Jeffrey, Ph.D., University of Geneva, Geneva, Switzerland
Botstein, David, Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts
Wensink, Peter, Ph.D., Brandeis University, Waltham, Massachusetts
Davis, Ronald, Ph.D., Stanford University, Stanford, California

ASSISTANTS

Lowenberg, Joel, B.S., Brandeis University, Waltham, Massachusetts
St. John, Tom, B.S., Stanford University, Stanford, California
Winston, Fred, A.B., Massachusetts Institute of Technology, Cambridge, Massachusetts

Students in the course carried out complete in vitro recombination experiments in which fragments of yea DNA were joined to *E. coli* plasmid vehicles by the terminal transferase method and to phage λ vehicles by th restriction endonuclease-polynucleotide ligase method. The in vitro recombinants were analyzed by methoc including colony hybridization to radioactive yeast ribosomal RNA, heteroduplex analysis in the electro microscope, and attempted genetic complementation of specific genetic defects in the bacterial hosts.

In order to make available a larger group of mutants suitable for complementation tests with in v recombinant DNA, insertion mutations (i.e., mutations caused by insertion of a translocatable drug resist

element) were isolated directly in highly transformable *E. coli* strains. One of these, *cys*: :Tn5 insertion mutation, appeared to be complemented by a yeast DNA fragment.

Concurrently with these experiments, students isolated and analyzed a large number of new nonsense suppressors with well-defined specificities of amino acid insertion. In addition, some of the students found some possibly interesting regulatory mutations affecting efficiency of nonsense suppression.

PARTICIPANTS

Allen, Robert G., Ph.D., General Foods Corp., White Plains, New York
Bach, Marie-Louise, Ph.D., Institute de Biologie, Strasbourg, France
Conrad, Susan E., B.S., California Institute of Technology, Pasadena
Crabeel, Majolaine, Ph.D., University of Brussels, Brussels, Belgium
Cunningham, Thomas S., B.S., Temple University, Philadelphia, Pennsylvania
DiLauro, Roberto, M.D., National Cancer Institute, Bethesda, Maryland
Duck, Peter, Ph.D., National Research Council, Ottawa, Ontario, Canada
Duncan, Craig H., B.S., University of Rochester, Rochester, New York
Farber, Florence, Ph.D., Baylor College, Houston, Texas
Hynes, M.J., La Trobe University, Victoria, Australia
Jones, Lily A., Ph.D., Wayne State University, Detroit, Michigan
Liebke, E. Hope, Ph.D., University of Connecticut, Storrs
Ljungquist, Siv, B.S., Karolinska Institute, Stockholm, Sweden
Lowrie, Robert J., Ph.D., Food Research Institute, Ottawa, Ontario, Canada
Neugebauer, Kristina, M.S., University of Heidelberg, Heidelberg, Federal Republic of Germany
Patterson, Bruce, Ph.D., National Cancer Institute, Bethesda, Maryland
Royer, Hans-Dieter, M.D., Max-Planck-Institut, Tübingen, Federal Republic of Germany
Sherman, Fred, Ph.D., University of Rochester, Rochester, New York
Spandidos, Demetrios, Ph.D., McGill University, Montreal, Quebec, Canada
Wenzl, Wilfried, Ph.D., Hygiene Institut, Graz, Austria

SEMINARS

Botchan, M., Cold Spring Harbor Laboratory. *Structure of integrated SV40 DNA in transformed cells.*
Maniatis, T., Cold Spring Harbor Laboratory. *In vitro synthesis and molecular cloning of eukaryotic chains.*
Barklay, E., National Institutes of Health. *Application of physical containment methods in recombinant DNA experiments.*
Gartland, W., National Institutes of Health. *Summary of the draft NIH guidelines on recombinant DNA research.*

ANIMAL VIRUSES, July 6 – July 26

INSTRUCTORS

Diggelman, Heidi, M.D., Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland
Beard, Peter, Ph.D., Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland

ASSISTANT

Mueller, Verena, Technical Assistant, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland

The course consisted of morning lectures covering the major classes of animal viruses. The laboratory exercises held each afternoon included preparation of primary cell cultures, growth of continuous tissue-culture cell lines, cell fusion, preparation and purification of virus stocks, extraction and purification of viral nucleic acids and viral proteins, restriction-enzyme analysis of viral DNA and viral chromatin, translation of viral RNAs by cell-free extracts, kinetic-complexity analysis of different RNAs by hybridization, and isolation of viral proteins from infected cells using immunological techniques. A variety of evening lectures were held to review some particular aspects of modern animal virology.

In addition to the formal laboratory exercises, some individual research projects were carried out in collaboration with the permanent staff of the Cold Spring Harbor Laboratory.

PARTICIPANTS

Bell, Anne P., B.S., Cornell University, Ithaca, New York
Bellemann, Peter, Ph.D., University of Tübingen, Tübingen, Federal Republic of Germany
Besemer-Rosenwirth, Brigitte, Ph.D., University of Cologne, Köln, Federal Republic of Germany
Cohen, Maurice, Ph.D., California Institute of Technology, Pasadena
Corden, Jeffry L., B.S., Oregon State University, Corvallis
Dorsky, David J., B.A., Harvard Medical School, Boston, Massachusetts
Ecklund, Peter S., Ph.D., Children's Hospital, Detroit, Michigan
Erkinger, Sigrid, Ph.D., University of Graz, Graz, Austria
Graessmann, Monika, Ph.D., Institut für Molekularbiologie, West Berlin, Germany
Hsu, Wen-Tah, Ph.D., University of Chicago, Chicago, Illinois
Ibelgaufts, Horst, M.A., Max-Planck-Institut, Köln, Federal Republic of Germany

Johansson, Katarine, B.S., Uppsala University, Uppsala, Sweden
McClain, Donald A., B.A., Rockefeller University, New York, New York
Parmeggiani, Andrea, M.D., École Polytechnique, Palaiseau, France
Sarasin, Alain, Ph.D., Stanford University, Stanford, California
Scholla, Clara, B.S., Sloan-Kettering Institute, New York, New York
Weber, Lee A., Ph.D., State University of New York, Albany
Wiegand, Roger C., B.A., Yale University, New Haven, Connecticut

SEMINARS

Pollack, R., State University of New York, Stony Brook. *Cell growth and behavior.*
Beard, P., Swiss Institute for Experimental Cancer Research. *Papovavirus growth and cell transformation.*
Shatkin, A., Roche Institute. *Reovirus.*
Diggelmann, H., Swiss Institute for Experimental Cancer Research. *RNA tumor virus replication.*
Wimmer, E., State University of New York, Stony Brook. *Poliovirus.*
Stark, G., Stanford University. *Papovavirus tumor antigen.*
Lilly, F., Albert Einstein College of Medicine. *Genetic factors in murine viral leukemia.*
Hynes, R., Massachusetts Institute of Technology. *Cell surfaces of normal and transformed cells.*
Eckhart, W., Salk Institute. *Genetics of papovaviruses.*
Sambrook, J., Cold Spring Harbor Laboratory. *Genetics of adenovirus.*
Lewis, J., Cold Spring Harbor Laboratory. *Synthesis of adenovirus RNA and proteins in vivo and in vitro.*
Ward, D., Yale University. *Parvoviruses.*
Lenard, J., Rutgers University. *Envelopes of myxo and paramyxoviruses.*
Kolakofski, D., University of Utah. *Replication of myxo and paramyxovirus RNA.*
Joklik, W.K., Duke University. *Vaccinia virus.*
Pizer, L., University of Pennsylvania. *Herpesviruses.*
Sugden, W., University of Wisconsin. *Epstein-Barr virus.*
Davis, R., Cold Spring Harbor Laboratory. *In vitro recombination of DNA.*

EXPERIMENTAL TECHNIQUES IN NEUROBIOLOGY, July 6–July 30

INSTRUCTORS

Kehoe, JacSue, Ph.D., École Normale Superieure, Paris, France
Chiarandini, Dante, M.D., New York University, New York, New York
Stefani, Enrique, M.D., Instituto Politecnico Nacional, Mexico City, Mexico
Kado, Ray, Ph.D., Centre National de la Recherche Scientifique, Gif-sur-Yvette, France

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

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

PARTICIPANTS

Gaathon, Ariel, Ph.D., Columbia University, New York, New York
Gaensler, Karin, Harvard University, Cambridge, Massachusetts
Greenspan, Ralph, B.A., Brandeis University, Waltham, Massachusetts
Ko, Patrick, B.S., McGill University, Montreal, Quebec, Canada
Maxell, Gerald, Ph.D., Harvard Medical School, Boston, Massachusetts
Nass, Menasche, Ph.D., Brown University, Providence, Rhode Island
Reiness, C. Gary, Ph.D., Harvard Medical School, Boston, Massachusetts
Schatz, Genevieve, B.S., University of Geneva, Geneva, Switzerland
Schneps, Sue, B.S., Massachusetts Institute of Technology, Cambridge
Walicke, Patricia A., B.S., Harvard Medical School, Boston, Massachusetts

MOLECULAR BIOLOGY AND GENETICS OF YEAST, July 28 – August 17

INSTRUCTORS

Fink, Gerald, Ph.D., Cornell University, Ithaca, New York
Sherman, Fred, Ph.D., University of Rochester, Rochester, New York
Petes, Thomas, Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts

ASSISTANT

Hicks, Jim, Ph.D., Cornell University, Ithaca, New York

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

Studies on chromosomal and extrachromosomal DNA were pursued by electron microscopy. The cell division cycle was studied by fluorescence microscopy. These new techniques were integrated with genetic analysis in order to study the cell cycle.

PARTICIPANTS

Cross, Heide S., M.S., University of Wien, Wien, Austria
DeAngelo, Joseph, B.S., Massachusetts Institute of Technology, Cambridge
Foor, Forrest, Ph.D., Massachusetts Institute of Technology, Cambridge
Fukasawa, Toshio, Ph.D., Keio University, Tokyo, Japan
Gancedo, Juana M., Ph.D., Harvard Medical School, Boston, Massachusetts
Goewert, Robert, B.S., St. Louis University, St. Louis, Missouri
Hixon, Sharon, Ph.D., Fondation Curie, Orsay, France
Koh, Youh T., Ph.D., University of Basel, Basel, Switzerland
Miller, Mark J., Ph.D., University of California, San Diego
Rouslin, William, Ph.D., Rutgers University, New Brunswick, New Jersey
Schekman, Randy, Ph.D., University of California, La Jolla
Sinha, Navin K., Ph.D., Princeton University, Princeton, New Jersey
Solioz, Marc, Ph.D., University of Basel, Basel, Switzerland
Stephens, John, B.S., University of California, Irvine
Tatti, Kathleen, B.S., Wayne State University, Detroit, Michigan
Tye, Bik-Kwoon, Ph.D., Stanford University, Stanford, California

SEMINARS

Davis, R., Stanford University. *Cloning of yeast DNA.*
Hartwell, L. H., University of Washington. *Cell cycle of yeast.*
———. *Control of cell division in yeast.*
Byers, B., University of Washington. *Cytology of the yeast life cycle.*
Esposito, M. S., University of Chicago. *Genetic recombination and genes controlling sporulation of yeast.*
Petes, T. D., Massachusetts Institute of Technology. *Structure and replication of yeast RNA.*
Rosbash, M., Brandeis University. *Classes of yeast mRNA, especially ribosomal protein mRNA.*
McLaughlin, C. S., University of California, Irvine. *Mutations and antibiotics that affect protein synthesis in yeast.*
Mortimer, R. K., University of California, Berkeley. *Genetic mapping in yeast.*
———. *Gene conversion and postmeiotic segregation.*
Fink, G. R., Cornell University. *The yeast killer factor.*
Sherman, F., University of Rochester. *Nonsense suppression in yeast.*
———. *Regulation of the iso-cytochromes c.*
Butow, R. A., University of Texas, Dallas. *Genetic and biochemical aspects of mitochondrial biogenesis.*
Robinow, C. F., University of Western Ontario. *Light microscopy of nuclear behavior in yeasts.*
Marmur, J. and R. Needleman, Albert Einstein College of Medicine. *Studies of the yeast mitochondrial genome.*
Warner, J., Albert Einstein College of Medicine. *Are syntheses of ribosomal RNA and of ribosomal protein related?*

IMMUNOGENETICS AND TUMOR IMMUNOLOGY, July 28 – August 17

INSTRUCTORS

Fleissner, Erwin, Ph.D., Sloan-Kettering Institute, New York, New York
Nowinski, Robert, Ph.D., Fred Hutchinson Cancer Center, Seattle, Washington

ASSISTANTS

Ledbetter, Jeff, B.S., Fred Hutchinson Cancer Center, Seattle, Washington
Ellis, Ron, B.S., Sloan-Kettering Institute, New York, New York

The course focused on current research in immunogenetics as it relates to differentiation, tumor biology, and host-virus interactions, primarily in the mouse. Among the topics covered were cellular immunology, differentiation of lymphoid cells in vivo and in vitro, the H-2 and TL loci, the G_{IX} system, the T locus, and the physiology and genetics of type-C viruses. Approaches at the molecular level were stressed, particularly with respect to genetic specification of normal and malignant cell surfaces.

The course consisted of lectures by instructors and invited speakers, discussions, and laboratory experiments in tissue-culture systems and in specially constructed genetic crosses between inbred mouse strains.

PARTICIPANTS

Barnes, David, B.A., Vanderbilt University, Nashville, Tennessee
Baron, Diethard, Ph.D., Sidney Farber Cancer Center, Boston, Massachusetts
Brown, Joseph, Ph.D., Fred Hutchinson Cancer Center, Seattle, Washington
Burnette, Neal, Ph.D., Albert Einstein College, Bronx, New York
Chen, Betty, Ph.D., Johns Hopkins University, Baltimore, Maryland
Ganguly, Asok, Ph.D., National Institutes of Health, Bethesda, Maryland
Harrison, Earl, Ph.D., Columbia University, New York, New York
Klitzman, Jack, M.S., Fred Hutchinson Cancer Center, Seattle, Washington
Lewis, John, B.S., Sloan-Kettering Institute, New York, New York
Mastro, Andrea, Ph.D., Penn State University, University Park, Pennsylvania
Novis, Peter, Ph.D., Swiss Institute, Lausanne, Switzerland
Pauli, Georg, Ph.D., Institut Virologie, Giessen, Federal Republic of Germany
Pratt, Diane, M.A., Harvard Medical School, Boston, Massachusetts
Torano, Alfredo, Ph.D., Indiana University, Bloomington.

SEMINARS

Nowinski, R., Fred Hutchinson Cancer Center. *Basic aims and techniques of cell-surface immunogenetics.*
Lilly, F., Albert Einstein College of Medicine. *Genetic control of viral leukemogenesis in mice.*
Hämmerling, U., Sloan-Kettering Institute. *Cellular basis of humoral immunity.*
Bach, F., University of Wisconsin. *Cell-mediated immunity.*
Klein, J., University of Texas. *The major histocompatibility antigens.*
Cunningham, B., Rockefeller University. *Structures of immunoglobulins and histocompatibility antigens.*
Goldstein, G., Sloan-Kettering Institute. *Physiology of lymphoid cell differentiation.*
Waelsch, S., Albert Einstein College of Medicine. *The T locus.*
Martin, G., University of California at San Francisco. *Teratomas as models for differentiation.*
Flaherty, L., New York State Health Department. *The W and steel loci.*
Schachner, M., Harvard University. *Differentiation antigens of the nervous system.*
Scharff, M., Albert Einstein College of Medicine. *Somatic cell genetics of immunoglobulin production.*
Zinkernagel, R., Scripps Clinic. *Histocompatibility restriction in cell-mediated cytotoxicity.*
Hellstrom, I., Fred Hutchinson Cancer Center. *Mechanisms of tumor rejection.*
Fleissner, E., Sloan-Kettering Institute. *Biology of RNA tumor viruses.*
Vogt, P., University of Southern California. *Genetics of RNA tumor viruses.*
Duesberg, P., University of California, Berkeley. *Structure of RNA tumor virus genomes.*
Fleissner, E., Sloan-Kettering Institute. *Proteins of murine leukemia viruses.*
Stephenson, J., National Institutes of Health. *Polymorphism of murine leukemia viruses.*
Jaenisch, R., Salk Institute. *Experimental integration of leukemia virus genes in a mouse germ line.*
Nowinski, R., Fred Hutchinson Cancer Center. *Natural immunity to leukemia viruses and leukemia cells.*
Lerner, R., Scripps Institute. *Physiology of leukemia virus protein expression in normal mouse tissues.*

PHYCOMYCES, August 2 – August 23

INSTRUCTORS

Delbrück, Max, Ph.D., Sc.D., California Institute of Technology, Pasadena
Sutter, Richard P., Ph.D., University of West Virginia, Morgantown
Cerdá-Olmedo, Enrique, Ph.D., University of Seville, Seville, Spain
Cohen, Robert J., Ph.D., University of Florida, Gainesville
Lipson, Edward D., Ph.D., California Institute of Technology, Pasadena

ASSISTANT

Presti, David, M.S., California Institute of Technology, Pasadena

This course was designed for graduate students and research workers interested in the cellular and molecula biology of signal handling. Lectures, discussions, and laboratory exercises dealt with *Phycomyces,* a single celled, multinucleate organism which responds to a variety of environmental stimuli.

Experimental work included measurements of responses to light, gravity, obstacles, and wind; isolation o mutants; phenotypic characterization of mutants; qualitative and quantitative complementation, usin

heterokaryons produced by microsurgical fusion; genetic crosses and recombination; and biochemical variables (cAMP, chitin synthetase) related to the sensory transduction processes.

PARTICIPANTS

Bacher, Adelbert, M.D., Universität Hohenheim, Stuttgart, Federal Republic of Germany
Gauger, Wendell L., Ph.D., University of Nebraska, Lincoln
Koman, Ahmet S., B.S., Uppsala University, Uppsala, Sweden
Lapointe, David S., B.A., University of Florida, Gainesville
Lopez-Diaz, Isabel, M.S., University of Seville, Seville, Spain
Radin, David M., Ph.D., University of California, Berkeley
Torres-Martinez, Santiago, M.S., University of Seville, Seville, Spain

SEMINARS

Cerdá-Olmedo, E., University of Seville. *Mutagenesis of coenocytic organisms.*
———. *Mutants of* Phycomyces.
———. *Qualitative and quantitative complementation.*
———. *Phenotypic and genetic classification of behavioral mutants.*
———. *Nuclear interaction and recombination in the sexual cycle.*
Lipson, E., California Institute of Technology. *Phototropism and the light growth response.*
———. *Other sensory responses.*
———. *Adaptation.*
Cohen, R., University of Florida. *The avoidance response.*
Delbrück, M., California Institute of Technology. *The photoreceptor.*
Sutter, R., University of West Virginia. *Regulation of the first stage of sexual development.*

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

ORIGINS OF LYMPHOCYTE DIVERSITY

Some ten years ago the concept that antibody specificity resided in precise amino acid sequences became generally accepted. Serious thought then began as to whether this meant that a separate germ-line gene would exist for each of the very large number of light and heavy immunoglobulin chains that are used to construct the many thousands of different antibodies that a single vertebrate animal is capable of generating. Though much discussion on this topic occurred during our 1969 Symposium on antibodies, it was clear then that many new facts had to be obtained before the genetic origin(s) of antibody diversity would be understood.

Over the past few years many of the experimental methodologies necessary to give us these facts have emerged. Nucleic acid hybridization techniques, for example, now provide a direct way to count gene copies. So we thought the time was again ripe for a Symposium to discuss the diversity question and hopefully this time to come up with firm answers—but with the qualification that the answers might not be as simple as we once guessed.

Now the immunological response is no longer regarded as the simple calling into action by antigens of lymphocytes already predetermined for the synthesis of single antibodies. Not only are lymphocytes divisible into the broad B and T categories, but the B-cell response generally requires some form of interaction with the T lymphocyte. For reasons which are still unclear, a diversification of the lymphocytes themselves is necessary for most immunological responses. In this process, key roles are played by immune response genes that are coded by the same gene complex responsible for the major histocompatibility surface antigens.

We thus suspect that we may not understand antibody diversity at a fundamental level until we understand more deeply the underlying cellular processes which generate so many lymphocyte classes, each capable of finely tuned interaction with the others. So we decided to use this Symposium to discuss antibody diversity within the much broader field of lymphocyte diversity. This meant, however, that we would be looking over almost all of immunology, and that we would be bringing together people with widely different objectives and vocabularies. Choosing the right speakers was not simple, and we are much indebted for advice given by Baruj Benacerraf, Gerald Edelman, Herman Eisen, Avrion Mitchison, Martin Raff, and Matthew Scharff. The final program witnessed 87 formal presentations before a total audience of 326, most of whom stayed for the entire week.

As in the past, key financial support was provided by the National

Institutes of Health, the National Science Foundation, and the United States Energy Research and Development Administration. The very large number of speakers from abroad required still additional sources of funds, and we are most indebted to Merck Sharp and Dohme Research Laboratories and Hoffmann-La Roche, Inc., for their generous responses to our last-minute requests for assistance.

TUESDAY EVENING, June 1

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

Opening address: N.K. Jerne, Basel Institute for Immunology, Basel, Switzerland

LYMPHOCYTE FUNCTION

WEDNESDAY MORNING, June 2

Session 1: T-cell markers and differentiation

Chairperson: R. Good, Memorial Sloan-Kettering Cancer Center; New York, New York

G. Goldstein, M. Scheid, E.A. Boyse, A. Brand*, and D. Gilmour*, Memorial Sloan-Kettering Cancer Center; *New York University Medical Center, New York: Thymopoietin and bursopoietin: Induction signals regulating early lymphocyte differentiation.

I.L. Weissman, Department of Pathology, Stanford University Medical Center, California: Thymus and T-cell maturation.

H. Cantor and E.A. Boyse*, Department of Medicine, Harvard Medical School, Sidney Farber Cancer Center, Boston, Massachusetts; *Memorial Sloan-Kettering Cancer Center, New York: Regulation of the immune response by subclasses of T lymphocytes.

L.A. Herzenberg, L.A Herzenberg, R.D. Stout, K. Okumura, and M.R. Loken, Department of Genetics, Stanford University School of Medicine, California: Membrane antigens and receptors of the cells in immune response networks.

A.B. Gottlieb, M. Engelhard, and H.G. Kunkel, The Rockefeller University, New York: An unusual Ig (kappa) antigen on the membrane of T and B lymphocytes.

A.F. Williams, A.N. Barclay, M. Letarte-Muirhead, and R.J. Morris, MRC Immunochemistry Unit, Department of Biochemistry, Oxford University, England: Purification, chemical composition, and tissue distribution of the rat Thy-1 antigens.

D. Baltimore, P. Kung, A. Silverstone, T. Harrison, and R. McCaffrey, Center for Cancer Research, MIT, Cambridge, Massachusetts: Specialized DNA polymerases in lymphoid cells.

B.R. Bloom, Albert Einstein College of Medicine, New York: Interactions of viruses with lymphocytes.

WEDNESDAY EVENING, June 2

Session 2: B-cell differentiation and commitment

Chairperson: E. Diener, MRC Transplantation Group, University of Alberta, Edmonton, Alberta, Canada

J.T. Owen, Department of Anatomy, University of Newcastle upon Tyne Medical School, England: In vitro studies of T and B lymphocyte maturation in the mouse embryo.

M.D. Cooper, J.F. Kearney, P.M. Lydyard, C.E. Grossi, and A.R. Lawton, Departments of Pediatrics and Microbiology, University of Alabama, Birmingham: Comparative analysis of B-cell generation in mouse, man, and chicken.

Melchers and R.A. Phillips*, Basel Institute for Immunology, Switzerland; *Ontario Cancer Institute, Toronto, Canada: Immunoglobulin synthesis and turnover and mitogen reactivity of murine B lymphocytes during ontogeny.

Pernis, Basel Institute for Immunology, Switzerland: Synthesis of multiple immunoglobulin classes by single lymphocytes.

E.S. Vitetta, J. Cambier, J. Kettman, J. Forman, M.K. Sawyer, D. Spiva, and J.W. Uhr, Department of Microbiology, University of Texas Southwestern Medical School, Dallas: Functional properties of subsets of B lympbocytes bearing different receptor isotypes.

N.R. Klinman, N.H. Sigal, E.S. Metcalf, P.J. Gearhart, and S.K. Pierce, Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia: The interplay of evolution and environment in B-cell diversification.

J.J. Oebra, P.H. Gearhart, R. Kamat, S.M. Robertson, and J. Tseng, Department of Biology, The Johns Hopkins University, Baltimore, Maryland: Origin and differentiation of lymphocytes involved in the secretory IgA response.

J. Andersson, A. Coutinho, F. Melchers, and T. Watanabe, Department of Immunology, University of Uppsala, Sweden, and Basel Institute for Immunology, Switzerland: Growth and maturation of lymphocytes in culture.

P.W. Kincade and P. Ralph, Sloan-Kettering Institute for Cancer Research, Rye, New York: Regulation of clonal B-lymphocyte proliferation by anti-immunoglobulin or anti-Ia antibodies.

THURSDAY MORNING, June 3

Session 3A: Helper and suppressor T cells and their products

Chairperson: B.H. Waksman, Yale University School of Medicine, New Haven, Connecticut

R.K. Gershon, D.D. Eardley, K.F. Naiford, and W. Ptak, Department of Pathology, Yale University School of Medicine, New Haven, Connecticut: Factors involved in the generation of antigen-specific suppressor T cells in vitro.

A. Basten, R. Loblay, and E. Chia, Immunology Unit, University of Sydney, Australia: T-cell-dependent influences in antibody production.

H.N. Claman and J.W. Moorhead, Departments of Medicine and Microbiology, University of Colorado Medical School, Denver: Tolerance—Two pathways of negative immunoregulation in contact sensitivity to DNFB.

M. Feldmann, P.C.L. Beverley, M. Dunkley, P. Erb, S. Howie, A. Maoz, M. Mathies, I. McKenzie*, and J. Woody, ICRF Tumor Immunology Unit, Department of Zoology, University College, London; Department of Medicine, Austin Hospital, Victoria, Australia: Heterogeneity of T lymphocytes—Relationship to cell interactions in antibody production in vitro.

T. Tada and M. Taniguchi, Laboratories for Immunology, School of Medicine, Chiba University, Japan: The suppressive T-cell factor as an I-region gene product: Properties and the subregion assignment.

Session 3B: B-cell activation and tolerance

Chairperson: N.A. Mitchison, University College, London, England

G. Möller, Division of Immunobiology, Wallenberg Laboratory, Karolinska Institute, Stockholm, Sweden: The mechanism of B-cell activation.

G.J.V. Nossal, J.W. Stocker, B.L. Pike, and J.W. Goding, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia: Hapten-specific B lymphocytes—Enrichment, cloning, receptor analysis, and tolerance induction.

M.C. Raff, MRC Neuroimmunology Project, Department of Zoology, University College, London, England: Immunoglobulin receptors for antigen on B lymphocytes.

THURSDAY EVENING, June 3

Session 4: Receptors

Chairperson: H. Eisen, Massachusetts Institute of Technology, Cambridge, Massachusetts

J.J. Marchalonis, J.M. Decker. D. DeLuca, J.W. Goding, J.M. Moseley, and G.W. Warr, The Walter and Eliza Hall Institute, Parkville, Victoria, Australia: Lymphocyte surface immunoglobulin—Evolutionary origins and role in activation.

H. Binz and H. Wigzell, Department of Immunology, Uppsala University, Sweden: Naturally occurring antigen-binding, idiotypic T-cell receptors.

K. Eichmann, C. Berek, G. Hämmerling, S. Black, and K. Rajewsky, Institute for Immunology and Genetics, German Cancer Research Center, Heidelberg, and Institute for Genetics, University of Cologne, Federal Republic of Germany: Genetic control of T- and B-cell receptor specificity in the mouse.

K. Rajewsky, U. Krawinkel, G.J. Hammerling, K. Eichmann, M. Cramer, S.J. Black, and C. Berek, Institute for Genetics, University of Cologne, Federal Republic of Germany: Receptors and receptor interactions in the immune system.

M. Nabholz and V. Miggiano, Basel Institute for Immunology, Switzerland: The generation of T-cell specificity—A hypothesis.

D.H. Sachs, J.A. Berzofsky, A.N. Schechter, C.G. Fathman, D.S. Pisetsky, and R.H. Schwartz, National Institutes of Health, Bethesda, Maryland: The immune response to staphylococcal nuclease—A probe of cellular and humoral antigen-specific receptors.

P.A. Cazenave, D. Juy, and C. Bona, Institut Pasteur, Paris, France: Functional characterization of rabbit lymphocytes carrying Fc receptor.

H.M. Grey, C. Anderson, K. von Eschen, C. Heusser, and J. Chiller, National Jewish Hospital and Research Center, Denver, Colorado: Structural and functional heterogeneity of Fc receptors.

THE MAJOR HISTOCOMPATIBILITY COMPLEX

FRIDAY MORNING, June 4

Session 5: Structure of molecular products

Chairperson: O. Smithies, University of Wisconsin, Madison, Wisconsin

J. Bridgen, C. Barnstable*, W.F. Bodmer*, M.J. Crumpton†, P. Goodfellow*, and D. Snary†, Laboratory of Molecular Biology, Cambridge; *Genetics Laboratory, Oxford; †National Institute for Medical Research, London, England: Isolation and structure of histocompatibility (HL-A) antigens.

B.A. Cunningham, R. Henning, R.J. Milner, K. Reske, J.A. Ziffer, and G.M. Edelman, The Rockefeller University, New York: Structure of murine histocompatibility antigens.

S.G. Nathenson, J.L. Brown, B.M. Ewenstein, J.H. Freed, and D.W. Sears, Department of Microbiology and Immunology and Department of Cell Biology, Albert Einstein College of Medicine, New York: Studies on the structural properties of H-2K and H-2D transplantation antigens.

J.L. Strominger, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Biochemistry of products of the major histocompatibility complex on the human lymphocyte surface.

J. Silver, M. Cecka, M. McMillan, and L. Hood, Division of Biology, California Institute of Technology, Pasadena: Chemical characterization of products of the H-2 complex.

J.W. Uhr, H.D. Capra, E.S. Vitetta, J. Klein, D.G. Klapper, K. Artzt, E.A. Boyse, D. Bennett, and F. Jacob, Department of Microbiology, University of Texas Southwestern Medical School, Dallas; Department of Anatomy, Cornell University Medical College, New York; Immunology Division, Memorial Sloan-Kettering Cancer Center, New York; Laboratoire de Genetique Cellulaire, Institut Pasteur et College de France, Paris: Primary structure of products of chromosome 17 in the mouse.

L. Östberg, L. Rask, and P.A. Peterson, Institute of Medical and Physiological Chemistry, Biomedical Center, University of Uppsala, Sweden: β_2-Microglobulin and associated cell-surface antigens.

FRIDAY EVENING, June 4

Session 6: Genetics of the MHC and associated products

Chairperson: H.O. McDevitt, Stanford University School of Medicine, Stanford, California

D.C. Shreffler, C.S. David, S.E. Cullen, J.E. Neiderhuber, and J.A. Frelinger, Department of Genetics, Washington University, St. Louis, Missouri; Immunology Branch, National Cancer Institute, Bethesda, Maryland; Department of Microbiology, University of Michigan, Ann Arbor; Department of Microbiology, University of Southern California at Los Angeles: Serological and functional subdivision of the *I* region of the H-2 complex.

J. Klein, V. Hauptfeld, and M. Hauptfeld, Department of Microbiology, University of Texas Southwestern Medical School, Dallas: Number and relationship of H-2 loci coding for Ia and histocompatibility antigens.

F.H. Bach, M.L. Bach, B.J. Alter, H.W. Sollinger, and O. Kuperman, Immunobiology Research Center, University of Wisconsin, Madison: Major histocompatibility antigens in allograft immunity.

W.F. Bodmer, C. Barnstable. J.G. Bodmer, E. Jones, B. Arce-Gomez, D. Snary*, and M.J. Crumpton*, Genetics Laboratory, University of Oxford; National Institute for Medical Research, London, England: Genetics and serology of human HL-A linked Ia antigens.

B. Benacerraf, M.E. Dorf, J.A. Kapp, and J. Thèze, Department of Pathology, Harvard Medical School, Boston, Massachusetts: The control of specific immune responses and specific immune suppression by *I*-region genes.

R. Hyman and I. Trowbridge. The Salk Institute for Biological Studies, San Diego, California: Somatic genetic studies on cell-surface-antigen variants.

SATURDAY MORNING, June 5

Session 7: Role of histocompatibility gene products in T-cell cytolysis

Chairperson: J.F.A.P. Miller, The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia

R.M. Zinkernagel and P. C. Doherty*, Scripps Clinic and Research Foundation, La Jolla, California: *The Wistar Institute, Philadelphia, Pennsylvania: H-2 antigens and specificity of virus-immune T cells—Altered-self or physiological interaction?

G.M. Shearer, A.M. Schmitt-Verhulst, T.G. Rehn, and G. Cudkowicz, Immunology Branch, National Cancer Institute, Bethesda, Maryland, and Department of Pathology, State University of New York, Buffalo: Generation of F_1 hybrid anti-parent cytotoxicity in vitro.

M.J. Bevan, The Salk Institute for Biological Studies, San Diego, California: Recognition of minor histocompatibility antigens by cytotoxic T lymphocytes.

U. Kozinowski and H. Ertl, Institute of Microbiology, University of Göttingen, Federal Republic of Germany: Recognition of early vaccinia surface antigens by cytotoxic T lymphocytes (CTL) and dependence of syngeneic target cell lysis on SD antigen concentration.

H. von Boehmer, J. Sprent, and W. Hass, Basel Institute for Immunology, Switzerland: The influence of H-2 gene products on cell-mediated and humoral immune responses.

J.W. Schrader, R. Henning, R.J. Milner, and G.M. Edelman, The Rockefeller University, New York: Interaction of viral and H-2 antigens at cell surfaces.

SATURDAY EVENING, June 5

Session 8: Expression of Ia antigens

Chairperson: D.C. Shreffler, Washington University, St. Louis, Missouri

H.O. McDevitt, T.L. Delovitch, and J.L. Press, Division of Immunology, Department of Medicine, Stanford University School of Medicine, California: Cellular expression of Ia antigens.

D.B. Murphy, K. Okumura, L.A. Herzenberg, and H.O. McDevitt, Division of Immunology, Department of Medicine, Stanford University School of Medicine, California: A Unique set of *I*-region-controlled determinants on T but not B lymphocytes.

J.J. van Rood, A. van Leeuwen, M. Jonker, J. Keuning, and B. Bradley, Department of Immunohaematology, University Hospital, Leiden, The Netherlands: Polymorphic B-cell determinants in man.

SUNDAY MORNING, June 6

Session 9: Antigen presentation and T-B-cell interactions

Chairperson: B. Benacerraf, Harvard Medical School, Boston, Massachusetts

C.W. Pierce, J.A. Kapp, and B. Benacerraf, Department of Pathology, Harvard Medical School, Boston,

Massachusetts: Genetic restrictions in macrophage–lymphoid cell interactions in antibody responses in vitro.

W.E. Paul, E.M. Shevach, S. Pickeral, and A.S. Rosenthal, NIAID, National Institutes of Health, Bethesda, Maryland: Genetic restriction in T-lymphocyte activation by antigen-pulsed peritoneal exudate cells.

J.F.A.P. Miller and M.A. Vadas, Experimental Pathology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia: Activation of distinct T-cell subsets by different major histocompatibility complex gene products.

P. Lake and N.A. Mitchison, ICRF Tumour Immunology Unit, Department of Zoology, University College, London, England: Control of the immune response to cell-surface antigens.

S.L. Swain, P.E. Trefts, H. Tse, and R.W. Dutton, Department of Biology, University of California at San Diego: The significance of T-B collaboration across haplotype barriers.

D.H. Katz, Department of Pathology, Harvard Medical School, Boston, Massachusetts: The role of the histocompatibility gene complex in lymphocyte differentiation.

IMMUNOGLOBULINS

SUNDAY AFTERNOON, June 6

Session 10: Antibody combining site

Chairperson: E.A. Kabat, Columbia University, New York, New York

D.R. Davies and E.A. Padlan, NIAMDD, National Institutes of Health, Bethesda, Maryland: The three-dimensional structure of the antibody combining site.

R.J. Poljak, L.M. Amzel, B.L. Chen, Y.Y. Chiu, R.P. Phizackerley, and F. Saul, Department of Biophysics, The Johns Hopkins University School of Medicine, Baltimore, Maryland: Three-dimensional structure of immunoglobulins.

E. Haber, M.N. Margolies, and L.E. Cannon, Massachusetts General Hospital and Harvard Medical School, Boston: The combining site and framework structures of the variable domains of rabbit antipneumococcal polysaccharide antibodies.

M. Potter, S. Rudikoff, M. Vrana, and N.D. Rao, Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland: Primary structural differences in myeloma proteins that bind the same haptens.

D. Givol, M. Gavish, R. Zakut, and M. Wilcheck, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel: Synthetic approach to the problem of antibody diversity.

MONDAY MORNING, June 7

Session 11: Allotypes and idiotypes

Chairperson: R.M. Krause, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

R.G. Mage, G.O. Young-Cooper, J. Rejnek, A. Ansari, C. Alexander, E. Appella, M. Carta-Sorcini, C. Landucci-Tosi, and R. Tosi, NIAID and NCI, National Institutes of Health, Bethesda, Maryland, and Instituto Superiore Sanita and CNR Laboratory for Cell Biology, Rome, Italy: Rabbit immunoglobulin allotypes—Complexities of their genetics, expression, structural basis, and evolution.

B.A. Fraser, A.P. Johnstone, and T.J. Kindt, Laboratory of Immunology and Immunochemistry, The Rockefeller University, New York: Diversity in the rabbit antibody response to streptococcal immunization.

A. Nisonoff and S.-T. Ju, Department of Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts: Immune suppression of a cross-reactive idiotype and adoptive transfer fo private idiotypes by cells from spleen or ascites.

H. Cosenza, A.A. Augustin, and M.H. Julius, Basel Institute for Immunology, Switzerland: Involvement of idiotype and anti-idiotype interactions in immunoregulation.

J.L. Claflin, Department of Microbiology, University of Michigan, Ann Arbor: Uniformity in a clonal repertoire—A case for a germ-line basis of antibody diversity.

O. Mäkelä, K. Karjalainen, M. Julin, and A. Cattan, Department of Serology and Bacteriology, University of Helsinki, Finland: Characterization of V_H gene products by the fine-specificity and isoelectric focusing of antibodies.

W.R. Geckeler, B. Blomberg, C. de Preval, and M. Cohn, The Salk Institute for Biological Studies, San Diego, California: Response genetics to α-1.3 dextran in mice.

MONDAY AFTERNOON, June 7

Session 12: Generation of diversity. 1. Biological aspects

Chairperson: M. Cohn, The Salk Institute for Biological Studies, San Diego, California

B.A. Askonas, National Institute of Medical Research, London, England: Life style of B cells and the constancy of the Ig product during clonal expansion.

A.J. Cunningham, Department of Microbiology, John Curtin School, Australian National University, Canberra: Rapid variation among stimulated lymphocytes helps to generate the immune repertoire.

L. DuPasquier and M.R. Wabl, Basel Institute for Immunology, Switzerland: The ontogenesis of lymphocyte diversity in amphibians.

D.H. Margulies, B. Dharmgrongartama, S.L. Morrison, M.L. Geftner, W. Cieplinski, and M.D. Scharff, Department of Cell Biology, Albert Einstein College of Medicine, New York: Regulation of immunoglobulin expression in mouse myeloma cells.

C. Milstein, K. Adetugbo, D. Kerr, G. Köhler, and D.S. Secher, MRC Laboratory of Molecular Biology, Cambridge, England: Somatic cell genetics of antibody-secreting cells—Studies of clonal diversification and analysis by cell fusion.

T.M.D. Cotner and H.N. Eisen, Center for Cancer Research and Department of Biology, MIT, Cambridge, Massachusetts: Regulation of production of an immunoglobulin light chain.

P.D. Gottlieb and P.J. Durda, Center for Cancer Research and Department of Biology, MIT, Cambridge, Massachusetts: Genetic and structural studies of the I_B-peptide marker in mouse V_L regions and the genetically linked Ly-3 thymocyte surface antigen.

TUESDAY MORNING, June 8

Session 13: Generation of diversity. 2. Structural aspects

Chairperson: M.E. Koshland, University of California, Berkeley, California

L. Hood, P. Barstad, B. Eaton, V. Farnsworth, E. Loh, J. Hubert, J. Silver, and M. Weigert, Department of Biology, California Institute of Technology, Pasadena: Some genetic and evolutionary features of immunoglobulins and transplantation antigens.

R. Riblet and M. Weigert, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Diversity of mouse antibodies.

J.D. Capra, D.G. Klapper, A. Tung, and A. Nisonoff, Department of Microbiology, University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, and Rosenstiel Research Center, Brandeis University, Waltham, Massachusetts: Identical hypervariable regions in light chains of differing V_κ subgroups.

P. Leder, D. Swan, T. Honjo, and J. Seidman, NICHD, National Institutes of Health, Bethesda, Maryland: The origin of antibody diversity—Evidence from hybridization studies.

G.P. Smith, University of Missouri, Columbia: Significance of hybridization kinetic experiments for theories of antibody diversity.

S. Tonegawa, N. Hozumi, G. Matthyssens, and R. Schuller, Basel Institute for Immunology, Switzerland: Somatic changes in content and context of immunoglobulin genes.

Summary: G. Edelman, The Rockefeller University, New York, New York

SUMMER MEETINGS

DICTYOSTELIUM

arranged by
HARVEY LODISH, *Massachusetts Institute of Technology*
WILLIAM LOOMIS, *University of California at San Diego*

123 participants

WEDNESDAY EVENING, May 12

Session 1: Genes and their transcripts

Chairperson: J. Gross, Imperial Cancer Research Fund Laboratories, London, England

A. Cockburn, G. Frankel, R.A. Firtel, and M.J. Newkirk, Department of Biology, University of California at San Diego, La Jolla: Structural organization of *Dictyostelium* ribosomal genes.

N. Maizels, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Using cloned DNA to look at the structure of *Dictyostelium's* genome.

R.P. Dottin, A.M. Weiner, and H.F.Lodish, Massachusetts Institute of Technology, Cambridge: 5′-Terminal nucleotide sequences of the messenger RNAs of *Dictyostelium discoideum*.

A.M. Weiner, R.P. Dottin, and H.F. Lodish, Massachusetts Institute of Technology, Cambridge: The relationship between 5′-terminal blocking groups and messenger RNA processing in *Dictyostelium discoideum*.

K. Kindle, R.A. Firtel, W. Rowekamp, and G. Frankel, Department of Biology, University of California at San Diego, La Jolla: Transcription of single-copy genes in *Dictyostelium*.

A. Jacobson, C.D. Lane*, T. Alton†, A. Goldin, M. Rosbash**, C. Palatnik, B. Beck, R. Davis‡, M. Thomas‡, C. Mabie, and C. Wilkins, University of Massachusetts Medical Center, Worcester; *Medical Research Council, Mill Hill, London, England; †Massachusetts Institute of Technology, Cambridge; **Brandeis University, Waltham, Massachusetts; ‡Stanford University, Palo Alto, California: Fractionation and purification of slime mold RNAs and their homologous genomic DNAs.

C. Mabie, K. Lowney, M. Koomey, C. Wilkins, T. Pedersen*, A. Goldin, and A. Jacobson, University of Massachusetts Medical School, Worcester; *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: In vitro transcription of the *Dictyostelium* genome.

B.B. Young and H.F. Lodish, Massachusetts Institute of Technology, Cambridge: Characterization of rRNA precursors in *Dictyostelium discoideum*.

Session 2: Biochemical differentiation and pattern formation

Chairperson: M. Sussman, University of Pittsburgh, Pittsburgh, Pennsylvania

B.E. Wright, Boston Biomedical Research Institute, Massachusetts: Enzyme expression in *Dictyostelium discoideum*.

W.F. Loomis and S.R. Thomas, Department of Biology, University of California at San Diego, La Jolla: Computer modeling of biochemical differentiation.

I. Takeuchi, Department of Botany, Kyoto University, Japan: Cell differentiation in *Dictyostelium*, with and without morphogenesis.

P. Farnsworth, Department of Biology, University of California at San Diego, La Jolla: Quantitation of the spatial distribution of prespore vacuoles in pseudoplasmodia.

C.D. Town, R.R. Kay, and J.D. Gross, Imperial Cancer Research Fund, London, England: Analysis of stalk cell induction by cyclic AMP and the isolation of mutants with an altered response.

C.L. Rutherford, J.F. Harris, B.L. Jefferson, and J.B. Wilson, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg: Cell-specific events occurring during differentiation of *Dictyostelium discoideum*.

M.A. Clark, University of Texas, Arlington: Nonrandom distribution of wild-type cells in mixed populations of cellular slime molds.

K.A. Killick, Boston Biomedical Research Institute, Massachusetts: The effect of endogenous polyribonucleotide phosphorylase activity on the assay of trehalose-6-P synthetase from myxamoebae of *Dictyostelium discoideum*.

J. Sternfeld, Department of Biology, Princeton University, Princeton, New Jersey: Differentiation of *Dictyostelium* in submerged cultures.

THURSDAY EVENING, May 13

Session 3: Genetics and gene products

Chairperson: K. Raper, University of Wisconsin, Madison, Wisconsin

M.A. Wallace and K.B. Raper, Department of Bacteriology, University of Wisconsin, Madison: The bowre of blisse: Development of macrocysts in *Dictyostelium discoideum*.

R.L. Dimond and W.F. Loomis, Massachusetts Institute of Technology, Cambridge; University of California at San Diego, La Jolla: The role of β-glucosidase activity and relationships between the isozymes.

D.L. Welker and R.A. Deering, Department of Biochemistry and Biophysics, Pennsylvania State University, University Park: Genetic analysis of radiation-sensitive mutations in the slime mold.

T. Muroyama, Y. Hashimoto, M. Sameshima, and T. Yamada, Genetics Laboratory, Tokyo Metropolitan Isotope Research Institute, Tokyo, Japan: The chromosome of the cellular slime molds.

R.C. Higgins and M.E. Dahmus, Department of Biochemistry and Biophysics, University of California at Davis: Analysis of protein synthesis during development of *Dictyostelium discoideum*.

T. Alton and H.F. Lodish, Massachusetts Institute of Technology, Cambridge: Analysis of the pattern of protein synthesis during development of *discoideum* and assay of the specific mRNAs by cell-free translation.

S.J. Free*, R.T. Schimke†, and W.F. Loomis†, *Stanford University, California; †University of California at San Diego, La Jolla: The structural gene for α-mannosidase-1 in *Dictyostelium discoideum*.

T. Pederson, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Purification and composition of *Dictyostelium* chromatin.

FRIDAY MORNING, May 14

Session 4: Membranes

Chairperson: H. Hohl, University of Zurich, Zurich, Switzerland

B.H. Long and E.L. Coe, Biochemistry Department, Northwestern University Medical School, Chicago, Illinois: Fatty acid composition of lipids from vegetative cells and mature sorocarps of *Dictyostelium discoideum*.

G. Weeks, Department of Microbiology, University of British Columbia, Vancouver, Canada: Manipulation of the fatty acid composition of *Dictyostelium discoideum* and its effect on differentiation.

K.L. Poff and M. Skokut, MSU/ERDA Plant Research Laboratory, Michigan State University, East Lansing: Thermosensory transduction by *Dictyostelium discoideum*.

.D. Rosen, P.C. Haywood, and S.H. Barondes, Department of Psychiatry, University of California at San Diego, La Jolla: Isolation of receptor for pallidin, a cell-surface lectin from the cellular slime mold, *Polysphondylium pallidum*.

C.-H. Siu, S.T. Stolp, and R.A. Lerner, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: Expression of an endogenous lectin-ligand system during development of *Dictyostelium discoideum*.

H.R. Hohl, E. Wehrli, and M. Bühlmann, Institute of Plant Biology, Cytological Laboratory, University of Zurich, Switzerland: Ultrastructural and permeability changes of the plasma membrane following heat activation of *Dictyostelium discoideum*.

.E. Geltosky, C.-H. Siu, and R.A. Lerner, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: Con A binding proteins on the cell surface of *Dictyostelium discoideum* during development.

M.H. Hanna and E.C. Cox, Department of Biology, Princeton University, Princeton, New Jersey: Cell-surface changes in developing *Polysphondylium violaceum*.

.S. Eckert, R.W. Rubin, and R.H. Warren, Department of Biological Structure, University of Miami School of Medicine, Florida: Developmental changes in contractile protein content of *Dictyostelium* amoebae.

.C. Mockrin and J.A. Spudich, Department of Biochemistry, University of California at San Francisco: Control and assembly of contractile proteins from *Dictyostelium discoideum*.

FRIDAY AFTERNOON, May 14

Session 5: Growth

Chairperson: R. Deering, Pennsylvania State University, University Park, Pennsylvania

.T. Marin, Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island: Regulation of early development in *Dictyostelium discoideum* by amino acid starvation and cell contact.

.W. Parish and U. Müller, Institute of Plant Biology, Cytological Laboratory, University of Zurich, Switzerland: Studies on the phagocytic vacuoles of *Dictyostelium discoideum*.

. Kobilinsky, B.I. Weinstein, and D.S. Beattie, Department of Biochemistry, Mt. Sinai School of Medicine, New York, New York: Effects of ethidium bromide on mitochondrial biogenesis in the cellular slime mold *Dictyostelium discoideum*.

. Felenbok, F. Monier, M. Scrive, J. Leal, and J.F. Guespin-Michel, Institut de Microbiologie, Université de

Paris XI, Orsay, France: Effect of a thymidine analog, 5-bromodeoxyuridine, on *Dictyostelium discoideum* development.

M.D. Hanish, Biology Department, Hunter College, New York, New York: Effect of division inhibitor on macromolecular synthesis in *Dictyostelium discoideum* in vivo.

M. Sameshima, T. Muroyama, Y. Hashimoto, and T. Yamada, Tokyo Metropolitan Isotope Research Institute, Genetics Laboratory, Tokyo, Japan: The appearance of multinuclear cells in the life cycle of axenic strains of *Dictyostelium discoideum*.

H.M. Tsuchiya and J.F. Drake, Department of Chemical Engineering and Materials Science and Department of Microbiology, University of Minnesota, Minneapolis: Predation of *E. coli* by *Dictyostelium discoideum* in chemostat cultures.

R.A. Deering, Department of Biochemistry and Biophysics, Pennsylvania State University, University Park: Uptake of labeled precursors into nuclear and mitochondrial DNA during development of *Dictyostelium discoideum* NC-4: Changes induced by radiation.

J.H. Gregg and G.C. Karp, Department of Zoology, University of Florida, Gainesville: Incorporation of ^{3}H-fucose by *Dictyostelium discoideum*.

SATURDAY MORNING, May 15

Session 6: Aggregation

Chairperson: J. Bonner, Princeton University, Princeton, New Jersey

L. Kobilinsky and D.S. Beattie, Department of Biochemistry, Mt. Sinai School of Medicine, New York, New York: The induction of filopodia in *Dictyostelium discoideum* by cyclic adenosine monophosphate.

P. Wier, Department of Biology, Princeton University, New Jersey: The relation of cyclic AMP and its cell-bound phosphodiesterase to the duration of the interphase period in *Dictyostelium*.

P. Brachet, M. Darmon, C. Klein, J. Barra, P. Barrand, and L.H. Pereira da Silva, Department of Molecular Biology, Institut Pasteur, Paris, France: Intercellular communications and cell aggregation in *Dictyostelium discoideum*.

A.S. Tsang and M.B. Coukell, Department of Biology, York University, Downsview, Ontario, Canada: Genetic and biochemical evidence for the existence of multiple cAMP phosphodiesterases in *Dictyostelium purpureum*.

W.J. Kuo and E.L. Coe, Biochemistry Department, Northwestern University Medical School, Chicago, Illinois: Response of suspensions of *Dictyostelium discoideum* amoebae to additions of cAMP, cGMP, folic acid, and prostaglandins detected by light-scattering changes.

R.P. Futrelle, Department of Genetics and Development, University of Illinois, Urbana: Computer analysis of cell motion in *Dictyostelium* development. I. Simulations. II. The GALATEA film analysis system.

H.V. Rickenberg and H.J. Rahmsdorf, Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, and Department of Biophysics and Genetics, University of Colorado School of Medicine, Denver, Colorado: The inhibition of the formation of cAMP and of development in *Dictyostelium discoideum* by metabolizable sugars.

B. Wurster, Biozentrum der Universität Basel, Switzerland: Stimulation of cell development in *Dictyostelium discoideum* by folic acid pulses.

J. Sampson, Imperial Cancer Research Fund, Mill Hill, London, England: Protein kinase activity during the development of *Dictyostelium discoideum*.

E.F. Rossomando and M.A. Hesla, Department of Oral Biology, University of Connecticut, Farmington: Time-dependent changes in *Dictyostelium discoideum* adenylate cyclase activity upon incubation with ATP.

DNA INSERTIONS

arranged by
AHMAD I. BUKHARI, *Cold Spring Harbor Laboratory*
JAMES SHAPIRO, *University of Chicago*
SANKAR ADHYA, *National Institutes of Health*

126 participants

TUESDAY EVENING, May 18

Welcoming remarks: A. I. Bukhari, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Introduction: J. Shapiro, University of Chicago, Chicago, Illinois

Session 1: The IS-type sequences

Chairperson: M. Malamy, Tufts University, Boston, Massachusetts

H. Ohtsubo and E. Ohtsubo, Department of Microbiology, State University of New York at Stony Brook: Isolation of inverted repeat sequences, including IS1, IS2, and IS3 in the *E. coli* plasmids.

L.T. Chow, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: The organization of insertion sequences on the *E. coli* chromosome.

F. Schmidt, J. Besemer, and P. Starlinger, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: The isolation and characterization of IS1 and IS2 DNA.

H.-J. Reif and H. Saedler, Institut für Genetik der Universität Köln, Weyertal, and Institut für Biologie III, Universität Freiburg, Federal Republic of Germany: IS1-dependent deletion formation in the gel region of *E. coli.*

P. Nevers, H.-J. Reif, and H. Saedler, Institut für Biologie III, Universität Freiburg, Federal Republic of Germany: A mutant of *Escherichia coli* defective in IS1-mediated deletion formation.

H. Saedler, Institut für Biologie III, Universität Freiburg, Federal Republic of Germany: The role of the DNA elements IS1 and IS2 in the evolution of chromosomal and some plasmid DNAs.

A. Ahmed, Department of Genetics, University of Alberta, Edmonton, Alberta, Canada: Reversion of the *gal3* mutation of *E. coli.*

S. Mickel, E. Ohtsubo, and W. Bauer, Department of Microbiology, State University of New York at Stony Brook: Small plasmids generated from R-factor R12 by in vivo recombination at an IS1 sequence.

M. Van Montagu*†, Ch. Genetello†, G. Engler†, J.-P. Hernalsteens*, I. Zaenen†, N. van Larebeke†, A. de Picker†, and J. Schell*†, *Laboratorium Genetische Virologie, Vrije Universiteit Brussel; †Laboratoria Histologie en Genetika, Rijksuniversiteit Gent, Belgium: Inserts and gene transposition in a large plasmid of *Agrobacterium tumefaciens.*

Geisselsoder, L. Souza, A. Hopkins, and R. Calendar, Department of Molecular Biology, University of California at Berkeley: P4 phage carrying nonhomologous DNA.

WEDNESDAY EVENING, May 19

Session 2A: Drug resistance translocation elements

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

Mitsuhashi, H. Hashimoto, S. Iyobe, Y. Ike, and R. Katsumata, Department of Microbiology, School of Medicine, Gunma University, Maebashi, Japan: The formation of conjugative drug resistance (R) plasmid.

.A. Jacoby, A.E. Jacob*, and R.W. Hedges*, Massachusetts General Hospital, Boston: *Bacteriology Department, Royal Postgraduate Medical School, London, England: Recombination between *Pseudomonas aeruginosa* plasmids of incompatibility groups P-1 and P-2.

.E. Jacob, R.W. Hedges, N. Datta, N.J. Grinter, and P.T. Barth, Department of Bacteriology, Royal Postgraduate Medical School, London, England: Characterization of transposons determining resistance to ampicillin and streptomycin, and trimethoprim and streptomycin.

D.B. Clewell and Y. Yagi, University of Michigan, Ann Arbor: Amplification of the tetracycline resistance determinant on the *Streptococcus faecalis* plasmid pAMα1.

L.A. MacHattie and J.B. Jackowski, Department of Medical Genetics, University of Toronto, Canada: Structure and excision properties of an IS1-bracketed drug resistance determinant carried by phage λ*cam*.

L. Rosner and M. Gottesman*, Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland; *Department of Anatomy, Harvard Medical School, Cambridge, Massachusetts: Loss of the chloramphenicol-resistance gene from λ*cam*.

A.I. Bukhari and S. Froshauer, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Some properties of the TnC element.

D.E. Berg, Department of Biochemistry, University of Wisconsin, Madison: The transposable kanamycin resistance determinant Tn*K(1)*.

B. Allet and D. Berg, University of Geneva, Switzerland; University of Wisconsin, Madison: Deletions of the transposable element Tn*K(1)* and restriction-endonuclease and EM-heteroduplex mapping of Tn*K(1)*.

WEDNESDAY AFTERNOON, May 19

Session 2B: Drug resistance translocation elements

Chairperson: R. Novick, Public Health Research Institute of the City of New York, New York, New York

N. Kleckner, K. Reichardt, and D. Botstein, Department of Biology, Massachusetts Institute of Technology Cambridge: Imprecise excision of the translocatable tetracycline resistance element Tn*T(1)*.

R. Jorgensen, D. Berg, and B. Allet*, University of Wisconsin, Madison; *University of Geneva, Switzerland Restriction fragments and RNA polymerase binding sites of the Tn*T(1)* element.

J. Brevet, P. Nisen, D.J. Kopecko, and S.N. Cohen, Stanford University School of Medicine, California Promotion of insertions and deletions by translocating segments of DNA carrying antibiotic resistance genes.

F. Heffron, P. Bedinger, J. Champoux, and S. Falkow, Department of Microbiology, University of Washington, Seattle: Biochemical deletion mutations affecting transposition of Tn*A*.

G. Weinstock and D. Botstein, Massachusetts Institute of Technology, Cambridge: Specialized transduction of ampicillin resistance by phage P22.

J.-P. Hernalsteens, R. Villarroel-Mandiola, M. Van Montagu, and J. Schell, Laboratorium Genetische Virologie, Vrije Universiteit Brussel, Belgium: Insertion of Tn*A* in R factors.

P.J. Kretschmer and S.N. Cohen, Stanford University School of Medicine, California: Selection for replicons containing the Tn*A* translocation segment: General method for selected translocation.

Session 3: Nomenclature of insertion elements

Chairperson: H. Lewis, National Science Foundation, Washington, D.C.

Discussion

WEDNESDAY EVENING, May 19

Session 4: Bacteriophage Mu

Chairperson: A.L. Taylor, University of Colorado Medical Center, Denver, Colorado

A. Toussaint and M. Faelen*, Université Libre de Bruxelles, Départment de Biologie Moléculaire, Rhode St Genese, Belgium; *UNAM Instituto de Investigaciones Biomedicas Mexico, Mexico: Mu-mediated illegitimate recombination.

P. van de Putte, G.C. Westmaas, C.A. Wijffelman, and M. Giphart, Department of Biochemistry, University of Leiden, The Netherlands: On the *kil* gene of bacteriophage Mu.

A.I. Bukhari, S. Froshauer, L. Chow, E. Ljungquist, H. Khatoon, and F. DeBruijn, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Integration and excision of bacteriophage Mu.

B.T. Waggoner, M.L. Pato, and A.L. Taylor, Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, and Department of Microbiology, University of Colorado Medical Center, Denver: Characterization of heterogeneous circular (Hc) DNA from heat-induced bacteriophage Mu-1 lysogen.

L.T. Chow, R. Kahmann, and D. Kamp, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Electron microscopic studies of viable Mu phage containing deletions and substitutions.

E. Ljungquist and A.I. Bukhari, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Bacteriophage Mu—The problem of circles.

M.M. Howe, M. Schnos, and R.B. Inman, University of Wisconsin, Madison: Packaging of bacteriophage Mu DNA.

L.T. Chow and A.I. Bukhari, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: The invertible G sequence of phage Mu DNA is identical to the invertible sequence of phage P1 DNA.

D. Figurski, R. Meyer, D. Miller, and D.R. Helinski, Department of Biology, University of California at San Diego, La Jolla: Generation in vitro of deletions of the P-type plasmid RK2 using Mu insertions and a restriction endonuclease.

D.D. Moore, J.P. Schumm, M.M. Howe, and F.R. Blattner, University of Wisconsin, Madison: Insertion of Mu DNA fragments into phage λ in vitro.

M. Van Montagu*†, S. Van den Elsacker†, G. Engler†, J.P. Hernalsteens*, A. Silva†, F. Van Vliet*, and J. Schell*†, *Laboratorium Genetische Virologie, Vrije Universiteit Brussel; †Laboratoria Histologie en Genetika, Rijksuniversiteit Gent, Belgium: The translocation of chromosomal genes onto R plasmids with a wide host range.

THURSDAY MORNING, May 20

Session 5A: The eukaryotic systems

Chairperson: M. Meselson, Harvard University, Cambridge, Massachusetts

R.F. Baker and C.A. Thomas, Jr., Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Palindromes in the *Drosophila* genome.

R.F. Baker and D.G. Dickinson, Department of Biological Sciences, University of Southern California at Los Angeles: Methylation of palindromes in sea urchin embryo DNA.

R. Egel, Institut für Biologie III, Universität Freiburg, Federal Republic of Germany: Transposition of mating type genes in fission yeast.

B. McClintock, Carnegie Institution of Washington, Cold Spring Harbor, New York: Transposable chromosomal elements in maize.

I.M. Greenblatt, Genetics and Cell Biology Section, Biological Sciences Group, University of Connecticut, Storrs: Enhancement of crossing over by modulator in maize.

P.A. Peterson, Department of Agronomy, Iowa State University, Ames: The position hypothesis for controlling elements in maize.

Session 5B

Chairperson: J. Sambrook, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

M. Botchan, W. Topp, and J. Sambrook, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: The structure of simian virus 40 genes in transformed cells.

J. Skare and W.C. Summers, Yale University School of Medicine, New Haven, Connecticut: Nucleotide sequence arrangements in the genome of herpes simplex virus and their relation to insertion elements.

R.J. Jacob, G.S. Hayward, S.C. Wadsworth, and B. Roizman, Department of Microbiology, University of Chicago, Illinois: The inverted repeat regions of herpes simplex virus DNA. I. A structural characterization.

G.S. Hayward, T.G. Buchman, R.J. Jacob, and B. Roizman, Microbiology Department, University of Chicago, Illinois: Inverted repeat regions in the DNA of herpes simplex virus. II. Sequence diversity in relation to various inter- and intramolecular recombination events.

Session 6: Influence of insertion elements on gene expression

Chairperson: R. Weisberg, National Institutes of Health, Bethesda, Maryland

S. Adhya, D. Court, A. Das, M. Gottesman, S. Hilliker, and O. Reyes, National Institutes of Health, Bethesda, Maryland: Influence of insertion elements on gene expression.

A. Das, D. Court, and S. Adhya, National Institutes of Health, Bethesda, Maryland: Insertional polarity is mediated by the transcription termination factor rho.

N.C. Franklin, Department of Biological Sciences, Stanford University, California: Possible polar insertions in the *N* operon of λ.

P.K. Tomich and D.I. Friedman, Department of Microbiology, University of Michigan, Ann Arbor: Mutations in an insertion sequence that relieve polarity.

E. Mosharrafa, W. Pilacinski, J. Zissler, M. Fiandt*, W. Szybalski*, Department of Microbiology, University of Minnesota, Minneapolis; *McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Polar and antipolar insertion sequences IS2 near the gene for prophage λ excision.

J. Besemer, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: Suppression of insertion mutations within the *gal* operon of *E. coli*.

S. Torti, F. Halpern, and M. Malamy, Department of Biology, Tufts University School of Medicine, Boston, Massachusetts: Effect of external suppressor on insertion polarity.

B. Rak, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: IS2 carries a promoter and a signal for specific RNA cleavage.

S.R. Jaskunas and M. Nomura, Institute for Enzyme Research, University of Wisconsin, Madison: Use of insertion mutations to study the organization of ribosomal protein genes in *E. coli*.

FRIDAY MORNING, May 21

Session 7: Integrative recombination

Chairperson: A. Campbell, Stanford University, Palo Alto, California

R.A. Weisberg, Laboratory of Molecular Genetics, NICHHD, National Institutes of Health, Bethesda, Maryland: What we think we know about site-specific recombination in phage λ.

L.W. Enquist and R.A. Weisberg, Laboratory of Molecular Genetics, NICHHD, National Institutes of Health, Bethesda, Maryland: Genetic studies of site-specific recombination in phage λ.

H.A. Nash, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland: The biochemistry of site-specific recombination of phage λ.

H.I. Miller and D.I. Friedman, Department of Microbiology, University of Michigan, Ann Arbor: Isolation of *E. coli* mutants unable to support λ site-specific recombination.

A. Campbell and L. Heffernan, Department of Biological Sciences, Stanford University, California: Regulation of λ integrase.

D. Court and S. Adhya*, National Institute of Mental Health and *National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Regulation of the genes for λ integration and excision.

G. Schulz and M. Stodolsky, Department of Microbiology, Loyola University, Stritch School of Medicine, Maywood, Illinois: Specialized P1*arg*F transducing phage.

K. Shimada, S. Maeda, and Y. Takagi, Department of Biochemistry, Kyushu University School of Medicine, Fukuoka, Japan: Translocation of ampicillin resistance from R1*drd* onto ColE1-*gua* plasmids.

Summary: S. Brenner, University of Cambridge, Cambridge, England

FRIDAY AFTERNOON, May 21

Special session: Use of insertion elements for in vivo genetic engineering

RNA TUMOR VIRUSES

arranged by
THOMAS AUGUST, *Albert Einstein College of Medicine*

224 participants

WEDNESDAY EVENING, May 26

)pening Address: Thomas August, Albert Einstein College of Medicine, Bronx, New York

ession 1: Endogenous viruses

hairperson: H. Temin, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin

.V. Prochownik, S. Panem, and W.H. Kirsten, Departments of Pathology and Pediatrics, University of Chicago, Illinois: Induction of infectious type-C virus from clonal populations of normal human cells by 2-deoxy-D-glucose and 5-iodo-2'-deoxyuridine.

J.R. Rapp*, W. Mark†, and R. Callahan*, *National Institutes of Health, Bethesda, Maryland, and †McArdle Laboratory, University of Wisconsin, Madison: Endogenous ecotropic C-type viruses from non-transformed and transformed cells of AKR, C3H, and BALB/c.

. Wong-Staal, D. Gillespie, and R. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Proviral sequences related to a baboon endogenous type-C RNA virus in DNA from human leukemic tissues.

. Moroni, N. Teich, and J. Schumann, Friedrich Miescher Institut, Basel, Switzerland; Imperial Cancer Research Fund, London, England; Research Department, Pharma Division, CIBA-Geigy Ltd., Basel, Switzerland: Mitogen induction of endogenous C-type viruses.

.O. Warnaar, J. te Velde, G. van Muijen, F. Prins, and J.C.H. de Man, Department of Pathology, University Medical Center, Leiden, The Netherlands: Biochemical and electron-microscopical evidence for the presence of oncornaviruses in spleen tissue of two patients with hematological malignancies.

H.L. Niman*, J.R. Stephenson†, M.B. Gardner**, and P. Roy-Burman*, Departments of **Pathology and *Biochemistry, University of Southern California School of Medicine at Los Angeles; †Viral Carcinogenesis Branch, National Cancer Institute, Bethesda, Maryland: Analysis of feline RNA-tumor-virus-specific gene expressions in various types of cat neoplasia.

R.C. Gallo, F. Wong-Staal, R.E. Gallagher, P. Markham, F. Ruscetti, R.G. Smith, D.H. Gillespie, and M.S. Reitz, National Cancer Institute and Litton Bionetics, Bethesda, Maryland: Distribution of replicating type-C virus, proviral DNA, and viral RNA in different tissues of a gibbon ape (*H. lar*) with "spontaneous" leukemia-lymphoma.

V. Klement, M.L. Bryant, M.B. Gardner, and M.F. Dougherty, Departments of Pediatrics, Microbiology, and Pathology, University of Southern California School of Medicine at Los Angeles: Wild mouse leukemia virus complex.

W.N. Drohan, D. Colcher, and J. Schlom, Meloy Laboratories, Inc., Springfield, Virginia, and National Cancer Institute, Bethesda, Maryland: Natural distribution of Mason-Pfizer monkey virus sequences in the primate population.

.J. Sherr, R.E. Benveniste, M.M. Lieber, and G.J. Todaro, Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland: Type-C viruses containing endogenous primate and Kirsten-sarcoma-related gene sequences.

.E. Benveniste, C.J. Sherr, and G.J. Todaro, Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland: Baboon type-C viral genes—A study of primate evolution and interspecies viral transfer.

. Ikawa, Y. Uchiyama, M. Aida, and M. Obinata, Laboratory of Viral Oncology, Cancer Institute, Tokyo, Japan: Differentiation and virus release among several Friend leukemia cell lines of different origin.

.C. Chen and P.K. Vogt, Department of Microbiology, University of Southern California School of Medicine at Los Angeles: New endogenous RNA tumor viruses isolated from normal avian cells.

P. Bentvelzen, J. Brinkhof, and J.J. Haaijman, Radiobiological Institute TNO, Rijswijk, The Netherlands: Genetic and epigenetic control of the expression of endogenous murine mammary tumor viruses.

R. Callahan, R.E. Benveniste, G.J. Todaro, and C.J. Sherr, Viral Leukemic and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland: Isolation of infectious type-B and type-C viruses from the Asian rodent *Mus cervicolor*.

K. Huebner, C.L. Green, and C.M. Croce, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Induction of endogenous BALB viruses in mouse-human hybrids segregating mouse chromosomes.

THURSDAY MORNING, May 27

Session 2: Virus-cell interaction

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

D. Tsuei and C. Friend, Center for Experimental Cell Biology, Mt. Sinai School of Medicine of the City University of New York: Synthesis of virus in Friend leukemia cells in vitro.

N. Rosenberg and D. Baltimore, Center for Cancer Research, MIT, Cambridge, Massachusetts: In vitro transformation by Abelson murine leukemia virus.

J.S. Rhim and R.J. Huebner, Microbiological Associates, Inc., and National Cancer Institute, NIH, Bethesda, Maryland: Transformation of rat epithelial cells by Kirsten sarcoma virus.

G. Calothy and B. Pessac, Institut du Radium, Orsay, France: Growth stimulation of chick embryo neuroretinal cells infected with Rous sarcoma virus and morphological transformation: Further genetic analysis.

R.L. Heberling, S.S. Kalter, J.W. Eichberg, and B. McCullough, Southwest Foundation for Research and Education, San Antonio, Texas: Fibrosarcomas in dogs and nonhuman primates induced by a baboon-type-C-virus–murine-sarcoma pseudotype.

J.W. Gautsch, H.G. Bedigian, and H. Meier, The Jackson Laboratory, Bar Harbor, Maine: A short-term quantitative XC assay for murine leukemia virus and the influence of steroid hormones on syncytia formation.

H.W. Snyder, E. Fleissner, M. Fox, and T. Pincus, Memorial Sloan-Kettering Cancer Center, New York, and Stanford University Medical Center, California: Antigenic specificities of antibodies in normal human sera reactive with antigens in preparations of mammalian type-C viruses.

G.J. Vosika and B.J. Kennedy, University of Minnesota, Minneapolis: Antibody to C-type RNA viral proteins in human sera.

T. Aoki, M. Liu, M.J. Walling, G.S. Bushar, and K.C. Hsu, Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, Maryland, and Department of Microbiology, Columbia University, New York: Human natural antibody to type-C viruses from primates.

G.B. Rossi, A. Dolei, L. Cioé, G.P. Matarese, A. Benedetto, and G. Rita, Section of Virology, Instituto Superiore Sanita, and Institute of Virology, University of Rome, Italy: Differentiation inhibition of Friend leukemia cells by interferon.

B.J. Weimann*, J. Schmidt*, W. Ostertag†, J.C. Krieg†, N. Kluge†, P. Swetly**, and J. Pragnell‡, *Basel Institute for Immunology, Switzerland; †Max-Planck-Institut für Experimentelle Medizin, Göttingen, Federal Republic of Germany; **Ernst-Böhringer-Institut für Arzneimittelforschung, Wien, Austria; ‡Beatson Institute for Cancer Research, Glasgow, Scotland: Intracisternal A-type particles in virus-positive and -negative mouse and human cells in culture.

B.A. Wolf and A.R. Goldberg, The Rockefeller University, New York: Isolation of Rous sarcoma virus transformed cells having low levels of plasminogen activator activity.

V. Mautner, R. Eisenman, P.W. Robbins, and R.O. Hynes, Center for Cancer Research and Department of Biology, MIT, Cambridge, Massachusetts: Immunological detection of cell surface alterations in virus transformed fibroblasts.

E. Wang and A.R. Goldberg, The Rockefeller University, New York: Changes in surface topography and microfilament organization upon transformation of chick embryo fibroblasts with Rous sarcoma virus.

M. Okayama, S. Roth, J. Chi, M. Muto, M. Yoshimura, and A. Kaji, Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia: Transformation of chicken chondrocytes by Rous sarcoma virus.

Session 3: Viral proteins

Chairperson: Thomas August, Albert Einstein College of Medicine, Bronx, New York

P. O'Donnell, E. Stockert, and A. DeLeo, Memorial Sloan-Kettering Cancer Center, New York: Induction of G_{IX} and GCSA cell-surface antigens after infection by ecotropic and xenotropic MuLVs in vitro.

M. Barbacid, J.R. Stephenson, and S.A. Aaronson, National Cancer Institute, Bethesda, Maryland: The gag gene of mouse type-C RNA viruses.

O.N. Witte and I.L. Weissman, Department of Pathology, Stanford University Medical Center, California: Cellular maturation of oncornavirus glycoproteins—Topological arrangement of two forms in cellular membranes.

A. Sen and G.J. Todaro, National Institutes of Health, Bethesda, Maryland: Type specificity of binding of viral p12 core protein to homologous viral RNA in the gibbon–woolly monkey type-C virus group.

R. Eisenman, R. Shaikh, and M. Linial*, Center for Cancer Research, MIT, Cambridge, Massachusetts; *Fred Hutchinson Cancer Research Center, Seattle, Washington: Aberrant precursor polypeptides in cells infected with cloned recombinants of RAV-O and PR-RSV.

S. Hino, J.R. Stephenson, and S.A. Aaronson, National Cancer Institute, NIH, Bethesda, Maryland: Glycoproteins of endogenous mouse type-C viruses.

B.M. Gallis*, R.N. Eisenman†, and H. Diggelmann*, *Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland; †Center for Cancer Research, MIT, Cambridge, Massachusetts: Synthesis of the precursor to avian RNA tumor virus internal structural proteins early after infection.

P. Roy-Burman, E.G. Hayman, H. Niman, and B.K. Pal, Departments of Pathology and Biochemistry, University of Southern California School of Medicine at Los Angeles: Phosphoproteins of RNA tumor viruses: Further structural and biological studies.

M.M.C. Lai and M.J. Hayman, Department of Microbiology, University of Southern California School of Medicine at Los Angeles: On the structure of envelope glycoproteins in Rous sarcoma virus.

R.B. Naso, R.B. Arlinghaus, L.J. Arcement, G.A. Jamjoom, and W. L. Karshin, Department of Biology, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: A model for the synthesis and cleavage of Rauscher leukemia virus proteins.

M.S. Halpern and R.N. Leamnson, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: The subunit structure of the glycoprotein complex of avian tumor virus.

E.J. Smith, J. Ignjatovic, and L.B. Crittenden, Regional Poultry Research Laboratory, East Lansing, Michigan: Expression of avian leukosis sarcoma virus antigens in chicken lines.

G. Schochetman and J. Schlom, Meloy Laboratories, Inc., Springfield, Virginia, and National Cancer Institute, NIH, Bethesda, Maryland: Independent polypeptide chain initiation sites for the synthesis of different classes of mouse mammary tumor virus proteins.

S.K. Ruscetti, E.M. Scolnick, and W.P. Parks, National Cancer Institute, NIH, Bethesda, Maryland: Characterization of the feline oncornavirus-associated cell membrane antigen.

W.G. Robey*, M.K. Oskarrson*, M. Scherer†, C. Long†, P.J. Fischinger**, and G.F. Vande Woude*, *Laboratory of DNA Tumor Viruses, National Cancer Institute, NIH, Bethesda, Maryland; †Flow Laboratories, Rockville, Maryland; **Laboratory of Viral Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland: Properties of m1 Moloney murine sarcoma virus P60.

G.F. Okasinski and L.F. Velicer, Department of Microbiology and Public Health, Michigan State University, East Lansing: The synthesis of precursor polypeptides of FeLV structural proteins.

J.-S. Tung, J.H. Elder, R.A. Lerner, E. Fleissner, and E.A. Boyse, Memorial Sloan-Kettering Cancer Center, New York, and Scripps Clinic and Research Foundation, La Jolla, California: Polymorphism of MuLV gp70 envelope proteins expressed in mouse cells.

FRIDAY MORNING, May 28

Session 4: Mutant or defective viruses

Chairperson: H. Hanafusa, The Rockefeller University, New York, New York

J.G. Levin, Laboratory of Molecular Genetics, NICHHD, NIH, Bethesda, Maryland: Persistent expression of murine leukemia virus messenger RNA in actinomycin D-treated cells: Synthesis of proteins associated with virions lacking high-molecular-weight viral RNA.

D. Galehouse and P. Duesberg, Department of Molecular Biology, University of California at Berkeley: Differences in the glycoproteins of avian tumor virus recombinants: Evidence for intragenic crossing-over.

M.S. Reitz, C.D. Trainor, A.M. Wu, and R.C. Gallo, National Cancer Institute, NIH, Bethesda, Maryland: Loss of viral genetic information in A revertants of mouse fibroblasts nonproductively transformed with Kirsten murine sarcoma virus.

H.M. Murphy, Imperial Cancer Research Fund Laboratories, London, England: Genetic analysis of avian sarcoma viruses defective in replication.

A.E. Frankel and P.J. Fischinger, Laboratory of Viral Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland: The nature and distribution of the Moloney sarcoma virus genome.

S. Kawai, H. Hanafusa, and P.H. Duesberg, The Rockefeller University, New York, and Department of Molecular Biology, University of California at Berkeley: Transformation defective mutants of RSV—Formation of wild-type sarcoma virus by recombination with a temperature-sensitive mutant.

S. Kawai and H. Hanafusa, The Rockefeller University, New York: Recombination between a temperature-sensitive mutant and a deletion mutant of Rous sarcoma virus.

J.A. Bilello, M. Strand, and J.T. August, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Phenotypic and genotypic mixing during rescue of murine sarcoma virus transformed cells.

D. Myerson, R. Rongey*, V. Klement*, and J.T. August, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York; *University of Southern California School of Medicine at Los Angeles: A defective Kirsten murine sarcoma virus—woolly monkey virus pseudotype.

W.S. Mason and C. Yeater, The Institute for Cancer Research, Philadelphia, Pennsylvania: A mutant of Rous sarcoma virus with a conditional defect in the determinant(s) of viral host range.

F. Tato, J.A. Beamand, and J.A. Wyke, Department of Tumor Virology, Imperial Cancer Research Fund Laboratories, London, England: Early function affected in the RSV temperature-sensitive mutant *ts* LA30m PR-A.

D.A. Zarling and H.M. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Spontaneous mutation of avian sarcoma virus B77.

E.M. Scolnick, D. Williams, J. Maryak, and R.J. Goldberg, National Cancer Institute, NIH, Bethesda, Maryland: Naturally occurring and exogenously produced nonproducer cells containing the rat sequences of Kirsten sarcoma virus.

P.T. Peebles, E.M. Scolnick, and R.S. Howk, Laboratory of Tumor Virus Genetics, National Cancer Institute, NIH, Bethesda, Maryland: Increased sarcoma-virus-specific RNA in cells transformed by usually non-transforming RNA leukemia type-C viruses: A model for leukemogenesis.

FRIDAY EVENING, May 28

Session 5: RNA structure and sequence

Chairperson: P. Vogt, University of Southern California, Los Angeles, California

P.T. Peebles, M. Strand, and J.T. August, Laboratory of Tumor Virus Genetics, National Cancer Institute, NIH, Bethesda, Maryland, and Department of Molecular Biology, Albert Einstein School of Medicine, Bronx, New York: Moloney leukemia virus gp69/71 and p30 protein expression of the Moloney sarcoma virus and models for a common gene map for oncogenic mammalian type-C viruses and for generation of their deletion mutant sarcoma viruses.

E. Hunter, M. Hayman, and A. Tereba, Department of Microbiology, University of Alabama, Birmingham, and Department of Microbiology, University of Southern California at Los Angeles: A mechanism for genetic recombination in the avian RNA tumor viruses.

P.H. Duesberg, L.-H. Wang, P. Mellon, W.S. Mason*, and P.K. Vogt†, Department of Molecular Biology and Virus Laboratory, University of California at Berkeley; *Institute for Cancer Research, Philadelphia,

Pennsylvania; †Department of Microbiology, University of Southern California at Los Angeles: Towards a complete genetic map of Rous sarcoma virus.

D. Dina, K. Beemon, and P. Duesberg, Department of Molecular Biology and Virus Laboratory, University of California at Berkeley: The 28S Moloney sarcoma virus RNA contains leukemia virus-specific nucleotide sequences.

G. Shanmugam, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Cleavage of murine leukemia virus 35S RNA and double-stranded polynucleotides by microsome-associated nuclease.

R. Joho, E. Stoll, R. Friis*, M. Billeter, and C. Weissmann, Institut für Molekularbiologie I, Universität Zürich, Switzerland; *Institut für Virologie, Giessen, Federal Republic of Germany: Partial genetic map of Rous sarcoma virus RNA.

M.J. Hayman and P.K. Vogt, Department of Microbiology, University of Southern California School of Medicine at Los Angeles: Subgroup-specific antigenic determinants of avian RNA tumor virus structural proteins—Analysis of virus recombinants.

P.E. Neiman, D. MacDonnell, S. Das, and C. McMillin, University of Washington and Fred Hutchinson Cancer Research Center, Seattle: Distribution of "exogenous" nucleotide sequences in polyadenylated subunits of RSV RNA.

P. Balduzzi, J.R. Christensen, and Y.M. Pearson, Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, New York: Provisional genetic mapping of temperature-sensitive mutants of avian sarcoma virus defective in transformation.

W. Bender, S. Hu, Y.H. Chien, P. Chandler, S. Dube, H.J. Kung, N. Davidson and J. Maisel*, Department of Chemistry, California Institute of Technology, Pasadena; *Department of Molecular Biology, University of California at Berkeley: A common structure for the high-molecular-weight RNAs of all type-C viruses?

S. Hu, N. Davidson, M.O. Nicolson, and R.M. McAllister, Department of Chemistry, California Institute of Technology, Pasadena, and Childrens' Hospital, University of Southern California School of Medicine, Los Angeles: A heteroduplex study of the sequence relations between RD-114 and BKD RNAs.

R.P. Junghans, S. Hu, C.A. Knight, and N. Davidson, Department of Molecular Biology, University of California at Berkeley, and Department of Chemistry, California Institute of Technology, Pasadena: Heteroduplex studies of avian RNA tumor virus genomes.

M. Bondurant, S. Hashimoto, and M. Green, Institute for Molecular Virology, St. Louis University Medical School, Missouri: Methylation of Moloney murine leukemia virus RNA subunits.

K. Bister, W. Bender*, Y. Chien*, N. Davidson*, H.E. Varmus†, E. Hunter, and P.K. Vogt, Department of Microbiology, University of Southern California School of Medicine at Los Angeles; *California Institute of Technology, Pasadena; †Department of Microbiology, University of California at San Francisco: Studies on the inactivation of RNA tumor viruses by UV irradiation.

SATURDAY MORNING, May 29

Session 6: *Provirus*

Chairperson: P. Neiman, University of Washington, Seattle, Washington

V.M. Zhdanov, D.I. Ivanovsky Institute of Virology, Moscow, USSR: Double and triple integrations of viral genomes by cell DNA.

F. Yoshimura, D. Smotkin, and R.A. Weinberg, Department of Biology and Center for Cancer Research, MIT, Cambridge, Massachusetts: Restriction endonuclease mapping of the proviral DNA of Moloney MuLV.

E. Fritsch, N. Battula, and H.M. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Formation and properties of the provirus of spleen necrosis virus.

K.J. van den Berg, K. Nooter, J.B. de Vries, and P. Bentvelzen, Radiobiological Institute TNO, Rijswijk, The Netherlands: Transfection of murine hemopoietic cells with proviral DNA of the Rauscher leukemia virus.

M.A. Baluda, M. Shoyab, M.N. Dastoor, and R.M. Evans, Department of Microbiology and Immunology, University of California School of Medicine at Los Angeles: Studies on integration site of avian oncornavirus proviral DNA.

M. Hatanaka, H. Okabe, J. DuBuy, and R.V. Gilden, National Cancer Institute, NIH, Bethesda, Maryland; Flow Laboratories Inc., Rockville, Maryland, and The Frederick Cancer Research Center, Seattle, Washington: A possible factor determining the persistent production of RNA tumor viruses from cells.

G. Ringold, K. Yamamoto, and H.E. Varmus, Departments of Microbiology and Biochemistry, University of California at San Francisco: Infection of rat hepatoma cells with mouse mammary tumor virus—Characterization of viral DNA and studies on the mechanism of glucocorticoid action.

P.R. Shank, J.M. Bishop, and H.E. Varmus, Department of Microbiology, University of California Medical Center, San Francisco: A cytoplasmic precursor to covalently closed circular avian sarcoma virus DNA.

N. Mueller-Lantzsch, L.E. Hatlen, and H. Fan, The Salk Institute for Biological Studies, San Diego, California: Immunoprecipitation of murine leukemia virus-specific polyribosomes—Identification of virus-specific messenger RNA.

D. Spector, T. Padgett, H.E. Varmus, J.M. Bishop, and C. Moscovici*, Department of Microbiology, University of California Medical Center, San Francisco; *Tumor Virology, Veterans Administration Hospital, Gainesville, Florida: RNA related to the transforming gene(s) of avian sarcoma viruses in normal and chemically transformed avian cells.

W.S. Hayward and H. Hanafusa, The Rockefeller University, New York: Expression of avian RNA tumor virus genes.

D.J. Fujita, J. Tal, H.E. Varmus, J.M. Bishop, S. Kawai, and H. Hanafusa, Department of Microbiology, University of California at San Francisco, and The Rockefeller University, New York: $cDNA_{gp}A$, a probe specific for the envelope glycoprotein gene of avian tumor viruses.

SATURDAY AFTERNOON, May 29

Session 7: Host control

Chairperson: R. Risser, NIAID, National Cancer Institute, Bethesda, Maryland

A. Ishimoto, W.P. Rowe, and J.W. Hartley, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland: Host-range restriction in mouse cells of xenotropic MuLV genome with ecotropic virus coat.

H.S. Tucker, J.H. Weens, P. Tsichlis, R. Khiroya, and R.S. Schwartz, Hematology Service, New England Medical Center Hospital, Boston, Massachusetts: Genetic resistance to MuLV related to the H-2 complex.

R. Risser, M. Cancro, M. Potter, and W.P. Rowe, NIAID and National Cancer Institute, NIH, Bethesda, Maryland: Genetic control of susceptibility to Abelson murine leukemia virus.

N. Hopkins, K. Whalen, and P. Traktman, Department of Biology and Center for Cancer Research, MIT, Cambridge, Massachusetts: N-tropic variants obtained after coinfection with N- and B-tropic murine leukemia viruses.

P. Jolicoeur* and D. Baltimore, Department of Biology and Center for Cancer Research, MIT, Cambridge, Massachusetts: Effect of Fv-1 gene product on accumulation of virus-specific DNA and on integration of proviral DNA in permissive and nonpermissive murine cells infected with MuLV.

A.F. Gazdar, J.E. Delarco, H.K. Oie, and J.D. Minna, Veterans Administration Hospital, Washington, D.C. and the National Cancer Institute, NIH, Bethesda, Maryland: Expression of surface receptors for murine leukemia virus strains on hamster × mouse hybrid cells.

W.K. Yang*, R.W. Tennant* and A. Brown†, *Biology Division, Oak Ridge National Laboratory, Tennessee; †Department of Microbiology, University of Tennessee, Knoxville: Cellular RNA specific for transfer of Fv-1 locus restriction of murine leukemia viruses.

M. Linial, Department of Medicine, Division of Oncology, University of Washington School of Medicine and The Fred Hutchinson Cancer Research Center, Seattle, Washington: Infection of C/B chicken cells by subgroup B Rous sarcoma virus.

S.K. Datta and R.S. Schwartz, Hematology Service, New England Medical Center Hospital, Boston, Massachusetts: Genetics of expression of xenotropic virus and autoimmunity in NZB mice.

R. Soeiro and M.M. Sveda, Departments of Medicine and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Host restriction of Friend leukemia virus.

S.V.S Kashmiri*, A. Rein*, R.H. Bassin†, and B.I. Gerwin†, *Litton Bionetics, Inc. and †National Cancer Institute, Bethesda, Maryland: NB-tropic MuLV phenotypically acquires B-tropism during mixed infection with B-tropic MuLV.

G. Duran-Troise*, R.H. Bassin*, B.I. Gerwin*, and A. Rein†, *National Cancer Institute, NIH and †Litton Bionetics, Inc., Bethesda, Maryland: Abrogation of Fv-1 restriction in BALB/3T3 cells following infection with N-tropic MuLV.

H.L. Robinson, Division of Infectious Diseases, Stanford University School of Medicine, California: An intracellular restriction on the growth of induced subgroup E avian C-type viruses in chicken cells.

S.J. O'Brien, J.M. Simonson, and C.W. Boone, National Cancer Institute, NIH, Bethesda, Maryland: A new genetic locus which restricts endogenous type-C virus production in feline × mouse hybrid cells.

SUNDAY MORNING, May 30

Session 8: Reverse transcriptase

Chairperson: K. Moelling, Max Planck Institute for Molecular Genetics, West Berlin, Germany

W.K. Yang*, A.M. Wu†, and R.C. Gallo**, *Biological Division, Oak Ridge National Laboratory, Tennessee; †Bionetic Research Laboratory, Bethesda, Maryland; **Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, Maryland: Cellular proline tRNAs—Specific interaction with high-molecular-weight-form (HMW) reverse transcriptase and 30–40S RNA of MuLV.

D.P. Nayak, A.R. Davis, and B.S. Vayuvegula, Department of Microbiology and Immunology, University of California School of Medicine at Los Angeles: Studies on RNA templated DNA polymerase reactions in human bladder tumor cells in culture.

D.P. Grandgenett and A.J. Faras*, Institute for Molecular Virology, St. Louis University Medical Center, Missouri, and *Department of Microbiology, University of Minnesota, Minneapolis: Different states of avian myeloblastosis virus DNA polymerase and their binding capacity to primer $tRNA^{Trp}$.

G.F. Gerard, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Multiple ribonuclease H activities in mammalian RNA tumor viruses.

K. Moelling and R.R. Friis, Max Planck Institute for Molecular Genetics, West Berlin, and Institute for Virology, University of Giessen, Federal Republic of Germany: Comparative studies between avian and murine viral reverse transcriptase-RNase H complex and analysis of the DNA polymerase of two avian temperature-sensitive mutants.

W.A. Haseltine* and D. Baltimore, Department of Biology and Center for Cancer Research, MIT, Cambridge, Massachusetts: Ordered transcription of RNA tumor virus genomes.

I. Hizi and W.K. Joklik, Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Isolation, characterization, and enzymic activities of the β polypeptide chain of avian sarcoma virus DNA polymerase.

J. Leis and R. Smith, Departments of Surgery and Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: DNA transcription by processive AMV polymerase with 60S and 34S virus RNA.

A.M. Wu*, W.-K. Yang†, T. LeBon, and R.C. Gallo**, *Bionetics Research Laboratory and **Laboratory of Tumor Cell Biology, NCI, Bethesda, Maryland; †Biological Division, Oak Ridge National Laboratory, Tennessee: Functional significance of high-molecular-weight-form (HMW) mammalian virus reverse transcriptase.

B. Cordell, H.M. Goodman, and J.M. Bishop, Department of Microbiology, University of California at San Francisco: The nucleotide sequence of primer required for binding to the avian sarcoma virus genome and for the initiation of DNA synthesis.

M.-H. Lai and I.M. Verma, Tumor Virology Laboratory, The Salk Institute, San Diego, California: Structural relationship of subunits of reverse transcriptase from avian myeloblastosis virus.

MOLECULAR ASPECTS OF *lac* OPERON CONTROL

arranged by
WILLIAM REZNIKOFF, *University of Wisconsin*
JEFFREY MILLER, *University of Geneva*

117 participants

WEDNESDAY EVENING, June 30

A Tribute to Jacques Monod: Melvin Cohn, The Salk Institute, San Diego, California

Session 1: Genetic studies of the repressor

Chairperson: S. Bourgeois, The Salk Institute, San Diego, California

J.H. Miller, C. Coulondre, U. Schmeissner, A. Schmitz, and P. Lu*, Department of Molecular Biology, University of Geneva, Switzerland; *Department of Chemistry, University of Pennsylvania, Philadelphia: Genetic analysis of the *lac* repressor.

M. Schlotmann, N. Geisler, and K. Beyreuther, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: Biochemical characterization of mutant *lac* repressors.

M. Pfahl, The Salk Institute, San Diego, California: Tight-binding repressors and consequences of an increased affinity of a repressor for DNA.

J.R. Sadler and J.L. Betz, Department of Biophysics and Genetics, University of Colorado Medical Center, Denver: Tight-binding mutants of the repressor.

A. Schmitz, C. Coulondre, and J.H. Miller, Department of Molecular Biology, University of Geneva, Switzerland: Altered repressors which bind operator more tightly resulting from suppressed nonsense mutations.

J.R. Sadler, C.R. Norwood, J. Miwa, M. Tecklenburg, T.F. Smith, and J.L. Betz, Department of Biophysics and Genetics, University of Colorado Medical Center, Denver: The modularity of the lactose repressor operator interaction.

THURSDAY MORNING, July 1

Session 2: Structural studies on the lac *repressor*

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

S. Bourgeois*, R.L. Jernigan**, E.A. Kabat†, S.C. Szu**, and T.T. Wu‡, *The Salk Institute, San Diego, California; **Laboratory of Theoretical Biology, National Cancer Institute, Bethesda, Maryland; †National Cancer Institute, Bethesda, Maryland and Columbia University, New York; ‡Department of Engineering Sciences, Northwestern University, Evanston, Illinois: Composite predictions of secondary structure of *lac* repressor.

K. Weber*, N. Geisler*, and J.G. Files†, *Max Planck Institute for Biophysical Chemistry, Göttingen, Federal Republic of Germany; †Department of Cell Biology, University of Colorado, Boulder: Studies on repressor fragments.

K. Beyreuther, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: Analysis of *lac* repressor conformations by proteolysis—The mechanisms of induction.

K.S. Matthews, A.A. Burgum, B.E. Friedman, R.B. Gorman, and C.F. Sams, Department of Biochemistry, Rice University, Houston, Texas: Chemical studies on *lac* repressor.

P. Lu, M. Jarema, M. Lillis, H. Sommer, and J. Miller*, Department of Chemistry, University of Pennsylvania, Philadelphia; *Department of Molecular Biology, University of Geneva, Switzerland: Spectroscopic studies on *lac* repressor.

C.-W. Wu, F.Y.-H. Wu, and P. Bandyopadhyay, Department of Biophysics, Albert Einstein College of Medicine, New York: Allosteric transition of *lac* repressor from *Escherichia coli.*

J.C. Maurizot, M. Charlier, F. Culard, and C. Hélène, Centre de Biophysique Moléculaire, Orléans, France: Interaction of inducers and anti-inducers with *lac* repressor—A circular dichroism study.

M.T. Record, Jr., T.M. Lohman, and P.L. de Haseth, Department of Chemistry, University of Wisconsin, Madison: Interpretation of ion effects on *lac* repressor–DNA interactions.

O.G. Berg and C. Blomberg, Department of Theoretical Physics, Royal Institute of Technology, Stockholm, Sweden: Mass action relations in vivo.

THURSDAY EVENING, July 1

Session 3: The lac *operator and its recognition by the repressor*

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

W. Gilbert, Harvard University, Cambridge, Massachusetts: The contacts between *lac* repressor and DNA.

C.P. Bahl*, R. Dickerson†, K. Itakura†**, S. Lin**, S.A. Narang‡, A.D. Riggs**, J. Rosenberg† and R. Wu*, *Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York; †California Institute of Technology, Pasadena; **City of Hope National Medical Center, Duarte, California; ‡Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario: Studies on *lac* repressor recognition of synthetic *lac* operator molecules.

M.H. Caruthers, D.V. Goeddel, R. Loder, D. Cribbs, and D. Yansura, Department of Chemistry, University of Colorado, Boulder: Synthesis of *lac* operator DNA sequences.

P.H. von Hippel, A.P. Butler, R.B. Winter, A. Revzin, A. Wang, and Y.K. Huang, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene: Interaction of *lac* repressor with non-operator DNA.

M. Charlier, J.C. Maurizot. F. Culard, and C. Hélène, Centre de Biophysique Moléculaire, Centre Nationale de Recherches Scientifiques, Orléans, France: Circular dichroism, fluorescence, and photochemical studies of *lac* repressor–inducer–DNA interactions.

J. Rosenberg*, C.P. Bahl†, H. Boyer**, R.E. Dickerson*, H.M. Goodman**, H. Heyneker**, K. Itakura*‡, O. Kallai*, M. Kopka*, S. Lin‡, S.A. Narang§, A.D. Riggs‡, R. Scheller*, J. Shine**, and R. Wu†, *California Institute of Technology, Pasadena; †Cornell University, Ithaca, New York; **University of California at San Francisco; ‡City of Hope National Medical Center, Duarte, California; §National Research Council of Canada, Ottawa, Ontario: The insertion of chemically synthesized *lac* operator DNA into plasmid DNA and demonstration of biological activity.

M. Pfahl and S. Bourgeois, The Salk Institute, San Diego, California: Isolation of "super-operator" mutants.

FRIDAY MORNING, July 2

Session 4: More on the R-O interaction and studies on the lac I *gene promoter*

Chairperson: W. Reznikoff, University of Wisconsin, Madison, Wisconsin

T.J. Richmond and T.A. Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Crosslinking of *lac* repressor to poly[d(A·U-HgX)]—The size of the DNase protected oligonucleotides.

R.T. Ogata, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Cross-linking of *lac* repressor to BrdU-substituted *lac* operator.

P. Farabaugh, The Biological Laboratories, Harvard University, Cambridge Massachusetts: The secondary repressor binding site acts as an attenuator in vivo.

A. Maxam, W. Gilbert, G. Copenhaver, H. Donis-Keller, N. Rosenthal, and D. States, Department of Biochemistry and Molecular Biology and Department of Biology, Harvard University, Cambridge, Massachusetts: Extended nucleotide sequences in the *lac* operon.

H.W. Chan and R.D. Wells, Department of Biochemistry, University of Wisconsin, Madison: Mung bean nuclease causes a specific single-stranded nick at the control region of the lactose operon.

U. Schmeissner, D. Ganem, and J.H. Miller, Department of Molecular Biology, University of Geneva, Switzerland: Deletion analysis of the *I* gene and its regulatory region.

D.A. Steege, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Nucleotide sequence of the 5′ end of *E. coli* lactose repressor mRNA.

B. Müller-Hill, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: Promoter mutations of the *I* gene.

FRIDAY EVENING, July 2

Session 5: The lac *promoter and its recognition by RNA polymerase and CAP*

Chairperson: A. Riggs, City of Hope National Medical Center, Duarte, California

W. Reznikoff, W. Barnes, R. Dickson, and J. Abelson, Department of Biochemistry, University of Wisconsin, Madison, and Department of Chemistry, University of California at San Diego: The *lac* promoter and its recognition by RNA polymerase.

J. Majors and L. Johnsrud, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Interactions between CAP factor, RNA polymerase, and the *lac* promoter.

R. Musso, R. DiLauro, B. de Crombrugghe, and I. Pastan, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland: Structure and function of the regulatory region of the galactose operon of *E. coli.*

A. Ullmann, Departement de Biochimie et Génétique Microbienne, Unité de Biochimie Cellulaire, Institut Pasteur, Paris, France: Catabolite modulating compound—A possible mediator of catabolite repression.

E. Eilen, C. Pampeno, and J.S. Krakow, Department of Biological Sciences, Hunter College of the City University of New York: Studies on proteolytic and sulfhydryl modification of cAMP receptor protein of *E. coli*

W.J. Schrenk and D.E. Morse, Department of Biological Sciences, University of California, Santa Barbara: Suppression of leaky mutations by deletion of the *lon* gene.

S. Mitra, G. Zubay, and A. Landy*, Department of Biological Sciences, Columbia University, New York; *Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island: Restriction fragments—A model system for studying the regulation of the *lac* operon.

SATURDAY MORNING, July 3

Session 6: β-Galactosidase and other lac *systems*

Chairperson: I. Zabin, University of California, Los Angeles, California

A.V. Fowler, A.J. Brake, and I. Zabin, Department of Biological Chemistry, UCLA School of Medicine, and Molecular Biology Institute, University of California at Los Angeles: The structure of β-galactosidase.

F. Celada, Departments of Biological Chemistry and Bacteriology, University of California at Los Angeles and Laboratorio di Biologia Cellulare, Italian Research Council, Rome: Immunological studies of β-galactosidase.

C.C. Jones, R.S.T. Loeffler, M.L. Sinnott, P.J. Smith, I.J.L. Soucbard, and S.G. Withers, Department of Organic Chemistry, University of Bristol, United Kingdom: Catalytic mechanism of β-galactosidase.

J.M. Yon, O.M. Viratelle, and P.J. Deschavanne, Laboratoire d'Enzymologie Physico-Chimique Moléculaire, Université de Paris-Sud, Orsay, France: Enzymatic properties of β-galactosidase from *E. coli*. Comparison between the wild-type enzyme and a point mutant—C.Z.P. protein.

R.E. Huber, R. Sept, and M. Babiak, Department of Biochemistry, University of Calgary, Alberta, Canada: Evidence for partially separate mechanisms of β-galactosidase *(E. coli)* for allolactose production and for galactose and glucose production.

J. Manley, Cold Spring Harbor Laboratory, New York: Abnormal polypeptides encoded by the *lac Z* gene.

N. Guiso and A. Ullmann, Département de Biochimie et Génétique Microbienne, Unité de Biochimie Cellulaire, Institut Pasteur, Paris, France: Expression and regulation of lactose genes carried by plasmids.

E.C.R. Reeve, Institute of Animal Genetics, Edinburgh, Scotland: Differences between the *lac* operons of *Klebsiella* and *Escherichia coli*.

B.G. Hall, Faculty of Medicine, Memorial University of Newfoundland, Canada: Regulation of the second *E. coli* β-galactosidase *ebg* enzyme.

SATURDAY AFTERNOON, July 3

Session 7: β-Galactoside permease and the y gene

T.H. Wilson and J. Flagg, Department of Physiology, Harvard Medical School, Boston, Massachusetts: Lactose transport and its energetics.

W.D. Nunn and J.E. Cronan, Jr., Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Integration of the permease and membrane lipid synthesis.

R.C. Tuttle, M.J. Newman, and M.H. Saier, Jr., Department of Biology, University of California at San Diego, Allosteric regulation of lactose and melibiose transport in *Escherichia coli* cells and membrane vesicles.

A.C. Hobson, D. Gho, and B. Müller-Hill, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: Genetical analysis of the *lac y* gene.

A.L. Koch and D.R. Purdy, Microbiology Department, Indiana University, Bloomington: The energy cost of galactoside transport to *Escherichia coli*.

Yariv, Department of Biophysics, Weizmann Institute of Science, Rehovot, Israel: Alkylation of β-galactosidase and of β-galactoside permease with a specific label.

J. Andrews and E.C.C. Lin, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Thiogalactoside transacetylase as a detoxification enzyme in *Escherichia coli* K-12.

SUNDAY MORNING, July 4

Session 8: Uses of the lac *system*

Chairperson: J. Beckwith, Harvard Medical School, Boston, Massachusetts

Casadaban, T. Silhavy, L. Guarente, H. Shuman, and J. Beckwith, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: The use of *lac* operon fusions for studying biological processes.

Kania, G. Heidecker, D. Brown, and B. Müller-Hill, Institut für Genetik der Universität Köln, Weyertal, Federal Republic of Germany: Gene fusions in the *trp-lac* systems of *E. coli* produce active chimeric proteins.

Ptashne, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Using *lac* to study λ.

Coulondre and J.H. Miller, Department of Molecular Biology, University of Geneva, Switzerland: Mutagenic specificity in *E. coli* determined using the *lac* system.

Van Brunt and G. Edlin, Department of Genetics, University of California at Davis: Use of *lac* transposition strains to probe the mutagenic topography of the *E. coli* chromosome.

F. Kung, C. Spears, and H. Weissbach, Department of Biochemistry, Roche Institute of Molecular Biology, Nutley, New Jersey: DNA-directed in vitro synthesis of β-galactosidase in a partially purified system.

Kennell, H. Riezman, J.E. Schneider, and L.W. Lim, Department of Microbiology and Immunology, Washington University, St. Louis, Missouri: Ribosome initiation frequencies, decay *lac* messages, and natural polarity.

SV40, POLYOMA, AND ADENOVIRUSES

arranged by
TERRI GRODZICKER, *Cold Spring Harbor Laboratory*
MICHAEL BOTCHAN, *Cold Spring Harbor Laboratory*

262 participants

WEDNESDAY EVENING, August 18

Session 1: Genetics

Chairperson: T. Grodzicker, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

C. Cole, T. Landers, S. Goff, S. Manteuil-Brutlag, and P. Berg, Department of Biochemistry, Stanford University Medical Center, California: Deletion mutants of SV40.

Y. Gluzman and E. Winocour, Department of Virology, Weizmann Institute of Science, Rehovot, Israel: Marker rescue of SV40 *ts* mutants passaged on transformed permissive monkey cells.

M. Fluck, R. Staneloni, J. Feunteun, L. Sompayrac, and T. Benjamin, Department of Pathology, Harvard Medical School, Boston, Massachusetts: Relationships between nontransforming mutants of polyom virus.

W. Eckhart, Tumor Virology Laboratory, The Salk Institute, San Diego, California: Complementation between polyoma mutants defective in cell transformation.

J. Rabek, V. Zakian, and A.J. Levine, Princeton University, New Jersey: SV40-mediated suppression of an adenovirus DNA-negative mutant.

J. Sambrook, J. Hassell, S. Zain, and E. Lukanidin, Cold Spring Harbor Laboratory, New York: Adenovirus-SV40 hybrids. I. New adenovirus genomes that contain SV40 sequences and SV40 genomes that contain adenovirus sequences.

G. Fey, J. Sambrook, J. Lewis, and E. Lukanidin, Cold Spring Harbor Laboratory, New York: Adenovirus-SV40 hybrids. II. Expression of the SV40 sequences.

S. Goff and P. Berg, Department of Biochemistry, Stanford University Medical Center, California: Construction, propagation, and expression of a hybrid virus containing SV40 and lambda DNA segments.

D.H. Hamer, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: SV40 carrying a bacterial suppressor tRNA gene.

THURSDAY MORNING, August 19

Session 2: Integrated genomes

Chairperson: P. Berg, Stanford University Medical Center, Stanford, California

C.M. Croce, The Wistar Institute, Philadelphia, Pennsylvania: Chromosome assignment of the integrated SV40 genome in different SV40-transformed human cells.

M. Botchan, W. Topp, and J. Sambrook, Cold Spring Harbor Laboratory, New York: The structure of simian virus 40 genes in transformed cells.

D.M. Anderson and W.R. Folk, Department of Biological Chemistry, University of Michigan, Ann Arbor: Analysis of polyoma virus DNAs rescued from transformed BKH 21 cell lines.

I. Prasad, D. Zouzias, and C. Basilico, Department of Pathology, New York University School of Medicine, New York: The state of viral DNA in rat cells transformed by polyoma and SV40.

K. Yoshiike and V. Defendi, Department of Pathology, New York University School of Medicine, New York: Addition of extra DNA sequences to SV40 DNA in vivo.

M.F. Singer, M. Garner, G.R.K. Rao, M. Rosenberg, and S. Segal, National Cancer Institute, Bethesda, Maryland: Characterization of the monkey repetitive DNA sequences in a substituted defective SV40 genome.

K.C. Lee and S. Mak, Biology Department, McMaster University, Hamilton, Ontario, Canada: Adenovirus-type-12 genomes in hamster tumor cells.

C. Tyndall, H.B. Younghusband, and A.J.D. Bellett, John Curtin School of Medical Research, Australian National University, Canberra, and National Institute for Medical Research, London, England: Integration of adenovirus DNA into cell DNA.

K. Baczko, J. Groneberg, and W. Doerfler, Institute of Genetics, University of Cologne, Federal Republic of Germany: Characterization of integrated viral sequences in adenovirus-infected and -transformed cells.

THURSDAY AFTERNOON, August 19

Session 3: SV40 and polyoma--Poster session

M. Berebbi, G. Meyer, and R. Cramer, INSERM, Marseille, France: Viral inhibiting factor in polyoma-transformed cells.

C. Chang, S.W. Luborsky, J. Anderson, and R.G. Martin, Macromolecular Biology Section, NCI, and Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland: Comparative behavior of SV40 T antigen and specific surface antigen(s) during partial purification.

J.Y. Chou, Section on Developmental Enzymology, Laboratory of Biomedical Sciences, NICHHD, National Institutes of Health, Bethesda, Maryland: Simian virus 40 mutants carrying two temperature-sensitive mutations.

R. Dhar, K. Subramanian, B. Thimmappaya, S. Zain, J. Pan, and S. Weissman, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Nucleotide sequence of SV40 DNA.

A. Gonenne, P. Asekawa, and T. Friedmann, Department of Pediatrics, University of California at San Diego: Altered virion protein VP2 components in polyoma ts3.

P. Gruss and G. Sauer, Insitut für Virusforschung, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: A new class of replicating linear SV40–host DNA sequences.

L. Hallick, J. Bartholomew, and J. Hearst, Departments of Chemistry and Chemical Biodynamics, University of California, Berkeley: Cross-linking as a probe for the structure of SV40 chromatin.

P.M. Howley, G. Khoury, K.K. Takemoto, and M.A. Martin, Laboratory of Pathology, NCI, and Laboratory of the Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland: Polynucleotide sequences common to the genomes of simian virus 40 and the human papovaviruses JC and BK.

W.F. Mangel*, R. Hewick, S.T. Bayley, T. Wheeler, and A.E. Smith, *Departments of Cell Regulation, Protein Chemistry and Molecular Virology, Imperial Cancer Research Fund, London, England: The in vitro synthesis and identification of the virus-coded polypeptides of the late polyoma genes.

R. Martin, C. Edwards, and S. Stein, Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland: The cell cycle and origins of DNA synthesis in SV40-transformed cells.

E. May, N.P. Salzman, J.V. Maizel, and M. Sullivan, Laboratory of Molecular Genetics, NICHHD, National Institutes of Health, Bethesda, Maryland: Mapping of SV40 viral mRNA by electron microscopy.

C. Mueller, M. Graessmann, and A. Graessmann, Institut für Molekularbiologie und Biochemie der Freien Universität Berlin, West Berlin, Germany: The biological activity of specific SV40 DNA fragments produced by Hpa I and Hpa II restriction endonucleases.

F.J. O'Neill and D. Carroll, Veterans Administration Hospital, Research Service, and Department of Microbiology, University of Utah, Salt Lake City: Variant viral DNA following SV40 propagation in human glioblastoma cells.

J.A. Robb, Department of Pathology, University of Calfornia, San Diego: Identification of SV40 U antigen.

S. Rozenblatt, M. Gorecki, R.C. Mulligan, and B.E. Roberts, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts: Direct biochemical mapping of SV40 DNA utilizing a linked transcription-translation system.

G. Sauer and P. Gruss, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: Infectious linear SV40–host DNA sequences—Properties of superhelical and linear progeny.

M. Shani, E. Birkenmeier, E. May, and N.P. Salzman, Laboratory of Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland: Properties of simian virus 40 transcriptional intermediates isolated from nuclei of permissive cells.

A.J. Buckler-White and V. Pigiet, Department of Biology and McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland: Presence of T antigen and polyoma DNA species on the nuclear matrix.

E. Yakobson, C. Prives, J. Hartman, E. Winocour, and M. Revel, Virology Department, Weizmann Institute of Science, Rehovot, Israel: Inhibition of SV40 DNA and protein synthesis by interferon treatment late in the lytic cycle.

N. Yamaguchi, K. Segawa, K. Yamamoto, S. Kito, and K. Oda, Department of Tumor Virus Research, Institute of Medical Science, Tokyo, Japan: Association of SV40 T antigen with ribosomes.

Session 4: Adenoviruses—Poster session

L. Aiello, K. Huebner, C.M. Croce, F.L. Graham, and R. Weinmann, The Wistar Institute, Philadelphia, Pennsylvania, and McMaster University, Hamilton, Ontario, Canada: Adenovirus-5 sequences associated with specific human chromosomes in human-mouse hybrids.

S.G. Baum, Department of Cell Biology, Albert Einstein College of Medicine, New York, New York: Studies of the biology of adenovirus-SV40 enhancement.

G.E. Blair and W.C. Russell, National Institute for Medical Research, Mill Hill, London, England: Polypeptide phosphorylation in adenovirus infection.

G. Chinnadurai, Y.-H. Jeng, and Z. Gilead, Institute for Molecular Virology, St. Louis University, Missouri: Early polypeptides induced by adenovirus 12.

G. Chinnadurai, H.M. Rho, and M. Green, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Messenger RNA from the transforming segment of the adenovirus-2 genome in productively infected and transformed cells.

L. Delbecchi, L. Raptis, D. Bourgaux-Ramoisy, and P. Bourgaux, Département de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada: Replication of adenovirus-type-2 DNA.

R. Dunker and D.T. Brown, Institute of Genetics, University of Cologne, Federal Republic of Germany: A tubular morphological variant of adenovirus.

E. Fanning and W. Doerfler, Institute of Genetics, University of Cologne, Federal Republic of Germany: Viral DNA sequences in hamster cells abortively infected and transformed with adenovirus type 12.

D. Galloway and J. Sambrook, Cold Spring Harbor Laboratory, New York: The arrangement of viral DNA in adenovirus-2-transformed cells.

S. Hashimoto, K. Brackmann, and M. Green, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Methylation studies of adenovirus-specific messenger and nuclear RNA at early stages of productive infection.

Y. Jeng, M. Harter, W. Wold, and M. Green, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Identification of adenovirus-2-induced early proteins.

Y. Jeng, W. Wold, K. Sugawara, and Z. Gilead, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Posttranslation modification of the adenovirus-2 DNA binding protein.

J.K. Mackey, P.M. Rigden, and M. Green, Institute for Molecular Virology, St. Louis University, Missouri: Do highly oncogenic group-A human adenoviruses cause human cancer?—Analysis of human tumors for adenovirus-12-transforming DNA sequences.

K. Mann, T. Hunter, and G. Walter, Tumor Virology Laboratory, The Salk Institute, San Diego, California: Tryptic peptide analysis of SV40-specific proteins in HeLa cells infected with nondefective adenovirus 2–SV40 hybrid viruses.

H.C. Meissner, J. Meyer, J.F. Maizel, Jr., and H. Westphal, Laboratory of Molecular Genetics, NICHHD, National Institutes of Health, Bethesda, Maryland: Visualization of large nuclear adenovirus RNA.

K. Raska, Jr. and F. Varricchio*, CMDNJ-Rutgers Medical School, Piscataway, New Jersey; *Memorial Sloan-Kettering Cancer Center, New York, New York: Synthesis of VA RNA in productive and abortive infections with adenovirus type 12.

K.H. Scheidtmann, J. Ortin, R. Greenberg, M. Westphal, and W. Doerfler, Institute of Genetics, University of Cologne, Federal Republic of Germany: Transcription maps of adenovirus-type-12 DNA in infected and transformed cells.

K. Shiroki, H. Shimojo, and K. Yamaguchi, Institute of Medical Science, University of Tokyo, Japan: T inclusions and viral DNA synthesis in adenovirus-12-infected cells.

J.R. Smiley and S. Mak, Biology Department, McMaster University, Hamilton, Ontario, Canada: Transcription of adenovirus-type-12 DNA.

J. Weber*, G. Khitto*, and J. Hassell†, *Department of Microbiology, Centre Hospitalier Universitaire, University of Sherbrooke, Quebec, Canada; †Cold Spring Harbor Laboratory, New York: An adenovirus-2 *ts* mutant defective for DNA encapsidation.

R. Weinmann, The Wistar Institute, Philadelphia, Pennsylvania: Adenovirus-specific low-molecular-weight RNA species.

THURSDAY EVENING, August 19

Session 5: Virus-cell interactions

Chairperson: W. Eckhart, The Salk Institute, San Diego, California

J.K. McDougall and L.B. Chen, Cold Spring Harbor Laboratory, New York: Adenovirus oncogenicity and LETS protein

G.C. Fareed, M.A. Gimbrone, Jr.*, and K.K. Takemoto†, Molecular Biology Institute and Department of Microbiology and Immunology, University of California at Los Angeles; *Department of Pathology, Peter Bent Brigham Hospital, and Harvard Medical School, Boston, Massachusetts; †NIAID, National Institutes of Health, Bethesda, Maryland: Interaction of SV40 and human papovaviruses, BK and JC, with human vascular endothelial cells.

W.W. Brockman, Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland: Temperature sensitivity of the transformed phenotype of BALB 3T3 cells transformed by different SV40 *tsA* mutants.

D. Tenen*, J. Anderson†, D. Livingston*, and R. Martin†, *Sidney Farber Cancer Center, Harvard Medical School, Boston, Massachusetts; †Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland: T antigen in Chinese hamster lung cell lines transformed by *tsA* mutants and their revertants.

R. Pollack, Department of Microbiology, State University of New York at Stony Brook: SV40 T antigen is necessary for the cytoskeletal changes characteristic of tumorigenic transformed cells.

C. Chang, A. Schultz, L. Couvillion, and P. Mora, Macromolecular Biology Section, National Cancer Institute, Bethesda, Maryland: In vivo selection of cells for tumorigenicity and against SV40-specific antigens.

G.B. Zamansky, L.F. Kleinman, J.B. Little, P.H. Black, and J.D. Kaplan, Department of Medicine, Massachusetts General Hospital and Departments of Microbiology and Molecular Genetics and Medicine, Harvard Medical School, Boston, Massachusetts: The effect of caffeine on the ultraviolet-light induction of SV40 virus from transformed hamster cells.

. Graessman, M. Graessmann, and C. Mueller, Institut für Molekularbiologie und Biochemie der Freien Universität Berlin, West Berlin, Germany: Regulatory mechanism of simian virus 40 gene expression in permissive and in nonpermissive cells.

C. Norkin, Department of Microbiology, University of Massachusetts, Amherst: Cytocidal and persistent SV40 infections.

FRIDAY MORNING, August 20

ssion 6: SV40 and polyoma—Transcription and replication

hairperson: G. Khoury, NIAID, National Institutes of Health, Bethesda, Maryland

. Mertz, J.B. Gurdon, and E.M. DeRobertis, Medical Research Council Laboratory of Molecular Biology, Cambridge, England: Transcription of SV40 DNA in *Xenopus* oocytes: A model system for studying control of transcription in eukaryotes.

Condit, A. Cowie, and R. Kamen, Department of Molecular Virology, Imperial Cancer Research Fund, London, England: Purification and properties of a polyoma virus transcription complex.

.H. Green, J. Allan, and T.L. Brooks, Department of Biology, University of California at San Diego: The template for SV40 transcription.

E. Birkenmeier, E. May, M. Shani, and N.P. Salzman, Laboratory of Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland: Characterization of simian virus 40 TsA58 transcriptional intermediates at nonpermissive temperatures.

Y. Aloni, Y. Groner, O. Laub, and Y. Reuveni, Departments of Genetics and Virology, The Weizmann Institute of Science, Rehovot, Israel: Regulation of simian virus 40 gene expression.

M.W. Gutai and D. Nathans, Department of Microbiology, Johns Hopkins University, Baltimore, Maryland: The origin of SV40 DNA replication.

K.N. Subramanian, R. Dhar, and S.M. Weissman, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Nucleotide sequence at the origin of DNA replication in SV40.

G. Kaufmann, S. Anderson, and M.L. DePamphilis, Harvard Medical School, Boston, Massachusetts: The distribution and dynamics of RNA-DNA linkages in replicating SV40 DNA.

H.J. Edenberg and J.A. Huberman*, Department of Biology, MIT, Cambridge, Massachusetts; *Department of Medical Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York: In vitro SV40 DNA synthesis in a subnuclear system.

FRIDAY EVENING, August 20

Session 7: SV40 and polyoma—Proteins

Chairperson: D. Nathans, Johns Hopkins University, Baltimore, Maryland

D.M. Livingston, D. Jessel, and D. Tenen, The Sidney Farber Cancer Center, Harvard Medical School, Boston, Massachusetts: Interactions of SV40 T antigen with SV40 DNA.

J. Anderson, C. Chang, P. Mora, D. Livingston, and R. Martin, Laboratory of Molecular Biology, NIAMDD and Macromolecular Biology Section, NCI, National Institutes of Health, Bethesda, Maryland, and Sidney Farber Cancer Center, Harvard Medical School, Boston, Massachusetts: Relationship of SV40 tumor specific transplantation antigen (TSTA) to tumor antigen (TA).

K. Rundell, P. Tegtmeyer, P. Wright*, and G. DiMayorca*, Department of Microbiology, State University of New York, Stony Brook; *Department of Microbiology, University of Illinois Medical Center, Chicago: Comparison of SV40 and BKV protein A.

S.I. Reed, G.R. Stark, and J.C. Alwine, Department of Biochemistry, Stanford University Medical Center, California: Autoregulation of simian virus 40 gene *A*.

R. Weil, M. Lichaa, H. Türler, B. Allet*, and C. Ahmad-Zadeh*, Institute of Molecular Biology, University of Geneva; *Institut d'Hygiene, Geneva, Switzerland: Studies on SV40 T antigen.

R. Carroll, Department of Pathology, New York University School of Medicine, New York: The significance of the molecular-weight forms of T antigen in various SV40-infected permissive, semipermissive, and nonpermissive cell lines.

C. Prives, Y. Gluzman, E. Gilboa, M. Revel, and E. Winocour, Virology Department, Weizmann Institute of Science, Rehovot, Israel: The synthesis of early and late SV40 polypeptides.

K. Shah, H. Ozer, H. Ghazey, and T. Kelly, Jr., Department of Pathobiology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland; The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: A common structural antigen of papovaviruses of the SV40 polyoma subgroup.

W. Gibson, W. Eckhart, T. Hunter, and B. Cogen, Tumor Virus Laboratory, The Salk Institute, San Diego, California: Altered virion proteins of a late temperature-sensitive mutant of polyoma virus *ts*59

SATURDAY MORNING, August 21

Session 8: DNA-protein interactions

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

A.J. Robinson, D.M.K. Rekosh, A.J.D. Bellet, and W.C. Russell, Imperial Cancer Research Fund, London

England, and National Institute for Medical Research, London, England: Characterization of A protein bound to the ends of adenovirus-type-5 DNA.

P.A. Sharp, C. Moore, and J.L. Haverty, Center for Cancer Research, MIT, Cambridge, Massachusetts: The infectivity of adenovirus-5 DNA-protein complex.

T.R. Broker and L.T. Chow, Cold Spring Harbor Laboratory, New York: Visualization of the terminal proteins of adenovirus-2 DNA with ferritin-biotin:avidin labels.

M. Wu and H. Kasamatsu, Division of Chemistry and Chemical Engineering and Division of Biology, California Institute of Technology, Pasadena: A nicked DNA-protein complex isolated from simian virus 40.

B. Ponder and L. Crawford, Department of Molecular Virology, Imperial Cancer Research Fund, London, England: Positioning of the nucleosomes in polyoma and SV40 nucleoprotein complexes.

J. Corden and G. Pearson, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: Adenovirus chromatin.

A.A. Qureshi and P. Bourgaux, Département de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada: Polyoma and SV40 nucleoprotein complexes from productively infected cells.

M.P. DiLauro, D. Livingston, and R. Martin, Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland, and Sidney Farber Cancer Center, Harvard Medical School, Boston, Massachusetts: SV40 chromatin interaction with T antigen.

K. Segawa, N. Yamaguchi, and K. Oda, Department of Tumor Virus Research, Institute of Medical Science, Tokyo, Japan: Phosphorylation of chromatin- and ribosome-associated proteins in SV40-transformed cells.

SATURDAY AFTERNOON, August 21

Session 9: Adenoviruses—Proteins

Chairperson: A. Levine, Princeton University, Princeton, New Jersey

T. Hunter and G. Walter, Tumor Virology Laboratory, The Salk Institute, San Diego, California: In vitro synthesis of SV40-specific proteins.

D. Klessig, Cold Spring Harbor Laboratory, New York: Isolation and characterization of a variant of human adenovirus serotype 2 (Ad2) which multiplies efficiently on monkey cells.

A. Levinson, A.J. Levine, and J. Williams, Princeton University, New Jersey: Immunoprecipitation and identification of the group-C adenovirus tumor antigens.

W.W. Chin, A.M. Lewis, Jr., and J.V. Maizel, Jr., Laboratory of Molecular Genetics, NICHHD, and Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: The composition of adenovirus-2 T antigen.

A.K. Biron and K. Raska, Jr., CMDNJ-Rutgers Medical School, Piscataway, New Jersey: Purification of T antigen from adenovirus-type-12-transformed hamster cells.

D.M.K. Rekosh and W.C. Russell, National Institute for Medical Research, Mill Hill, London, England: Proteolytic cleavage of the major core protein precursor in adenovirus type 5.

E.B. Carstens and J. Weber, Department of Microbiology, Centre Hospitalier Universitaire, University of Sherbrooke, Quebec, Canada: Pleotropic effects of a hexon defective mutant.

Z. Gilead, K. Sugawara, Y. Jeng, H.M. Rho, M. Harter, W. Wold, and M. Green, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Tentative identification of adenovirus-2-induced early proteins involved in cell transformation.

K. Sugawara, Z. Gilead, and M. Green, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Studies on the adenovirus-2 coded DNA binding protein—Purification, characterization, and immunofluorescent visualization in infected and transformed cells.

SUNDAY MORNING, August 22

Session 10: Adenoviruses—Transcription and replication

Chairperson: P.A. Sharp, Massachusetts Institute of Technology, Cambridge, Massachusetts

M. Arens, T. Yamashita, R. Padmanabhan, T. Tsuruo, and M. Green, Institute for Molecular Virology, S Louis University School of Medicine, Missouri: Characterization of the in vitro products and enzym components of a soluble adenovirus-2 DNA replication complex.

B. Weingärtner, R. Schilling, and E.-L. Winnacker, Institute of Genetics, University of Cologne, Federa Republic of Germany: Strand-specific initiation and termination of adenovirus-type-2 DNA replication.

J. Pan, M. Celma, and S.M. Weissman, Department of Human Genetics, Yale University School of Medicine New Haven, Connecticut: Nucleotide sequences at the ends of VA RNA.

R.E. Gelinas and R.J. Roberts, Cold Spring Harbor Laboratory, New York: 5′-Terminal oligonucleotides fron adenovirus-2-infected cells.

N.W. Fraser and E. Ziff, Department of Molecular Cell Biology, The Rockefeller University, New York Analysis of 3′-terminal regions of late Ad2 mRNAs.

S.G. Zimmer, E.A. Craig, M. Sayavedra, and H.J. Raskas, Department of Pathology, Washington Universit School of Medicine, St. Louis, Missouri: In vivo and in vitro studies of adenovirus-2 nuclear RNAs.

S.J. Flint, S.M. Berget, and P.A. Sharp, Center for Cancer Research, MIT, Cambridge, Massachusetts: The siz of adenovirus-2 early and transformed cell mRNA species.

P. Neuwald, J. Meyer, J.V. Maizel, and H. Westphal, Laboratory of Molecular Genetics, NICHHD, Nationa Institutes of Health, Bethesda, Maryland: Mapping of R loops generated by adenovirus-type-2 messenge RNA.

W. Büttner, Z. Veres-Molnar, and M. Green, Institute for Molecular Virology, St. Louis University School o Medicine, Missouri: Preparative isolation and mapping of adenovirus-2 early messenger RNA species.

PHYCOMYCES

arranged by
RICHARD SUTTER, *West Virginia University*
MAX DELBRÜCK, *California Institute of Technology*

33 participants

SATURDAY AFTERNOON, August 21

Session 1

Chairperson: E. Cerdá-Olmedo, Universidad de Sevilla, Sevilla, Spain

H.I. Yaxán, Departamento de Genética, Facultad de Ciencias, Universidad de Sevilla, Spain: Prote variability in *Phycomyces* wild types.

A.P. Eslava and M.I. Alvarez, Max-Planck-Institut für Molekulare Genetik, West Berlin, German Mutagenesis in *Phycomyces*.

C. Pueyo and E. Cerdá-Olmedo, Departamento de Genética, Facultad de Ciencias, Universidad de Sevill Spain: Genetic classification of lethality induced by several fungicides, ICR-170, and heat.

F.J. Murillo and S. Torres-Martínez, Departamento de Genética, Facultad de Ciencias, Universidad d Sevilla, Spain: Mutational analysis of the carotene pathway.

M. Delbrück, Division of Biology, California Institute of Technology, Pasadena: The *dar* paradox.

K. Brunke and R. Mercurio, Department of Microbiology, University of Pennsylvania, Philadelphia: Proc dure for isolation of auxotrophic mutants in *Phycomyces*.

K. Bergman and W. Pieciak, Department of Biology, Northeastern University, Boston, Massachuset Biochemical genetics of a β-glucosidase from *Phycomyces*.

SUNDAY MORNING, August 22

Session 2

Chairperson: E. D. Lipson, California Institute of Technology, Pasadena, California

T. Ootaki, Biology Department, Yamagata University, Yamagata, Japan: Complementation tests between (+) *Phycomyces* mutants with abnormal phototropisms.

A.P. Eslava, M.I. Alvarez, E.D. Lipson, D.E. Presti, and K. Kong, Division of Biology, California Institute of Technology, Pasadena: Recombination between *Phycomyces* photomutants.

E.D. Lipson and S.M. Block, Division of Biology, California Institute of Technology, Pasadena: Kinetics of adaptation in *Phycomyces* light responses.

. Reissig and M. Delbrück, Division of Biology, California Institute of Technology, Pasadena: Riboflavin analog effects on *Phycomyces*.

E.D. Lipson and D.E. Presti, Division of Biology, California Institute of Technology, Pasadena: Light-induced absorbance changes in *Phycomyces* photomutants.

M. Delbrück, A. Katzir, and D. Presti, Divisions of Biology and Engineering, California Institute of Technology, Pasadena: Responses of *Phycomyces* indicating optical excitation of the lowest triplet state of riboflavin.

.J. Cohen, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville: Cyclic nucleotide responses of *Phycomyces*.

). La Pointe and Robert J. Cohen, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville: Polyamine levels during the light growth response of *Phycomyces*.

.P. Fischer, H. Lyle, and K.S. Thomson*, Division of Biology, California Institute of Technology, Pasadena; *Fachbereich Biologie, Universität Kaiserslautern, Federal Republic of Germany: The chitin synthetase system in *Phycomyces*.

.W. Foster, M. Pene, and H.C. Berg, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: The technology and photophysiology of *Halobacterium halobium*.

. Delbrück and D. Presti, Division of Biology, California Institute of Technology, Pasadena: Tropic behavior of *carB, carR,* and *carBcarR* mutants.

. Lafay and J. Matricon, Laboratoire Biologie Experimentale, Orsay, France: Reconsidering avoidance and house response.

MONDAY MORNING, August 23

ssion 3

airperson: R. P. Sutter, West Virginia University, Morgantown, West Virginia

. Whitaker and R.P. Sutter, Department of Biology, West Virginia University, Morgantown: Trisporic acid biosynthesis in *Phycomyces blakesleeanus*.

.E. Nelson and R.P. Sutter, Department of Biology, West Virginia University, Morgantown: Zygophore development in select mucoraceous fungi.

. Jockusch and T. Wurtz, Biocenter, Basel, Switzerland and Max-Planck-Institut, Tübingen, Federal Republic of Germany: Mutants of *Muco mucedo* affected in their gamone responses.

D. Bu'lock and B.E. Jones, Microbial Chemistry Laboratory, The University, Manchester, England: Cyclic AMP in *Mucor* spp.

L. O'Donnell, G.R. Hooper, and W.G. Fields, Northern Regional Research Center, ARS, USDA, Peoria, Illinois: Zygosporogenesis in *Phycomyces blakesleeanus* Burgeff.

.H. Schroeder and D. Tsongas, Division of Biology, California Institute of Technology, Pasadena: Some new observations during the sexual differentiation of *Phycomyces*.

Cerdá-Olmedo, Departamento de Genética, Universidad de Sevilla, Spain: Nuclear interaction in the sexual cycle.

. Gauger, School of Life Sciences, University of Nebraska, Lincoln: Recombinant progeny from immature zygotes of *Rhizopus stolonifer*.

Cerdá-Olmedo, Departamento de Genética, Universidad de Sevilla, Spain: Germspore formation.

BACTERIOPHAGE

arranged by
AHMAD I. BUKHARI, *Cold Spring Harbor Laboratory*
ELISABETH LJUNGQUIST, *Cold Spring Harbor Laboratory*

136 participants

TUESDAY EVENING, August 24

Session 1: P2-P4 interaction; Focus on the life cycles of P22, P1, and Mu

L. Tinelli, G. Dehó, and R. Goldstein, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Studies on the interference exerted by satellite phage P4 on its helper phage P2.

G. Dehó, L. Tinelli, and R. Goldstein, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Satellite phage P4—Characterization of a mutant affected in the ability to derepress helper prophage P2.

S. Valla and B.H. Lindqvist, Institute of Medical Biology, University of Tromso, Norway: P4-induced activation of P2 transcription in vitro.

D. Botstein and P. Grisafi, Department of Biology, Massachusetts Institute of Technology, Cambridge: Overproduction of *c2* repressor by *cly* mutants of phage P22.

M.M. Susskind, Department of Microbiology, University of Massachusetts Medical School, Worcester: Mechanism of action and control of P22 antirepressor.

J.R. Scott, M.M. Kropf, and R.H. Chesney, Department of Microbiology, Emory University, Atlanta, Georgia: A gene affecting maintenance of the plasmid state of P1 and P7 (ϕAmp).

R.H. Chesney and J.R. Scott, Department of Microbiology, Emory University, Atlanta, Georgia: Suppression of *dna*A⁻ by P1 and P7.

H. Khatoon and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: A mutant of bacteriophage Mu that is temperature sensitive for establishment but not for maintenance of lysogeny.

H. Khatoon and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: Excision of the *X* mutants of prophage Mu.

E. Ljungquist and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: Most of the parental Mu DNA retains its free ends after infection of host cells.

WEDNESDAY MORNING, August 25

Session 2: The λ life cycle—N gene function; Promotion and termination

M. Rosenberg and D. Court, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland: A nucleotide sequence common to p_R and p_L transcripts—A possible *N* recognition site.

C. Dambly, L. Desmet, and F. Salomon, Laboratory of Genetics, Department of Molecular Biology, Université libre de Bruxelles, Rhode St. Genèse, Belgium: The so-called paradox of λ*imm*21.

J.S. Salstrom, M. Fiandt, and W. Szybalski, McArdle Laboratory, University of Wisconsin, Madison: Isolation of λN^{21}*imm*434*nin*5 recombinants.

J.S. Salstrom and W. Szybalski, McArdle Laboratory, University of Wisconsin, Madison: Revertants of phage lambda *nut*L mutants.

J. Greenblatt and P. Malnoe, Department of Molecular Biology, University of Geneva, Switzerland: Studies on the gene *N* protein of bacteriophage λ.

G. Cesareni, S. Nasi, and E. Calef, Centro degli Acidi Nucleici, Consiglio Nazionale delle Ricerche, Rome, Italy: Ambers in the *Ai* gene.

R. Maurer and M. Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Function and control of the P_{RM} promoter of phage λ in vivo.

M. Rosenberg and D.L. Wulff,* National Cancer Institute, Bethesda, Maryland; *Department of Molecular

Biology and Biochemistry, University of California, Irvine: Termination of rightward transcription in the lambda *y* region.

D. Court, M. Rosenberg, B. de Crombrugghe, C. Brady, and D. Wulff,* Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland; *Department of Molecular Biology and Biochemistry, University of California, Irvine: Transcription termination at a Rho-dependent signal in vivo and in vitro.

P.J. Anevski, D.A. Wilder, H.A. Lozeron, and D. Apirion,* Department of Biochemistry, St. Louis University, Missouri; *Department of Microbiology, Washington University, St. Louis, Missouri: Supression of bacteriophage lambda gene *N* mutations in a polar suppressor host deficient in ribonuclease III activity.

WEDNESDAY EVENING, August 25

Session 3: The λ life cycle—Immunity establishment; Integration-excision

M. Jones and I. Herskowitz, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: *c*III-independent mutants of λ.

S. Hayes and C. Hayes, Department of Molecular Biology and Biochemistry, University of California, Irvine: Control of repressor establishment transcription.

A. Honigman, S.L. Hu, and W. Szybalski, McArdle Laboratory, University of Wisconsin, Madison: Antitermination of RNA leader sequences as the regulatory mechanism for the establishment of lysogeny by coliphage λ.

A.B. Oppenheim, A. Oppenheim, N. Katzir, and M. Belfort, Department of Microbiological Chemistry, The Hebrew University–Hadassah Medical School, Jerusalem, Israel: Regulation of the Int gene bacteriophage λ.

S. Chung and H. Echols, Department of Molecular Biology, University of California, Berkeley: Activation of integrative but not excisive recombination by the *c*II gene of phage λ.

S. Adhya,* D. Court,* H.A. Nash,† and L. Enquist,** *NCI, †NIMH, **NICHHD, National Institutes of Health, Bethesda, Maryland: Regulation of the genes for λ integration and excision.

M. Kotewicz, S. Chung, Y. Takeda, and H. Echols, Department of Molecular Biology, University of California, Berkeley: Int protein of phage λ—Purification and regulation.

K. Mizuuchi, M. Gellert, R. Weisberg, and H. Nash, National Institutes of Health, Bethesda, Maryland: Characterization of integrative recombination of phage λ DNA in vitro.

THURSDAY MORNING, August 26

Session 4: λ Proteins; Various λ and host functions

A. Folkmanis, Y. Takeda, and H. Echols, Department of Molecular Biology, University of California, Berkeley: In vitro studies of the λ*cro* product.

G. Sutcliffe, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Purified virions may contain a control protein bound to an operator.

C. Epp and M. Pearson, Department of Medical Genetics, University of Toronto, Canada: Identification of more λ early gene products.

P. Toothman and I. Herskowitz, Institute of Molecular Biology, University of Oregon, Eugene: The effect of Rex on λ development.

L. Debrouwere, M. Zabeau, J. Schell and M. Van Montagu, Laboratory of Genetics, State University of Gent, Belgium: *Ral* mutations in λ.

R.E. Malone, M.M. Stahl, D.K. Chattoraj, and F.W. Stahl, Institute of Molecular Biology, University of Oregon, Eugene: Chi recombination hot spot sites.

S. Gottesman, Department of Biology, Massachusetts Institute of Technology, Cambridge: Effects of *E. coli* *deg*⁻ mutants on lambda growth.

A. Das, B. Citron, D. Court, and S. Adhya, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland: Pleiotropic properties of rho mutants of *Escherichia coli*.

H.H. Liebke, A. Pao, and J. Speyer, Department of Biological Sciences, University of Connecticut, Storrs: A sigma mutant of RNA polymerase.

Session 5: A. Functional mRNA; λ DNA replication
B. Morphogenesis I

K.K. Mark, L.C. Tsui, Y.P. Yu, K.C. Luk, C.K. Lau, C.L. Choi, K.C. Li, and K.S. Ho, Department of Biology, New Asia College, The Chinese University of Hong Kong: Effect of multiplicity of infection on gene expression.

A. Oppenheim and A.B. Oppenheim, Department of Microbiological Chemistry, The Hebrew University–Hadassah Medical School, Jerusalem, Israel: Functional analysis of early lambda mRNA degradation.

T.J. Pollock and I. Tessman, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Decayons—Functional decay units of phage S13 messenger RNA.

M.E. Furth,* C. McLeester,* and J. Yates,† *McArdle Laboratory and †Enzyme Institute, University of Wisconsin, Madison: Recombinants of phages λ and ϕ80 or 82 with hybrid replication proteins.

M.E. Furth,* W.F. Dove,* B. Williams,† and F.R. Blattner,† *McArdle Laboratory and †Department of Genetics, University of Wisconsin, Madison: Mapping the origin of DNA replication of phage λ.

L.C. Gosule and D.K. Chattoraj, Institute of Molecular Biology, University of Oregon, Eugene: Compact form of DNA induced by polyamines.

M. Syvanen, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: DNA packaging steps with bacteriophage lambda.

N. Sternberg and R. Weisberg,* NICHHD, National Institutes of Health, Bethesda; *Frederick Cancer Research Center, Frederick, Maryland: A model for the packaging of λ DNA.

FRIDAY MORNING, August 27

Session 6: Morphogenesis II

A.R. Poteete and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: P22 proheads in in vitro DNA packaging.

P. Youderian and J. King, Massachusetts Institute of Technology, Cambridge: λ Tail length determination.

D. Shore, L. Tinelli, G. Dehó, J. Tsipis, and R. Goldstein, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Head size determination by satellite bacteriophage P4—Characterization of a P4 *sid* (size direction) mutant which makes large capsids equal in size to that of its helper P2.

M.K. Showe, E. Isobe, and L. Onorato, Department of Microbiology, Biozentrum der Universität Basel, Switzerland: T4 prehead proteinase—Identification and characterization of the purified enzyme and its cleavage from the T4 gene 21 protein.

J.R. Paulson and U.K. Laemmli, Department of Biochemical Sciences, Princeton University, New Jersey: The structure of the morphogenetic core of the T4 head.

S.C. Kayman and J. King, Massachusetts Institute of Technology, Cambridge: Distribution of suppressors of Ts phenotypes in the T4 baseplate.

P.B. Berget and J.A. King, Massachusetts Institute of Technology, Cambridge: Characterization of T4 baseplate assembly intermediates.

Y. Yamada, J. Silnutzer, and D. Nakada, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: A host mutation which blocks T7 phage assembly.

T. Isobe, L. W. Black, and A. Tsugita, Abteilung Microbiologie, Biozentrum der Universität Basel, Switzerland: Specificity of the assembly-dependent cleavage in bacteriophage T4.

FRIDAY EVENING, August 27

Session 7: DNA recombination-replication

P.J. Vlachopoulou and P.D. Sadowski, Department of Medical Genetics, University of Toronto, Canada: A physical assay for bacteriophage T7 recombination in vitro.

W.K. Holloman and C.M. Radding, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut: Promotion of a common step in genetic recombination by superhelical DNA and the *recA* gene.

H. Fujisawa and M. Hayashi, Department of Biology, University of California, San Diego: Functions of gene *C* and gene *D* products of bacteriophage ϕX174.

E.S. Tessman and P.K. Peterson, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Evidence for a complex composed of *rep* protein and ϕX174 F and A proteins which acts late in infection.

P.D. Baas, W. Keegstra, and H.S. Jansz, Institute of Molecular Biology and Laboratory for Physiological Chemistry, State University, Utrecht, The Netherlands: Mechanism of bacteriophage ϕX174 RF DNA replication.

E.S. Tessman and P.K. Peterson, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Gene *A* protein is required for single-stranded DNA synthesis of phage S13.

D.K. Chattoraj, Microbial Genetics Laboratory, Karolinska Institutet, Stockholm, Sweden: A strand-specific break near the origin of bacteriophage P2 DNA replication.

J. Glassberg and C.R. Stewart, Department of Biology, Rice University, Houston, Texas: Initiation and termination of SPO1 DNA replication.

K. Denniston-Thompson and W. Ginoza, Department of Microbiology and Cell Biology, The Pennsylvania State University, University Park: Multiple sites of initiation of T4 DNA synthesis.

SATURDAY MORNING, August 28

Session 8: T4

D. Belin and R.H. Epstein, Département de Biologie Moléculaire, Université de Genève, Switzerland: A temperature-sensitive mutation which affects the synthesis of the mutant gene product.

J.D. Childs, Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Ontario: T4 mutants unable to grow on streptomycin resistant hosts.

C.G. Goff, Biology Department, Haverford College, Pennsylvania: T4-induced ADP-ribosylation of *E. coli* RNA polymerase.

O. Sköld and C. Linder, Department of Microbiology, Faculty of Pharmacy, University of Uppsala, Sweden: Evidence for a diffusible T4 phage protein governing the initiation of delayed early RNA synthesis.

M.F. Mowrey and J.S. Wiberg, Department of Radiation Biology and Biophysics, University of Rochester, New York: Basis for decreased production of T4D *regA*$^{-}$ mutants at high temperature.

E. Kutter, L. Snyder,* B. Guttman, and R. Bestwick, The Evergreen State College, Olympia, Washington; *Michigan State University, East Lansing: Mutants permitting the production of viable phage in which C replaces HMC.

D. Bradley, E. Kutter, B. Guttman, and N. Mesta, The Evergreen State College, Olympia, Washington: Effects of cytosin in T4 DNA on T4 early-enzyme synthesis.

K.E. Abremski and L.W. Black, Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore: A possible role for bacteriophage T4 internal protein I in the infection of *E. coli* CT596.

SATURDAY AFTERNOON, August 28

ession 9: Manipulations of phage genomes; Physical mapping

).K. Chattoraj, Y.K. Oberoi, and G. Bertani, Microbial Genetics Laboratory, Karolinska Institutet, Stockholm, Sweden: *Eco*RI cleavage of bacteriophage P2 DNA in vivo and in vitro.

.W. Schumm,* D.D. Moore, F.R. Blattner, and M.M. Howe,* *Department of Bacteriology and Department of Genetics, University of Wisconsin, Madison: In vitro construction of λ-Mu hybrids.

\. Sternberg, D. Tiemeier, and L. Enquist, NICHHD, National Institutes of Health, Bethesda, and Frederick Cancer Research Center, Frederick, Maryland: In vitro packaging of *Eco*RI DNA fragments in a λ *D*am vector.

N. Sternberg, D. Tiemeier, and L. Enquist, LMG, NICHHD, National Institutes of Health, Bethesda, and Frederick Cancer Research Center, Frederick, Maryland: The in vitro construction of λP1 hybrids.

R.J. Mural, T. Yun, and D. Vapnek, Program in Genetics and Departments of Zoology, Biochemistry, and Microbiology, University of Georgia, Athens: Physical and genetic mapping of cloned P1 DNA fragments generated by restriction endonucleases.

F. DeBruijn and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: Insertion elements in mutants of bacteriophages Mu and P1.

L.T. Chow and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: The invertible G sequence of phage Mu DNA is identical to the invertible sequence of phage P1 DNA.

L.T. Chow, R. Kahmann, and D. Kamp, Cold Spring Harbor Laboratory, New York: Electron-microscopic studies of nondefective Mu phage containing deletions or substitutions in the G and/or β regions

SUNDAY MORNING, August 29

Session 10: PRR1; T1, P22, T7 mutants; Nucleotide reactions; EM techniques

P. Dhaese, J. Vandekerckhove, and M. Van Montagu, Laboratorium voor Histologie en Genetika, Rijksuniversiteit Gent, Belgium: Studies on PRR1, an RNA phage specific for broad-host-range P-group plasmids.

M.D. Roberts and H. Drexler, Department of Microbiology and Immunology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina: Transducing mutants of T1.

M. Ruddel and V. Israel, Medical College of Georgia, Augusta: Isolation of a bacteriophage P22 mutation reversibly temperature sensitive in both an early and a late step.

J.E. Mott and J. Speyer, Department of Biological Sciences, University of Connecticut, Storrs: The growth inhibition and release of inhibition of T7 and lambda on P2 lysogens.

J. Davison and F. Brunel, MRC Virology Unit and Beatson Institute for Cancer Research, Glasgow, Scotland: Restriction insensitivity in bacteriophage T5.

H.R. Warner, T.J. Mozer, and S. Rakow, Department of Biochemistry, University of Minnesota, St. Paul: Metabolism of deoxyribonucleotides after infection of *Escherichia coli* with bacteriophage T5.

A.R. Shaw and D.J. McCorquodale, Department of Biochemistry, Medical College of Ohio, Toledo: Decreased levels of nucleoside triphosphates after BF23-infection of *Escherichia coli* carrying Col Ib plasmids.

I. Tessman, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: A mechanism of UV reactivation.

T.R. Broker and L.T. Chow, Cold Spring Harbor Laboratory, New York: Visualization of the terminal proteins of *B. subtilis* phage ϕ29 DNA with ferritin-biotin:avidin labels.

K. Calame and G. Ihler, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: EM visualization of DNA-ribosome initiation complexes.

HERPESVIRUSES

arranged by
JOSEPH S. PAGANO, *University of North Carolina*
JOHN SUBAK-SHARPE, *University of Glasgow*

173 participants

TUESDAY EVENING, August 31

Session 1: DNA structure I

Chairpersons: P. Sheldrick, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France
P. Wildy, University of Cambridge, Cambridge, England

P. Sheldrick, N. Berthelot, and S. Chousterman, I.R.S.C., Villejuif, France: Channel catfish virus—A "minimal" herpesvirus?

D. Hayward, W. King, and E. Kieff, Department of Medicine, University of Chicago, Illinois: The DNA of Epstein-Barr viruses—Restriction-enzyme analysis.

J.E. Shaw and J.S. Pagano, Cancer Center Program and Departments of Medicine and Bacteriology, School of Medicine, University of North Carolina, Chapel Hill: Analysis of the Epstein-Barr-virus DNA synthesized in superinfected Raji cells.

Y. Yajima, Y.-S. Lee, and M. Nonoyama, Departments of Microbiology. Rush-Presbyterian–St. Luke's Medical Center and University of Illinois Medical Center, Chicago, Illinois: Characterization of Epstein-Barr-virus DNA obtained after superinfection of Raji cells with HRI EB virus.

G.W. Bornkamm,* H. Delius,† B. Fleckenstein,* C. Mulder,** and H. zur Hausen,* *Institute für Klinische Virologie, Universität Erlangen, Federal Republic of Germany; †EMBO Laboratorium, Heidelberg, Federal Republic of Germany; **University of Massachusetts Medical School, Worcester: Characterization of Epstein-Barr-virus DNA from P3HR-1 and B 95-8 cells.

A. Adams, G. Bjursell, C. Kaschka-Dierich, L. Falk, and T. Lindahl, Departments of Tumor Biology, Biochemistry, and Chemistry, Karolinska Institute, Stockholm, Sweden: Strain differences in Epstein-Barr virus.

M. Andersson, A. Adams, and T. Lindahl, Departments of Chemistry and Tumor Biology, Karolinska Institute, Stockholm, Sweden: Integrated sequences of Epstein-Barr-virus DNA in human lymphoid cell lines.

C. Mulder, B. Fleckenstein, G.W. Bornkamm, F.J. Werner, H. Delius, and J. Simonds, University of Massachusetts Medical School, Worcester; Institut für Klinische Virologie, Universität Erlangen, Max-Planck-Institut für Biochemie, Munich, and EMBO Laboratorium, Heidelberg, Federal Republic of Germany; National Cancer Institute, Bethesda, Maryland: Structural analysis of *Herpesvirus saimiri* and *Herpesvirus ateles* DNA.

WEDNESDAY MORNING, September 1

Session 2A: DNA structure II

Chairpersons: H. zur Hausen, Institut für Klinische Virologie, Erlangen, Federal Republic of Germany
P. Schaffer, Baylor College of Medicine, Houston, Texas

B.A. Kilpatrick and E.-S. Huang, Department of Bacteriology and Medicine, and Cancer Research Center, University of North Carolina, Chapel Hill: Structural analysis of cytomegalovirus genomes.

G.S. Hayward, T.G. Buchman, and B. Roizman, Department of Microbiology, University of Chicago, Illinois: Cleavage maps of HSV-1 and HSV-2 DNA molecules.

N.M. Wilkie, R. Cortini, D. Powell, and J.B. Clements, Institute of Virology, Glasgow, Scotland: Sequence arrangement and physical maps for herpesvirus DNAs.

J. Skare and W.C. Summers, Yale University School of Medicine, New Haven, Connecticut: Restriction-endonuclease cleavage maps of the DNA of the KOS strain of HSV-1.

N. Frenkel and H. Locker, Department of Biology, University of Chicago, Illinois: Origin and coding capacity of defective herpes simplex virus DNA.

H.C. Kaerner, C.H. Schröder, A. Ott, B. Stegmann, K. Munk, and G. Darai, Institute of Virus Research, German Cancer Research Center, Heidelberg, and Institute of Medical Virology, University of Heidelberg, Federal Republic of Germany: A defective genotype of HSV ANG.

Session 2B: Genetics

J.T. Jofre, V.C. Carter, M.C. Timbury,* and P.A. Schaffer, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas; *Institute of Virology, University of Glasgow, Scotland: Genetic studies with temperature-sensitive mutants of herpes simplex virus.

N.D. Stow, J.H. Subak-Sharpe, and N.M. Wilkie, Institue of Virology, University of Glasgow, Scotland: Rescue of selected genetic markers from restriction-endonuclease-produced fragments of herpes simplex virus type 1 DNA.

W.P. Summers, W.C. Summers, and K.J. Cremer, Yale University School of Medicine, New Haven, Connecticut: The thymidine kinase gene of HSV-1.

J. Bookout, D.J.M. Purifoy, P.A. Schaffer, and N. Biswal, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: Rescue of genetic information by temperature-sensitive mutants from purified defective DNA of HSV-1.

S. Moira Brown, D.A. Ritchie, A.T. Jamieson, and J.H. Subak-Sharpe, Institute of Virology, Glasgow, Scotland: The effect of 5-fluorodeoxyuridine on partial "HETS" and on recombination.

M.K. Howett and F. Rapp, The Pennsylvania State University College of Medicine, Hershey: Characterization of host-range temperature-sensitive mutants of herpes simplex virus type 2.

G.S. Read, S. Person, and P.M. Keller, Laboratory of Biophysics, Department of Biochemistry and Biophysics, Penn State University, University Park, Pennsylvania: Genetic analysis of cell fusion induced by syncytia-producing mutants of herpes simplex virus type 1.

WEDNESDAY EVENING, September 1

Session 3: Replication

Chairpersons: B. Hirt, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland
T. Ben-Porat, Vanderbilt University School of Medicine, Nashville, Tennessee

T. Ben-Porat and J.-H. Jean, Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee: Studies on the replication of parental pseudorabies (Pr) viral DNA.

D.J. O'Callaghan, J.C. Cohen, G.P. Allen, and C.C. Randall, Department of Microbiology, University of Mississippi Medical Center, Jackson: Studies of the replication and transcription of equine herpesvirus type 1.

R.J. Jacob and B. Roizman, Departments of Microbiology and Biophysics/Theoretical Biology, University of Chicago, Illinois: The structure of replicating herpes simplex DNA molecules.

I. Hirsch, N. Biswal, J. Roubal,* and V. Vonka,* Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas; *Institute of Sera and Vaccines, Prague, Czechoslovakia: Replicating DNA of herpes simplex virus type 1.

J. Shlomai, A. Friedmann, and Y. Becker, Laboratory for Molecular Virology, Hebrew University–Hadassah Medical School, and Department of Genetics, Hebrew University, Jerusalem, Israel: Replicative intermediates of herpes simplex virus DNA.

T. Seebeck and J.S. Pagano, Cancer Research Center, University of North Carolina, Chapel Hill: In vitro synthesis of Epstein-Barr-virus DNA.

Y. Becker, Y. Asher, and J. Shlomai, Laboratory for Molecular Virology, Hebrew University–Hadassah Medical School, Jerusalem, Israel: In vitro synthesis of viral DNA in nuclei isolated from cells infected with wild-type and a phosphonoacetic-acid-resistant mutant of herpes simplex virus.

M.L. Fenwick, Department of Microbiology and Biophysics/Theoretical Biology, University of Chicago, Illinois: Synthesis of virus-specific proteins in enucleated cells.

THURSDAY MORNING, September 2

Session 4: Protein I

Chairpersons: P. Spear, University of Chicago, Chicago, Illinois
S. Klein, Karolinska Institute, Stockholm, Sweden

D. Baron and J.L. Strominger, Sidney Farber Cancer Center, Harvard Medical School, Boston, Massachusetts: Heat stability of the Epstein-Barr nuclear antigen.

H. zur Hausen, K.O. Fresen, and B. Merkt, Institut für Klinische Virologie, Erlangen, Federal Republic of Germany: Heterogeneity of EBNA induction by P3HR-1 virus.

G. Klein, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden: EBV-cycle and surface marker studies on somatic-cell hybrids and on EBV-converted sublines of virus-DNA negative lymphomas.

M.F. Stinski, D.R. Thomsen, and E.S. Mocarski, Department of Microbiology, University of Iowa, Iowa City: Membrane glycoproteins of human cytomegalovirus.

L.F. Velicer and R.L. Witter,* Michigan State University and *USDA Regional Poultry Research Laboratory, East Lansing, Michigan: Physical, chemical, and immunological characterization of antigens from Marek's disease herpesvirus infected cells.

R.F. Naegele and A. Granoff, Laboratories of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: The detection of LHV-associated membrane antigen(s) on Lucké tumor cells.

R.A. Killington, R.E. Randall, and D.H. Watson, Department of Microbiology, School of Medicine, The University of Leeds, England: Studies on the type-specific antigens of herps simplex virus types 1 and 2.

H. Ludwig, G. Pauli, and M.D. Daniel,* Institute of Virology, Justus Liebig University, Giessen, Federal Republic of Germany; *New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts: Common antigenic components of different herpesviruses.

R.W. Knowles, S. Person, G.S. Read, T. Holland, and P.M. Keller, Biophysics Laboratory, Department of Biochemistry and Biophysics, Penn State University, University Park, Pennsylvania: A correlation between the inhibition of cell fusion and the appearance of surface glycoproteins in HSV-1-infected cells.

R.J. Courtney, J.W. Burek, and R. Eberle, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: Biochemical and immunological studies of herpes simplex virus type 1 and type 2 glycoproteins resolved by sodium dodecyl sulfate–hydroxylapatite chromatography.

J.C. Glorioso and J.W. Smith, Department of Microbiology, Louisiana State University Medical Center, New Orleans: Expression of cell-surface antigens in HSV-1 and HSV-2 infected BHK-21 cells.

H.S. Marsden, R.G. Hope, J. Hay, and M.C. Timbury, M.R.C. Virology Unit and Department of Virology, University of Glasgow, Scotland: The polypeptide profiles of temperature-sensitive mutants of HSV-2.

THURSDAY EVENING, September 2

Session 5A: Protein II

Chairpersons: D. Watson, University of Leeds, Leeds, England
J. Stevens, University of California Medical Center, Los Angeles, California

J. Hay, H.S. Marsden, and R.T. Hay, Institute of Virology, University of Glasgow, Scotland: DNA-binding proteins in herpesvirus-infected cells.

E.J. Bayliss and M. Nonoyama, Departments of Microbiology, Rush-Presbyterian–St. Luke's Medical Center and University of Illinois, Chicago: Superinfection of Raji cells with Epstein-Barr virus: The induction of DNA-binding proteins.

J.S. Kim, V. Sapienza, R.I. Carp, and H.M. Moon, Department of Microbiology and Animal Experimentation, New York State Institute for Research in Mental Retardation, Staten Island: Analysis of structural polypeptides of purified human cytomegalovirus.

M. Fiala,* W. Gibson†, R.W. Honess,** and L.B. Guze†,‡ *Martin Luther King Jr. General Hospital, Los Angeles, California; †The Salk Institute, La Jolla, California; **University of Leeds, England; †‡Veterans Administration (Wadsworth) and UCLA, Los Angeles, California: Structural polypeptides of cyto megalovirus virions, dense bodies, and nucleocapsids.

Session 5B: Latency

J.G. Stevens,* K.L. Lofgren,* H.S. Marsden,† and J.H. Subak-Sharpe,† *Department of Microbiology and Immunology, University of California, Los Angles; †Institute of Virology, University of Glasgow, Scotland: Temperature-sensitive mutants of herpes simplex virus differ in the capacity to establish latent infections.

K. Cheung, D.J. Lang, and E.S. Huang, Department of Pediatrics, Duke University Medical Center, Durham, and Department of Medicine, University of North Carolina, Chapel Hill: Detection of latent murine cytomegalovirus genome in tissues of commercially supplied laboratory mice.

F. Rapp and C. Reed, Department of Microbiology, The Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey: Interaction between superinfecting herpesviruses and endogenous type-C viruses.

A.S. Rubenstein and R.G. Duff, Department of Virology, Abbott Laboratories, North Chicago, Illinois: Effects of disodium phosphonoacetate on latent herpesvirus.

J.H. Joncas, J. Menezes, and E.S. Huang, Pediatric Research Center, Ste.-Justine Hospital, University of Montreal, Canada; Virology Center, Institut Armand-Frappier, University of Quebec, Montreal, Canada; Cancer Research Center, University of North Carolina, Chapel Hill: Persistence of less than one copy per cell of the CMV genome in a human lymphoblastoid cell line.

I.T. Magrath, P.A. Pizzo, and A.S. Levine, Pediatric Oncology Branch, National Cancer Institute, Bethesda, Maryland: Increased yield of Epstein-Barr virus and EBV DNA from P_3HR1 cells stimulated with corticosteroid at 32°C.

FRIDAY MORNING, September 3

Session 6: Transformation

Chairpersons: A. Nahmias, Emory University School of Medicine, Atlanta, Georgia
F. Rapp, Pennsylvania State University, College of Medicine, Hershey, Pennsylvania

V.L. Flannery, R.J. Courtney, and P.A. Schaffer, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: Expression of a herpes simplex virus-specific antigen in HSV-transformed cells.

C.D. Copple, L.B. Chen, and J.K. McDougall, Cold Spring Harbor Laboratory, New York: Properties of HSV-2-transformed hamster cells.

R. Buttyan, J. Leiden, and P.G. Spear, Department of Microbiology, University of Chicago, Illinois: Regulation of herpes simplex virus gene expression in transformed cells.

S. Bachetti and F.L. Graham, Departments of Pathology and Biology, McMaster University, Hamilton, Ontario, Canada: Transfer of the gene for thymidine kinase to human cells by means of purified herpes simplex viral DNA.

L. Geder, R. Lausch, and F. Rapp, Department of Microbiology, The Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey: Properties of human embryo lung fibroblasts transformed in vitro by human cytomegalovirus.

J.M. DeMarchi and A.S. Kaplan, Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee: Nonproductive infection in human embryonic lung cells stimulated by CMV to synthesize cellular DNA.

K. Nazerian, E.A. Stephens, J.M. Sharma, L.F. Lee, M. Gailitis, and R.L. Witter, Regional Poultry Research Laboratory, USDA, East Lansing, Michigan: A non-producer, transplantable lymphoblastoid cell line from Marek's disease tumor.

A.A. Newton, Department of Biochemistry, University of Cambridge, England: Growth stimulation of duck fibroblasts infected with Marek's disease virus.

E. Henderson, L. Heston, and G. Miller, Department of Pediatrics and Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut: Biologic activities of transforming strains of EBV differ in their sensitivity to UV inactivation.

J. Menezes, H. Dussault, and P. Patel, Department of Microbiology and Immunology and Pediatric Research Center, University of Montreal, Quebec, Canada: Epstein-Barr virus (EBV) strains—Further differences between lymphocyte-transforming (B95-8) and nontransforming (P3HR-1) EBV strains in regard to their ability to induce nuclear antigen (EBNA) and early antigen (EA).

G. Lenoir, A. Voller,* M.C. Berthelon, M.F. Lavoué, and G. de Thé, International Agency for Research on Cancer, Lyon, France; *The Zoological Society of London, England: Studies on the characterization of Epstein-Barr virus early antigens.

D.A. Thorley-Lawson, Sidney Farber Cancer Center, Harvard Medical School, Boston, Massachusetts: Studies on the early behavior of cellular DNA after infection of human lymphocytes by Epstein-Barr virus.

F. Deinhardt, L.A. Falk, M. Nonoyama, L. Wolfe, C. Bergholz, B. Lapin, L. Yakovleva, V. Agrba, G. Henle, and W. Henle, Department of Microbiology, Rush-Presbyterian–St. Luke's Medical Center, Chicago, Illinois; Institute of Experimental Pathology and Therapy, Sukhumi, USSR; The Children's Hospital of Philadelphia, Pennsylvania: Baboon lymphotropic herpesvirus related to Epstein-Barr virus.

F. Colbère-Garapin and F. Horodniceanu, Department of Virology, Pasteur Institute, Paris, France: Oncogenic transformation of hamster cells by herpes simplex virus DNA.

L.S. Kucera and J.P. Gusdon, Departments of Microbiology and Immunology and Obstetrics and Gynecology, Bowman Gray School of Medicine, Winston-Salem, North Carolina: Oncogenic transformation of rat embryo fibroblasts with photoinactivated herpes simplex virus type 2.

J.C.M. Macnab, B. Visser, J. Hay, and A.T. Jamieson, Institute of Virology, University of Glasgow, Scotland: Tumorigenicity of HSV-2-transformed cells in immunosuppressed and immunocompetent rats.

FRIDAY AFTERNOON, September 3

Session 7: Enzymes and PAA

Chairperson: A. Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey

J.A. Boezi, S.S. Leinbach, and L.F. Lee, Department of Biochemistry, Michigan State University, and USDA, ARS Regional Poultry Research Laboratory, East Lansing, Michigan: Mechanism of phosphonoacetate inhibition of herpesvirus-induced DNA polymerase: Alternate product or dead-end inhibitor?

E.-S. Huang, S.-M. Huong, S.-T Chen, C.-H. Huang, and J. S. Pagano, Department of Medicine and Cancer Research Center, University of North Carolina, Chapel Hill: DNA polymerases of various EBV-producing, nonproducing, and superinfected lymphoblastoid cells and their sensitivities to phosphonoacetic acid.

D.J.M. Purifoy and K.L. Powell, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: Herpes simplex virus DNA polymerase associated with a virus-induced polypeptide.

R.W. Honess, Department of Microbiology, School of Medicine, The University of Leeds, England: Physiology and genetics of herpes simplex virus resistance and sensitivity to phosphonoacetic acid.

S. Kit and D.R. Dubbs, Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas: Regulation of herpesvirus thymidine kinase in cells [LM (TK)/HSV-1] transformed by UV-irradiated herpes simplex virus.

J.E. Estes and E.-S. Huang, Department of Medicine and Cancer Research Center, University of North Carolina, Chapel Hill: Thymidine kinase activity in WI38 cells infected with human CMV.

Y.-C. Cheng, Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, New York: Deoxythymidine kinase induced in HeLa TK cells by herpes simplex virus type I and type II. Substrate specificity and kinetic behavior.

T.-S. Chan, Department of Physiology, University of Connecticut Health Center, Farmington: Induction of deoxycytidine deaminase activity in a mouse cell line by infection with herpes simplex virus.

Y. Langelier and G. Buttin, Unité de Génétique Cellulaire, I.R.B.M., Paris, France: Deoxycytidylate (dCMP)-deaminase activity in hamster cells infected by herpes simplex virus—Lack of evidence for a viral isozyme.

SATURDAY MORNING, September 4

Session 8: Transcription, translation, and regulation of host function

Chairperson: B. Roizman, University of Chicago, Chicago, Illinois

P.C. Jones, G.S. Hayward, and B. Roizman, Departments of Microbiology and Biophysics/Theoretical Biology, University of Chicago, Illinois: Templates for early functions (alpha mRNA) in HSV-1 DNA are located within both the L and S segments.

W. Bodemer, M. Bodemer, and E.E. Petersen, Institute of Virology, Zentrum Hygiene der Universität Freiburg, Federal Republic of Germany: Characterization of viral mRNA from cells lytically infected with herpes simplex virus type 2 and hybridization of mRNA to *Eco*I fragmented viral DNA.

J.B. Clements, B. Perbal, and N.M. Wilkie, Institute of Virology, University of Glasgow, Scotland: Herpes simplex virus-specific transcripts in infected BHK cells.

J.R. Stringer, L.E. Holland, M.K. Rice, R.I. Swanstrom, and E.K. Wagner, Department of Molecular Biology and Biochemistry, University of California, Irvine: Metabolism of HSV-specific mRNA in infected HeLa cells.

Y. Nishioka and S.J. Silverstein, Department of Microbiology, Columbia University, New York, New York: Fate of host mRNA sequences following productive infection with HSV-1.

L.I. Pizer and P. Beard,* Department of Microbiology, University of Pennsylvania, Philadelphia; *Swiss Institute for Experimental Cancer Research, Lausanne: The effect of herpesvirus infection on mRNA in polyoma-transformed cells.

L. Pereira, M. Wolff, and B. Roizman, Departments of Microbiology and Biophysics/Theoretical Biology, University of Chicago, Illinois: α-Infected cell polypeptides of HSV-1 and HSV-2—A comparative study of their synthesis, processing, and transport.

I.W. Halliburton, Department of Microbiology, School of Medicine, University of Leeds, England: Studies on the control of protein synthesis using *ts* mutants and recombinants of HSV.

ORIGINS OF HUMAN CANCER

arranged by
J.D. WATSON, *Cold Spring Harbor Laboratory*
H. H. HIATT, *Harvard University School of Public Health*
J.A. WINSTEN, *Harvard University School of Public Health*

237 participants

TUESDAY EVENING, September 7

Welcoming remarks: J.D. Watson, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
H. H. Hiatt, Harvard University School of Public Health, Boston, Mass.

Opening address: R. Doll, University of Oxford, Oxford, England

I. INCIDENCE OF CANCER IN HUMANS

Session 1: *Effects of geography and genetic background*

Chairperson: J. Clemmesen, Danish Cancer Registry, Copenhagen, Denmark

J.W. Berg, Department of Preventive Medicine, University of Iowa, Iowa City: Worldwide variations in cancer incidence—Known, presumptive, and possible causes.

W.J. Blot, T.J. Mason, R. Hoover, and J.F. Fraumeni, Jr., Environmental Epidemiology Branch, National Cancer Institute, Bethesda, Maryland: Cancer by county—Etiologic implications.

L. Chiazze, Jr., D.L. Levin, and D.T. Silverman, Georgetown University School of Medicine, Washington, D.C., and Biometry Branch, National Cancer Institute, Bethesda, Maryland: Recent changes in estimated cancer mortality.

A.G. Knudson, University of Texas Health Science Center, Houston: Genetic predisposition to cancer.

WEDNESDAY MORNING, September 8

Session 2A: *Changing patterns*

Chairperson: J. Berg, University of Iowa, Iowa City, Iowa

T. Hirayama, Epidemiology Division, National Cancer Center Research Institute, Tokyo: Changing patterns of cancer in Japan with special reference to the decrease in stomach cancer mortality.

B. Henderson and M. Pike, University of Southern California School of Medicine, Los Angeles: Hormonal basis of breast cancer.

J. Clemmesen, Danish Cancer Registry and Department of Pathology, Finsen Institute, Copenhagen: Correlation of sites.

Session 2B: Effects of occupation

Chairperson: N. Nelson, New York University Medical Center, New York, New York

N. Nelson, Institute of Environmental Medicine, New York University Medical Center, New York: The carcinogenicity of chloro ethers and related compounds.

C. Maltoni, Institute of Oncology and Tumor Center, Bologna, Italy: Vinyl chloride carcinogenicity—An experimental model for carcinogenesis studies.

I.J. Selikoff, Environmental Sciences Laboratory, Mount Sinai School of Medicine, City University of New York: Cancer risk of asbestos exposure.

S. Hernberg, Department of Epidemiology and Biometry, Institute of Occupational Health, Helsinki, Finland: Incidence of cancer in populations with exceptional exposure to metals.

T. Norseth, Institute of Occupational Health, Oslo, Norway: Industrial viewpoints on the effects of occupation on cancer caused by metals.

WEDNESDAY AFTERNOON, September 8

Public policy panel I: Influence of the media

Judith Crighton, CBS News
Judy Randal, New York Daily News
Jonathan Spivak, The Wall Street Journal
Morton Mintz, The Washington Post

WEDNESDAY EVENING, September 8

Session 3: Effects of drugs

Chairperson: J. Fraumeni, National Cancer Institute, Bethesda, Maryland

R. Hoover, Environmental Epidemiology Branch, National Cancer Institute, Bethesda, Maryland: Effects of drugs—Immunosuppression.

R.W. Miller, Clinical Epidemiology Branch, National Cancer Institute, Bethesda, Maryland: Prenatal factors.

H. Jick, Department of Medicine, Boston University Medical Center, Massachusetts: The magnitude of the problem of drug-induced cancer.

A.L. Herbst,* R.E. Scully, S.J. Robboy, and W.R. Welch, *Department of Obstetrics and Gynecology, University of Chicago, Illinois; Department of Pathology, Massachusetts General Hospital, Boston: Abnormal development of the human genital tract following prenatal exposure to diethylstilbestrol.

N.S. Weiss, Department of Epidemiology, School of Public Health and Community Medicine, University of Washington, Seattle: Exogenous estrogens and the incidence of cancer in tissues of Müllerian origin.

M. Pike and B. Henderson, University of Southern California School of Medicine, Los Angeles: Liver adenoma and oral contraceptives.

R.H. Adamson and S.M. Sieber, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Maryland: Antineoplastic agents as potential carcinogens.

E. Bueding and R. Batzinger, Johns Hopkins University, Baltimore, Maryland: Hycanthone and other antischistosomal drugs—Lack of obligatory association between chemotherapeutic effects and mutagenic activity.

P. Goldman, J.A. Ingelfinger, and P.A. Friedman, Clinical Pharmacology Division, Department of Pharmacology, Harvard Medical School, Boston, Massachusetts: Metronidazole, isoniazid, and the threat of human cancer.

Session 4A: Effects of radiation

Chairperson: A. Upton, State University of New York, Stony Brook, New York

A.C. Upton, Department of Pathology, State University of New York, Stony Brook: Carcinogenic effects of radiation.
G.B. Hutchison, Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts: Carcinogenic effects of medical irradiation.
W.H. Ellet and A.C.B. Richardson, Criteria and Standards Division, Office of Radiation Programs, Environmental Protection Agency, Washington, D.C.: Estimates of the cancer risk due to nuclear electric power generation.
E.L. Scott and M.L. Straf, Statistical Laboratory, University of California, Berkeley; Committee on Statistics, National Academy of Sciences, Washington, D.C.: Ultraviolet radiation as a cause of cancer.

Session 4B: Effects of diet

Chairperson: T. Sugimura, National Cancer Center, Tokyo, Japan

C.A. Linsell, International Agency for Research on Cancer, Lyon, France: Field studies to evaluate a dietary carcinogen.
B.K. Armstrong, Department of Medicine, University of Western Australia, Nedlands: Epidemiologic approaches to the role of diet in carcinogenesis as exemplified by endometrial cancer.
M.J. Hill, Central Public Health Laboratory, Colindale, London: The role of unsaturated bile acids in the etiology of large-bowel cancer.
J.H. Weisburger and E.L. Wynder, American Health Foundation, Naylor Dana Institute for Disease Prevention, Valhalla, New York: On the etiology and metabolic epidemiology of the main human cancers.

THURSDAY AFTERNOON, September 9

Public policy panel II: FDA —Diethylstilbestrol

Peter Hutt, Covington and Burling, Washington, D.C.
Thomas Jukes, University of California, Berkeley
Roy Hertz, George Washington Medical School, Washington, D.C.

II. MECHANISM OF CARCINOGENESIS

THURSDAY EVENING, September 9

Session 5A: Electrophilicity I

Chairperson: E. Miller, University of Wisconsin, Madison, Wisconsin

J.A. Miller and E.C. Miller, McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison: Ultimate chemical carcinogens as reactive mutagenic electrophiles.
P.H. Magee, Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania: Evidence for the formation of electrophilic metabolites from *N*-nitroso compounds.
D.M. Jerina, H. Yagi, M. Schaefer-Ridder, R. Lehr, D.R. Thakker, A.W. Wood,* A.Y.H. LU,* D. Ryan,* S. West,* W. Levin,* and A.H. Conney,* NIAMDD, National Institutes of Health, Bethesda, Maryland; *Department of Biochemistry and Drug Metabolism, Hoffman-LaRoche, Inc., Nutley, New Jersey: Bay-

region epoxides of dihydrodiols—A unifying concept which explains the mutagenic and carcinogenic activity of polycyclic aromatic hydrocarbon metabolites.

Session 5B: Genetics and cancer

Chairperson: A. Hollaender, Associated Universities, Inc., Washington, D.C.

B.N. Ames and J. McCann, Department of Biochemistry, University of California, Berkeley: Do chemical and physical carcinogens cause cancer through damage to DNA?

R. Peto, University of Oxford, England: Rate-determining causes of human cancer which are not caused by "Ames-type" mutagens.

R.B. Setlow, Biology Department, Brookhaven National Laboratory, Upton, New York: Xeroderma pigmentosum—Damage to DNA is involved in carcinogenesis.

J.B. Little, Department of Physiology, Harvard University School of Public Health, Boston, Massachusetts: Radiation carcinogenesis *in vitro*—Implications for mechanisms.

A.M. Sincock, The Galton Laboratory, University College London, England: In vitro chromosomal effects of asbestos and other materials.

FRIDAY MORNING, September 10

Session 6A: Viruses I

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

H.E. Varmus, D. Spector, D. Stehelin, T. Padgett, C.T. Deng, and J.M. Bishop, Department of Microbiology, University of California Medical School, San Francisco: Function and origin of the transforming gene of avian sarcoma virus.

G.J. Todaro, Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, Maryland: RNA tumor virus genes (virogenes) and the transforming genes (oncogenes)—Genetic transmission, infectious spread, and modes of expression.

P. Tegtmeyer, Department of Microbiology, State University of New York, Stony Brook: Gene function of tumor virus SV40.

J.K. McDougall, Cold Spring Harbor Laboratory, New York: Tumorigenicity of cell lines transformed in vitro by adenoviruses.

B. Roizman, N. Frenkel, E. Kieff, and P. Spear, University of Chicago, Illinois: Herpesviruses and human cancer—The relevance of the association.

Session 6B: Modifying factors

Chairperson: E. Reich, The Rockefeller University, New York, New York

O. Stutman, Department of Cellular Immunobiology, Memorial Sloan-Kettering Cancer Center, New York, New York: Immune surveillance.

I.B. Weinstein, M. Wigler, and C. Pietropaolo, Institute of Cancer Research and Departments of Medicine and Microbiology, Columbia University, New York, New York: The action of tumor-promoting agents in cell culture.

R.K. Boutwell, McArdle Laboratory, University of Wisconsin, Madison: On the role of the induction of ornithine decarboxylase in tumor promotion.

M.B. Sporn, Lung Cancer Branch, National Cancer Institute, Bethesda, Maryland: Prevention of epithelial cancer by vitamin A and its synthetic analogs (retinoids).

FRIDAY AFTERNOON, September 10

Public policy panel III: FDA—Cyclamates

Arnold Brown, Mayo Clinic, Rochester, Minnesota

George R. Thompson, International Flavors and Fragrances, Union Beach, New Jersey
Richard Bates, Food and Drug Administration, Rockville, Maryland

FRIDAY EVENING, September 10

Session 7A: *Electrophilicity II*

Chairperson: I.B. Weinstein, Columbia University, New York, New York

W. Levin, A.Y.H. Lu, D. Ryan, S. West, A.W. Wood, J. Kapitulnik, D.R. Thakker,* H. Yagi,* D.M. Jerina,* and A.H. Conney, Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche, Inc., Nutley, New Jersey; *NIAMDD, National Institutes of Health, Bethesda, Maryland: Properties of the liver microsomal monoxygenase system and epoxide hydrase—Factors influencing the metabolism and mutagenicity of benzo(a)pyrene.

L.W. Wattenberg, L.K.T. Lam, J.L. Speier, W.D. Loub, and P. Borchert, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis: Inhibitors of chemical carcinogenesis.

D.J. Brusick, K. Bakshi, D.R. Jagannath, and U. Weekes, Department of Genetics, Litton Bionetics, Inc., Kensington, Maryland: Organ specificity and the effects of microsomal enzyme modifiers on the metabolism of chemical carcinogens to active mutagens in mice and rats.

D.P.H. Hsieh, J.J. Wong, and Z.A. Wong, Department of Environmental Toxicology, University of California, Davis: Hepatic biotransformation of aflatoxin and its carcinogenic activity.

M.F. Rajewsky, L.H. Augenlicht, H. Biessmann, R. Goth, D.F. Hülser, O.D. Laerum, and L.Ya. Lomakina, Institut für Zellbiologie (Tumorforschung), Universität Essen, Federal Republic of Germany: Nervous-system-specific carcinogenesis by ethylnitrosourea in the rat—Molecular and cellular aspects.

Session 7B: *Aryl hydroxylase genetics*

Chairperson: D. Jerina, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland

R.E. Kouri and D.W. Nebert, Department of Biochemical Oncology, Microbiological Associates, Inc., and Developmental Pharmacology Branch, NICHHD, National Institutes of Health, Bethesda, Maryland: Genetic regulation of susceptibility to polycyclic hydrocarbon-induced tumors in the mouse.

G.H. Kellermann, Department of Human Oncology, University of Wisconsin Medical School, Madison: Hereditary factors in human cancer.

A. Poland and A. Kende, Departments of Pharmacology and Chemistry, University of Rochester, New York: The regulation of aryl hydrocarbon hydroxylase activity—Evidence for an induction receptor.

S.S. Thorgeirsson, A.R. Boobis, P.J. Wirth, and C.E. Reinhold, Development Pharmacology Branch, NICHHD, National Institutes of Health, Bethesda, Maryland: Genetic regulation of metabolism, mutagenicity, and carcinogenicity of 2-acetylaminofluorene and related compounds in mice.

III. HUMAN RISK ASSESSMENT

SATURDAY MORNING, September 11

Session 8: *Animal cancer tests*

Chairperson: S. Weinhouse, Temple University School of Medicine, Philadelphia, Pennsylvania

S. Weinhouse, Fels Research Institute and Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania: Problems in the assessment of human risk of carcinogenesis by chemicals.

U. Saffiotti, Experimental Pathology Branch, Carcinogenesis, DCCP, National Cancer Institute, Bethesda Maryland: Identifying and defining chemical carcinogens.

W.G. Flamm, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland: Animal tests and human hazards.

L. Tomatis, International Agency for Research on Cancer, Lyon, France: Value of long-term testing for the implementation of primary prevention.

H. Popper, C. Maltoni, I.J. Selikoff, R.A. Squire, and L.B. Thomas, Mount Sinai School of Medicine, New York; Institute of Oncology, Bologna, Italy; National Cancer Institute, Bethesda, Maryland: Comparison of neoplastic hepatic lesions in man and experimental animals.

D.P. Rall, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Species differences in carcinogenesis testing.

D.G. Hoel, National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina: Some problems in low-dose extrapolations.

N. Mantel, Biostatistics Center, George Washington University, Washington, D.C.: Aspects of the Hartley-Sielken approach for setting "safe doses" of carcinogens.

SATURDAY AFTERNOON, September 11

Session 9: Short-term assays—Predictive value

Chairperson: B. Ames, University of California, Berkeley, California

J. McCann, C. Sawyer, and B.N. Ames, Department of Biochemistry, University of California, Berkeley: The *Salmonella*/microsome test—Predictive value for animal carcinogenicity.

P. Moreau, A. Bailone, and R. Devoret, Section de Radiobiologie, Laboratoire d'Enzymologie du CNRS, Gif-sur-Yvette, France: A new test for potential carcinogens—Prophage Y induction in *E. coli* K12 *env*A *uvr*B.

K. Russell and M. Meselson, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Quantitative measures of carcinogenic and mutagenic potency.

E. Vogel, Department of Radiation, Genetic and Chemical Mutation, University of Leiden, The Netherlands: Identification of carcinogens by mutagenicity testing in *Drosophila*.

H.F. Stich, R.H.C. San, P. Lam, J. Koropatnick, and L. Lo, Cancer Research Centre and Department of Medical Genetics, The University of British Columbia, Vancouver, Canada: Unscheduled DNA synthesis as a short-term assay for chemical carcinogens.

C. Heidelberger, Cancer Center, University of Southern California, Los Angeles: Oncogenic transformation by chemical carcinogens of rodent cell lines.

E. Huberman, Biology Division, Oak Ridge National Laboratory, Tennessee: Mutability of different genetic loci in mammalian cells by metabolically activated chemical carcinogens.

T. Kakunaga, National Cancer Institute, Bethesda, Maryland: Chemical transformation of rodent and human cells.

W.R. Bruce and J.A. Heddle, Ontario Cancer Institute, Toronto, Canada: The comparison of the micronucleus and sperm abnormality assays for the mutagenic activity of 61 different agents.

SUNDAY MORNING, September 12

Session 10: Industrial and agricultural chemicals

Chairperson: L. Golberg, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina

J.R. Allen and D.H. Norback, Department of Pathology and Regional Primate Research Center, University of Wisconsin, Madison: Carcinogenic potential of the polychlorinated biphenyls.

P.J. Gehring, P.G. Watanabe, and J.D. Young, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical, Midland, Michigan: The relevance of dose-dependent pharmacokinetics and biochemical alterations in the assessment of carcinogenic hazard by chemicals.

P.F. Infante and J.K. Wagoner, National Institute for Occupational Safety and Health, Cincinnati, Ohio: Chloroprene—Observations of carcinogenesis and mutagenesis.

J.R. Withey, Bureau of Chemical Safety, Health Protection Branch, Health and Welfare, Ottawa, Canada: Mutagenic, carcinogenic, and terratogenic hazards arising from human exposure to plastics additives.

S.S. Epstein, Department of Occupational and Environmental Medicine, School of Public Health, University of Illinois, Chicago: Halogenated hydrocarbons—Pesticides.

Y. Shirasu, M. Moriya, H. Tezuka, and S. Teramoto, Institute of Environmental Toxicology, Kodaira-shi, Tokyo: Mutagenicity screening on pesticides.

B. Ames, Biochemistry Department, University of California, Berkeley: Other suspicious chemicals from in vitro experiments.

SUNDAY AFTERNOON, September 12

Public Policy Panel IV: EPA—Dieldrin

Walter Appleby, Shell Chemical Company, San Ramon, California
John Kolojeski, Environmental Protection Agency, Washington, D.C.

SUNDAY EVENING, September 12

Session 11: *Air and water pollutants (additives)*

Chairperson: R. Doll, Oxford University, Oxford, England

T. Mack, M. Pike, and B. Henderson, University of Southern California School of Medicine, Los Angeles: Epidemiological methods for human risk assessment.

P.J. Lawther and R.E. Waller, MRC Environmental Hazards Unit, St. Bartholomew's Hospital Medical College, London, England: Air pollutants and lung cancer—Recent trends in England and Wales.

R.E. Albert, Institute of Environmental Medicine, New York University Medical Center, New York: Carcinogenic atmospheric pollutants.

D.H. Fine, D.P. Rounbehler, R. Ross, and T. Fan, Cancer Research Department, Thermo Electron Research Center, Waltham, Massachusetts: Human exposure to preformed *N*-nitroso compounds.

R.H. Harris, T. Page, and N. Reiches, Environmental Defense Fund, Resources for the Future, Inc., Washington, D.C., and Department of Preventive Medicine, Ohio State University, Columbus: Drinking-water contamination and its relations to human cancers.

T.A. DeRouen and J.E. Diem,* University of Washington, Seattle; *Tulane University, New Orleans, Louisiana: Relationships between cancer mortality in Louisiana, drinking-water source, and other possible causative agents.

C.R. Buncher, Division of Epidemiology and Biostatistics, Department of Environmental Health, University of Cincinnati, College of Medicine, Ohio: Drinking water as an epidemiologic risk factor for cancer.

D.R. Taves, Department of Pharmacology and Toxicology, University of Rochester, New York: Fluoridation and cancer mortality.

MONDAY MORNING, September 13

Session 12: *Viruses II*

Chairperson: G. Todaro, National Cancer Institute, Bethesda, Maryland

M. Essex,* J.R. Stephenson,† W.D. Hardy, Jr.,** S.M. Cotter,* and S. A. Aronson,† *Harvard School of Public Health, Boston, Massachusetts; †National Cancer Institute, Bethesda, Maryland; **Memorial Sloan-Kettering Cancer Center, New York, New York: Leukemia, lymphoma, and fibrosarcoma of cats as models for similar diseases of man.

W.H.F. Jarrett, Department of Veterinary Pathology, University of Glasgow Veterinary School, Scotland: Development of vaccines against feline leukemia.

M.J. Van Der Maaten and J.M. Miller, U.S. Department of Agriculture, National Animal Disease Center, Ames, Iowa: Current assessment of human health hazards associated with bovine leukemia virus.

M.B. Gardner, R.M. McAllister, V. Klement, S. Rasheed, R.W. Rongey, B.E. Henderson, and H.P. Charman, Departments of Pathology, Pediatrics, and Microbiology, University of Southern California School of Medicine and Children's Hospital, Los Angeles; Frederick Cancer Research Center, Frederick, Maryland: Search for type-C virus in humans.

R.C. Gallo, National Cancer Institute, Bethesda, Maryland: Some ideas on the origin of leukemia in man and recent evidence for the presence of acquired and endogenous type-C-viral-related sequences.

C. McGrath, H.D. Soule, E.J. Marianeau, L.J. Larson, and M.A. Rich, Michigan Cancer Foundation, Detroit: A biological rationale for isolation of endogenous viruses from human breast carcinoma.

G. de Thé, International Agency for Research on Cancer, Lyon, France: Origins of human cancers—Can epidemiology help in searching for a viral etiology?

W.E. Rawls, Department of Pathology, McMaster University, Hamilton, Ontario, Canada: Herpes simplex viruses and human malignancies.

MONDAY AFTERNOON, September 13

Session 13A: Viruses III

Chairperson: B. Hirt, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland

G. Khoury, M. Brown, C.-J. Lai, P. Howley, M. Israel, and M. Martin, National Cancer Institute and NIAID, National Institutes of Health, Bethesda, Maryland: Human papovaviruses and their relationship to SV40.

G. Orth, Institute Gustave Roussy, Villejuif, France: Papillomaviruses—Possible role in human cancer.

M. Green and J. Mackey, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Are oncogenic human adenoviruses associated with human cancer? Analysis of human tumors for adenovirus-transforming gene sequences.

Session 13B: Possible dietary carcinogens

Chairperson: P. Magee, Temple University Medical School, Philadelphia, Pennsylvania

T. Sugimura, T. Matsushima, H. Matsumoto, T. Kawachi, M. Nagao, S. Sato, T. Yahagi, Y. Seino, M. Sawamura, and A. Shirai, National Cancer Center Resarch Institute and Institute of Medical Science, Tokyo University, Japan: Mutagen-carcinogen and food.

W. Lijinsky, Chemical Carcinogenesis, Frederick Cancer Research Center, Frederick, Maryland: Nitrosamines and their precursors in food.

H. Endo, Division of Chemistry, Cancer Research Institute, Kyushu University Medical School, Fukuoka, Japan: Survey of mutagens formed from environmental materials under physiological conditions.

S.R. Tannenbaum, M.C. Archer, and J.S. Wishnok, Department of Nutrition and Food Science, MIT, Cambridge, Massachusetts: Nitrates, nitrites, *N*-nitroso compounds, and human cancer.

W.R. Bruce, A.J. Varghese, and R. Furrer, Ontario Cancer Institute, Toronto, Canada: A mutagen in the feces of normal humans.

MONDAY EVENING, September 13

Remarks: Theodore W. Cooper, Assistant Secretary for Health, Department of Health, Education, and Welfare, Washington, D.C.

Session 14: Further strategies and standard setting

Chairperson: S. M. Wolfe, Public Citizen's Health Research Group, Washington, D.C.

S.M. Wolfe, Public Citizen's Health Research Group, Washington, D.C.: Standard setting—Preventive or after the fact?
W. Nicholson, Mount Sinai School of Medicine, City University of New York, New York: Standards for carcinogens and their effectiveness.
J.K. Wagoner and P.F. Infante, National Institute for Occupational Safety and Health, Cincinnati, Ohio: Vinyl chloride—The use of laboratory assay for regulatory control.
W. Lijinsky, Frederick Cancer Research Center, Frederick, Maryland: Standard setting for nitrates and nitrosamines.
E.C. Hammond, American Cancer Society, New York, New York: Smoking.

Summary: J. Cairns, Imperial Cancer Research Fund, London, England

IN-HOUSE SEMINARS

:old Spring Harbor in-house seminars were initiated to provide a emiformal avenue for communication between the various research roups at the laboratory. They are particularly useful for research ersonnel who have joined the laboratory during the summer. The eminars also afford a necessary opportunity for the graduate students nd postgraduate staff to develop their skills in defending, organizing, nd presenting their research. In addition to those listed below, semi-ars were given by many others involved in research at this laboratory.

ctober

4th. Drs. Ada L. and Donald E. Olins, Oak Ridge National Laboratory, Tennessee: *The "nu" structure of chromatin.*

ovember

5th. Dr. James Lake, New York University Medical Center, New York: *Ribosome structure—Localization of specific proteins by electron microscopy of antibody labels.*

4th. Dr. John Carbon, University of California, Santa Barbara: *Biochemical construction of hybrid ColE1 plasmids—Making and using an* E. coli *gene bank.*

9th. Dr. Marshall Hurwitz, Albert Einstein College of Medicine, Bronx, New York: *Adenovirus DNA synthesis.*

0th. Dr. George Fey, Cold Spring Harbor Laboratory, New York: *Peptide analysis of structural proteins from SV40* ts *mutants.*

1st. Dr. Jerry Both, Roche Institute of Molecular Biology, Nutley, New Jersey: *Factors influencing the efficiency of mRNA-ribosome interactions.*

4th. Dr. Ahmad Bukhari, Cold Spring Harbor Laboratory, New York: *Mutants of* E. coli *unable to survive in animals—Safe hosts for DNA recombinant molecules.*

5th. Dr. Victor Ling, Ontario Cancer Institute, Toronto, Canada: *A cell-surface glycoprotein in modulating drug permeability in Chinese hamster ovary cell mutants.*

ecember

3rd. Dr. Masayori Inouyé, State University of New York at Stony Brook: *Biosynthesis and assembly of a lipoprotein of the* E. coli *outer membrane.*

th. Dr. Timothy Hall, University of Wisconsin, Madison: *Studies on the aminoacylation property of RNA from several plant viruses.*

th. Dr. Vivian Mautner, The Cancer Center, Massachusetts Institute of Technology, Cambridge: *Immunological studies of cell-surface components in normal and transformed cells.*

th. Dr. Yung Chi Cheng, Roswell Park Memorial Institute, Buffalo, New York: *Comparisons of purified thymidine kinases from herpesvirus-infected and noninfected human cells.*

th. Dr. Tim Hunt, Cambridge University, England: *The control of protein synthesis by reversible phosphorylation of an initiation factor.*

th. Dr. Gerry Rubin, Stanford School of Medicine, California: *Arrangement of sequences in cloned segments of* Drosophila *DNA.*

uary

st. Dr. Joan Kaplan, Massachusetts General Hospital, Boston: *The induction of SV40 virus from transformed hamster cells.*

th. Dr. A. Mirzabekov, Molecular Biology Institute, Moscow, USSR: *Chromatin structure and DNA-protein interaction as studied by the biochemical approach.*

h. Dr. Ronald H. Cohn, Stanford University, California: *The organization of histone genes.*

th. Dr. Suzanne Emerson, University of Virginia, Charlottesville: *Identification of the proteins involved in transcribing the RNA genome of vesicular stomatitis virus.*

February

3rd. Dr. Jane Flint, Massachusetts Institute of Technology, Cambridge: *Ordered transcription of RNA tumor virus genomes in vitro.*

12th. Dr. Warren Maltzman, University of California, Berkeley: *Regulation of phage lambda DNA synthesis.*

13th. Dr. Beatrice Mintz, The Institute of Cancer Research, Philadelphia, Pennsylvania: *Normalized gene expression in teratocarcinoma cell differentiation.*

18th. Dr. George Miller, Yale Medical School, New Haven, Connecticut: *Host-cell factors and transformation by Epstein-Barr virus.*

25th. Dr. S. Muthukrishnan, Roche Institute of Molecular Biology, Nutley, New Jersey: *mRNA methylation and protein synthesis in eukaryotes.*

27th. Dr. William Haseltine, Massachusetts Institute of Technology, Cambridge: *Ordered transcription of RNA tumor virus genomes in vitro.*

March

1st. Dr. Ernest Winocour, Weizmann Institute, Rehovot, Israel: *Recombination between SV40 and the host genome.*

2nd. Dr. Eiichi Ohtsubo, State University of New York at Stony Brook: *Structure and function of* E. coli *sex and resistance factors as determined by EM heteroduplex analysis and restriction-enzyme mapping.*

5th. Dr. Philip Serwer, California Institute of Technology, Pasadena: *Phage T7 DNA packaging—Biophysical analysis using iothalimate density gradients.*

9th. Dr. Fred Blattner, University of Wisconsin, Madison: *Efforts to construct "safe" lambda vectors for cloning DNA.*

12th. Dr. John Bridgen, University of Alabama, Birmingham: *New microsequencing technique—Application to membrane proteins.*

16th. Dr. Maxine Singer, National Institutes of Health, Bethesda, Maryland: *Interaction of histone with superhelical DNA.*

19th. Dr. Nicholas Maroudas, Imperial Cancer Research Institute, London, England: *Physical chemistry of nonspecific cell adhesion and spreading on synthetic solid substrata.*

25th. Dr. Wayne Barnes, Medical Research Council at Cambridge, England: *Manipulating a DNA plasmid to study the histidine operon.*

26th. Dr. J.P. Trinkhaus, Yale University, New Haven, Connecticut: *Modes of cell locomotion in vivo.*

30th. Dr. David Denhardt, McGill University, Montreal, Quebec, Canada: *DNA replication, with special emphasis on* ϕ*X174.*

April

1st. Dr. Guenter Albrecht-Buehler, Cold Sprıng Harbor Laboratory, New York: *A quantitative description of locomotory surface activities in spreading 3T3 cells.*

8th. Dr. James Manley, Cold Spring Harbor Laboratory, New York: *Metabolism of abnormal polypeptides by the* lacZ *gene of* E. coli.

13th. Dr. Dick Fine, Boston University, Massachusetts: *Coated vesicles—What they tell us about* Ca^{++} *regulation in nonmuscle cells.*

15th. Dr. James McDougall, Cold Spring Harbor Laboratory, New York: *Clonal derivatives of a herpes-type-2-transformed hamster cell line (333-8-9)—Cytogenic analysis and virus messenger-RNA detection.*

20th. Dr. Vincenzo Enea, The Rockefeller University, New York: *Deletion mutants of phage* F_1*—Mutants with no genes.*

23rd. Dr. Bob Wayland, Institute Pasteur, Paris, France: *Protein synthesis and actin heterogeneity in cultured muscle cells.*

May

7th. Dr. Robert Tjian, Harvard University, Boston, Massachusetts: *Regulatory proteins of phage SPO1.*

11th. Dr. Gunther Blobel, The Rockefeller University, New York: *Early events in the secretory pathway.*

14th. Dr. David Ward, Yale University, New Haven, Connecticut: *Structure and replication of nondefective parvoviruses–Antineoplastic agents (?).*

17th. Dr. Robert Goldman, Carnegie-Mellon Institute, Pittsburgh, Pennsylvania: *Actomyosinlike contractile structures and the control of normal and virus-transformed cell behavior.*

18th. Dr. Douglas Berg, University of Wisconsin, Madison: *A transposable segment of prokaryotic DNA which encodes resistance to kanamycin.*

25th. Dr. M.V. Nermut, Medical Reseach Council, London, England: *Structural elements in adenovirus cores.*

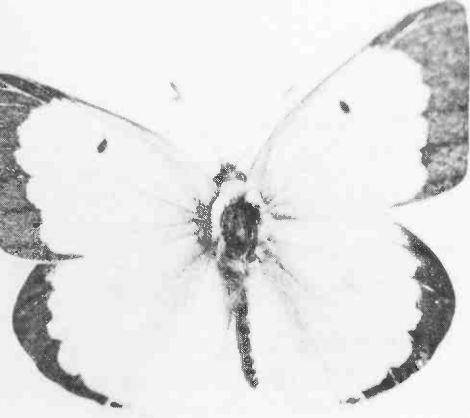
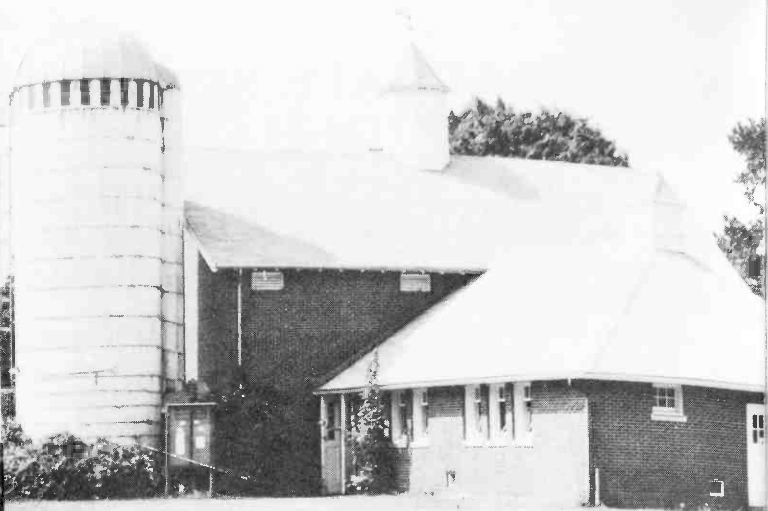
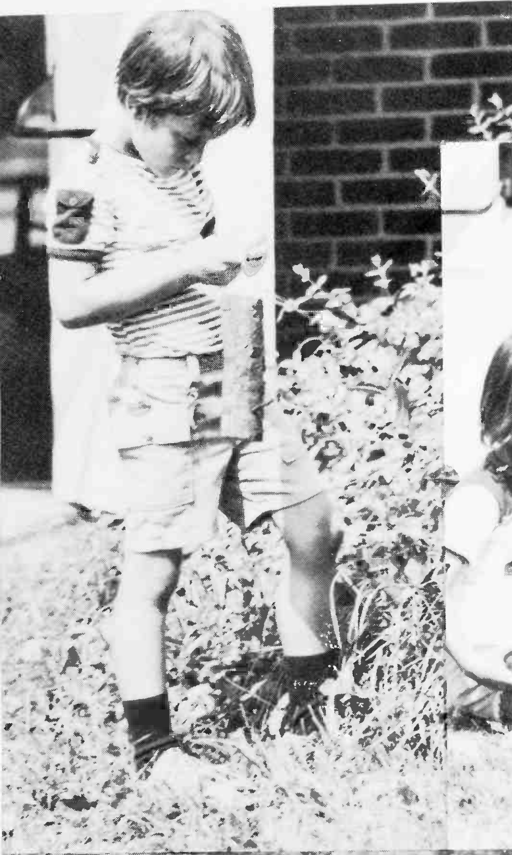
UNDERGRADUATE SUMMER RESEARCH PROGRAM

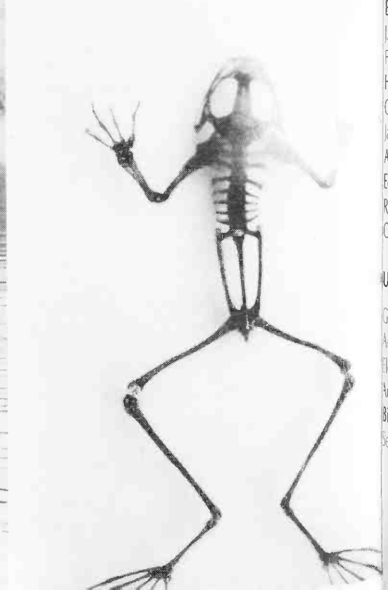
Another aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year 175 students have completed the course, and many have gone on to creative careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology, (2) an increased awareness of major problem areas under investigation, (3) an increased awareness of the physical and intellectual tools for modern research and the pertinence of this information to future training, and (4) a personal acquaintance with research, research workers, and centers for study.

Ten college undergraduates, selected from a large number of applicants, take part in the program, which is now mainly supported by Laboratory funds. This year's students are listed below, with their laboratory sponsors and topics of research. We gratefully acknowledge the generosity of Central General Hospital, Plainview, New York, which provided fellowships to support the work of Mark Glen, Franklin Moser, and Beth Weinstein.

Student	Research topic
Mark Dulaney Glen, University of Pennsylvania *Supervisor:* M. Mathews	The nucleotide sequence of the gene for adenovirus-2 virus-associated RNA
Marion Gold, University of California, Berkeley *Supervisor:* C.G. Miller	In vitro studies of protein degradation in *Escherichia coli*
Robert Craig Gudor, University of California, Berkeley *Supervisor:* A. I. Bukhari	Interaction of the genomes of bacteriophages Mu and P1 in *Escherichia coli*
Francine Bryanne Hanberg, Yale University *Supervisor:* R. J. Roberts	Screening bacterial strains for new restriction endonucleases
Nancy Harris, Yale University *Supervisor:* J. Manley	Transcription of adenovirus-2 DNA by wheat-germ RNA polymerases
Franklin G. Moser, Yale University *Supervisor:* L.B. Chen	Distribution of cell-surface LETS protein in cocultures of normal and transformed cells
Phyllis Barbara Moses, The Johns Hopkins University *Supervisor:* R. Kahmann	Structure and biological properties of hybrids made in vitro between pMB9 and the c-terminal *Hind* III fragment of bacteriophage Mu
James Albert Rhodes, Harvard College *Supervisor:* K. Burridge	Direct gel analysis of glycoproteins from cultured fibroblasts and epithelial cells
James M. Roberts, Amherst College *Supervisors:* T. Broker and L. Chow	A cytoplasmic RNA transcript map of adenovirus 2 determined by electron microscopy of RNA:DNA hybrids
Beth Weinstein, Cornell University *Supervisor:* J. Broach	Search for operon for mutants in the galactose system of yeast



NATURE STUDY PROGRAM

The Nature Study Program is designed for elementary and high school students who wish to achieve a greater understanding of their environment. In summer and fall of 1976, 376 students participated in this program. When weather permitted, most of the courses were held outdoors on the laboratory grounds or at the Uplands Farm Nature Preserve of the Nature Conservancy. The Laboratory has built and equipped classroom-laboratories at Uplands Farm for the study of field specimens collected by students.

This fall a Marine Environmental Science course was conducted as a joint project with the Marine Sciences Research Center at the State University at Stony Brook. The instructors were advanced graduate students and well-known specialists holding university appointments and actively engaged in research in their specific subject areas. An all-day trip to Stony Brook and a cruise on the center's research vessel Onrust were included.

Program director: Sanford Kaufman, M.S., M.P.A., biology teacher, Hewlett High School.

TEACHERS

Linda Bovich, M.S., science teacher, Rocky Point High School
Donn Dunn, M.S., art and photography teacher, Hewlett High School
Steven Englebright, M.S., curator of geology, SUNY at Stony Brook
George Hechtel, Ph.D., Department of Biology, SUNY at Stony Brook
Barbarajean Kaufman, M.S., Ph.D. candidate, SUNY at Stony Brook
J.L. McHugh, Ph.D., Marine Sciences Research Center, SUNY at Stony Brook
Fred Maasch, M.Ed., biology teacher, Islip High School
Harold O'Connors, Ph.D., Marine Sciences Research Center, SUNY at Stony Brook
C. Donald Powers, Ph.D., Marine Sciences Research Center, SUNY at Stony Brook
James Romansky, M.S., biology teacher, Bay Shore High School
Anthony Taormina, New York State Department of Conservation
Edward Tronolone, M.S., science teacher, Lynbrook High School
Robert Wilson, Ph.D., Marine Sciences Research Center, SUNY at Stony Brook
Charles Wurster, Ph.D., Marine Sciences Research Center, SUNY at Stony Brook

COURSES

General Nature Study
Advanced Nature Study
Elementary Geology
Advanced Geology
Bird Study
Seashore Life
Vertebrate Biology
Reptiles and Amphibians
Aquatic Biology
Marine Biology
Nature Photography I and II
Fresh Water Life I and II

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NOVEMBER 1976

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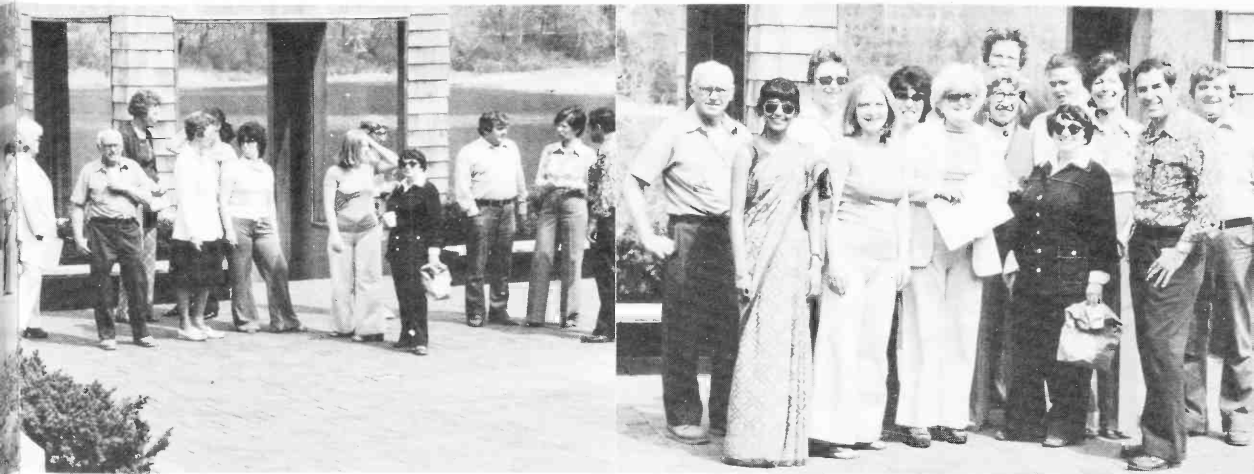

FINANCIAL STATEMENT

BALANCE SHEET
year ended October 31, 1976
with comparative figures for 1975

ASSETS

	1976	1975
CURRENT FUNDS		
Unrestricted		
Cash	$ (49,845)	$ 126,878
Marketable securities	93,148	–
Accounts receivable	66,739	70,059
Inventory of books	212,839	167,085
Prepaid expenses	33,921	8,796
Due from restricted fund	214,654	36,595
Total unrestricted	571,456	409,413
Restricted		
Grants receivable	728,974	605,651
Total current funds	1,300,430	1,015,064
PLANT FUNDS		
Investment	85,037	39,197
Due from unrestricted fund	293,468	176,156
Land and improvements	595,954	581,496
Buildings	2,315,592	1,980,099
Furniture, fixtures, and equipment	614,980	476,789
Books and periodicals	365,630	365,630
Construction in progress	17,211	276,993
	4,287,872	3,896,360
Less allowance for depreciation and amortization	867,187	686,331
Total plant funds	3,420,685	3,210,029
BANBURY EDUCATIONAL CENTER		
Cash	200	–
Prepaid expenses	100	–
Due from unrestricted fund	42,951	–
	43,251	–
Land	772,500	–
Buildings	245,000	–
Furniture, fixtures, and equipment	113,343	–
Construction in progress	3,954	–
	1,134,797	–
Less allowance for depreciation	14,221	–
	1,120,576	–
Total Banbury Educational Center	1,163,827	–

LIABILITIES AND FUND BALANCES

	1976	1975
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 49,097	$ 58,347
Due to plant funds	293,468	176,156
Due to Banbury Educational Center	42,951	–
Fund balance	185,940	174,910
Total unrestricted	571,456	409,413
Restricted		
Due to unrestricted funds	214,654	36,595
Fund balance	514,320	569,056
Total restricted	728,974	605,651
Total current funds	1,300,430	1,015,064
PLANT FUNDS		
Fund balance	3,420,685	3,210,029
BANBURY EDUCATIONAL CENTER		
Fund balance	1,163,827	–

CURRENT REVENUES, EXPENDITURES, AND TRANSFERS
year ended October 31, 1976
with comparative figures for 1975

COLD SPRING HARBOR LABORATORY

	1976	1975
REVENUES		
Grants	$1,918,414	$1,661,421
Indirect cost allowance on grants	783,683	856,344
Contributions	170,799	62,072
Robertson Research Fund contribution	300,000	275,000
Summer programs	175,658	151,235
Laboratory rental	19,754	19,633
Marina rental	35,430	31,300
Investment income	10,363	19,990
Publications sales	289,698	382,221
Dining hall	220,783	160,021
Rooms and apartments	143,823	133,576
Other sources	11,762	24,747
Total revenues	4,080,167	3,777,560
EXPENDITURES		
Research*	1,722,024	1,593,093
Summer programs*	371,888	179,861
Library	82,927	56,361
Operation and maintenance of plant	626,047	502,136
General and administrative	446,570	387,595
Publications sales*	225,863	224,145
Dining hall*	190,354	188,786
Total expenditures	3,665,673	3,131,977
TRANSFERS TO PLANT FUNDS	403,464	507,950
Total expenditures and transfers	4,069,137	3,639,927
Excess of revenues over expenditures and transfers	11,030	137,633

**Reported exclusive of an allocation for operation and maintenance of plant, general and administrative, and library.*

BANBURY EDUCATIONAL CENTER

	1976	1975
REVENUES		
Contributions		
(including land, buildings, and furnishings)	$1,230,843	–
Rooms and Apartments	11,646	–
Total revenues	1,242,489	–
EXPENDITURES		
Operation and maintenance of plant	65,361	–
General and administrative	13,301	–
Total expenditures	78,662	–
Excess of revenues over expenditures	1,163,827	–

NOTE: Copies of the complete financial statements, certified by our independent auditors, Peat, Marwick, Mitchell & Co., are available upon 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 from the termination of "private" foundations.

The Laboratory depends upon the generous contributions of its sponsors, participating institutions, and friends for central institutional needs and capital improvements. In addition, the development of any new programs, such as year-round research in neurobiology 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

(1) Your broker or bank may sell the securities and remit the proceeds directly to the Laboratory.

(2) If you wish to send stock directly to the Laboratory, either (a) endorse the certificate(s) by signing your name on the back, leave the space for the transferee's name blank, have your signature guaranteed on the certificate(s) by your bank or broker, and send the certificate(s) by *registered mail* to the Laboratory, or (b) send unsigned certificate(s) with a covering letter and send under separate cover a stock power executed in blank, with signature guarantee, for each certificate, and also a copy of the covering letter (use first-class mail). Depreciated securities should be sold to establish a tax loss, then the contribution to the Laboratory should be made by check.

Bequests

Probably most wills need to be updated. Designating the Cold Spring Harbor Laboratory as a beneficiary ensures that a bequest will be utilized as specified for continuing good.

Appreciated real estate or personal property

Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Conversion of private foundation to "public" status on termination

This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation could be accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a "supporting organization of 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 516-692-6660.

GRANTS

November 1, 1975—October 31, 1976

NEW GRANTS

Grantor	Principal investigator and program	Total award	Duration of grant
National Institutes of Health	Dr. Watson – general research support	$ 46,495	4/1/76 – 3/31/77
	Contract – advanced bacterial genetics workshop	25,325	7/1/76 – 6/30/77
	Dr. Watson – herpesvirus workshop	22,000	6/30/76 – 6/29/77
	Dr. Watson – conference on origins of human cancer	60,000	6/30/76 – 6/29/77
	Dr. Maniatis – research subcontract from Harvard	26,850	7/1/76 – 12/31/76
National Science Foundation	Dr. Bukhari – research	120,000	8/1/76 – 7/31/78
	Dr. Albrecht-Buehler – research	10,000	6/1/76 – 11/30/77
	Dr. Bukhari – conference on DNA insertions	3,000	3/15/76 – 2/28/77
	Dr. Zain – research	12,100	3/1/76 – 5/31/77
	Dr. Watson – herpesvirus workshop	5,000	5/15/76 – 4/30/77
	Dr. Gesteland – workshop on *Dictyostelium*	4,600	4/15/76 – 9/30/76
Rita Allen Foundation	Dr. Lewis – research	150,000	8/1/76 – 7/31/81
Anna Fuller Fund	Dr. Manley – fellowship	10,000	9/1/76 – 8/31/77
Interboro Leukemia Organization	Dr. Watson – equipment support	4,000	4/1/76 – 6/30/76
Muscular Dystrophy Association	Dr. Chen – fellowship	13,500	1/1/76 – 12/31/76
Merck, Sharp & Dohme Research Laboratories	Dr. Watson – Symposium support	5,000	6/1/76 – 6/8/76
Hoffman-La Roche, Inc.	Dr. Watson – Symposium support	6,000	6/1/76 – 6/8/76

CONTINUING GRANTS

Grantor	*Principal investigator and program*	*Total award*	*Duration of grant*
National Institutes of Health	Dr. Watson – general research support	$ 145,638	1/1/75 – 3/31/76
	Dr. Bukhari – career development	110,000	5/1/75 – 4/30/80
	Dr. Hassell – fellowship	26,000	10/1/75 – 9/30/77
	Dr. Gelinas – fellowship	26,000	7/3/74 – 7/2/76
	Dr. Watson – Cancer Research Center	7,789,000	1/1/72 – 12/31/76
	Dr. Watson – Symposium support	96,000	4/1/74 – 3/31/79
	Dr. Zipser – research	350,000	5/1/74 – 4/30/79
	Dr. Gesteland – summer workshops	194,895	4/1/74 – 3/31/77
	Dr. Watson – neurobiology training	152,172	5/1/74 – 4/30/77
National Science Foundation	Dr. Albrecht-Buehler – research	76,300	7/1/75 – 6/30/77
	Dr. Gesteland – research	45,000	1/1/75 – 12/31/77
	Dr. Bukhari – research	60,000	8/1/74 – 1/31/77
	Dr. Roberts – research	50,000	6/15/74 – 11/30/76
	Dr. Zipser – research	160,000	1/1/74 – 2/28/78
	Dr. Gesteland – research	41,400	9/1/74 – 2/29/76
	W. Udry – Symposium support	5,000	5/15/76 – 4/30/77
Helen Hay Whitney Foundation	Dr. Daniell – fellowship	17,583	3/1/74 – 12/31/75
Volkswagen Foundation	Dr. Watson – training support	73,085	1/1/71 – 12/31/76
Energy Research and Development Administration	Dr. Watson – Symposium support	8,000	6/1/76 – 6/8/76
Grass Foundation	Dr. Watson – neurobiology training	5,000	6/1/76 – 9/30/76
American Cancer Society	Dr. Endow – fellowship	10,333	1/1/75 – 2/29/76
	Dr. Lewis – research	74,100	7/1/75 – 6/30/77
…pley Foundation	Dr. Watson – neurobiology	10,000	1/1/76 – 12/31/76
…a Fuller Fund	Dr. Burridge – fellowship	18,100	6/1/75 – 5/31/77
Cystic Fibrosis Foundation	Dr. Bukhari – research	29,000	1/1/75 – 12/31/76
Alfred P. Sloan Foundation	Dr. Watson – neurobiology	165,000	1/1/75 – 12/31/78
…ne Coffin Childs Memorial Fund for Cancer Research	Dr. Bothwell – fellowship	10,500	7/1/75 – 6/30/76

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LONG ISLAND BIOLOGICAL ASSOCIATION

he new Williams House, built with LIBA contributions, nears completion in spring 1977.

he Laboratory was founded in 1890 by several local philanthropists and the Brooklyn Institute of Arts and ciences. The first chairman of the Board of Managers of the Laboratory was Eugene G. Blackford, who served om 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 pporters of the research formalized their efforts by incorporating as the Long Island Biological Association. lonel T. S. Williams became the first Chairman of the new group. Jointly with the Carnegie Institution of ashington, 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 sponsibilities. During the past 14 years, LIBA's chief function has been to widen the interest of the community the Laboratory and to help support it financially.

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