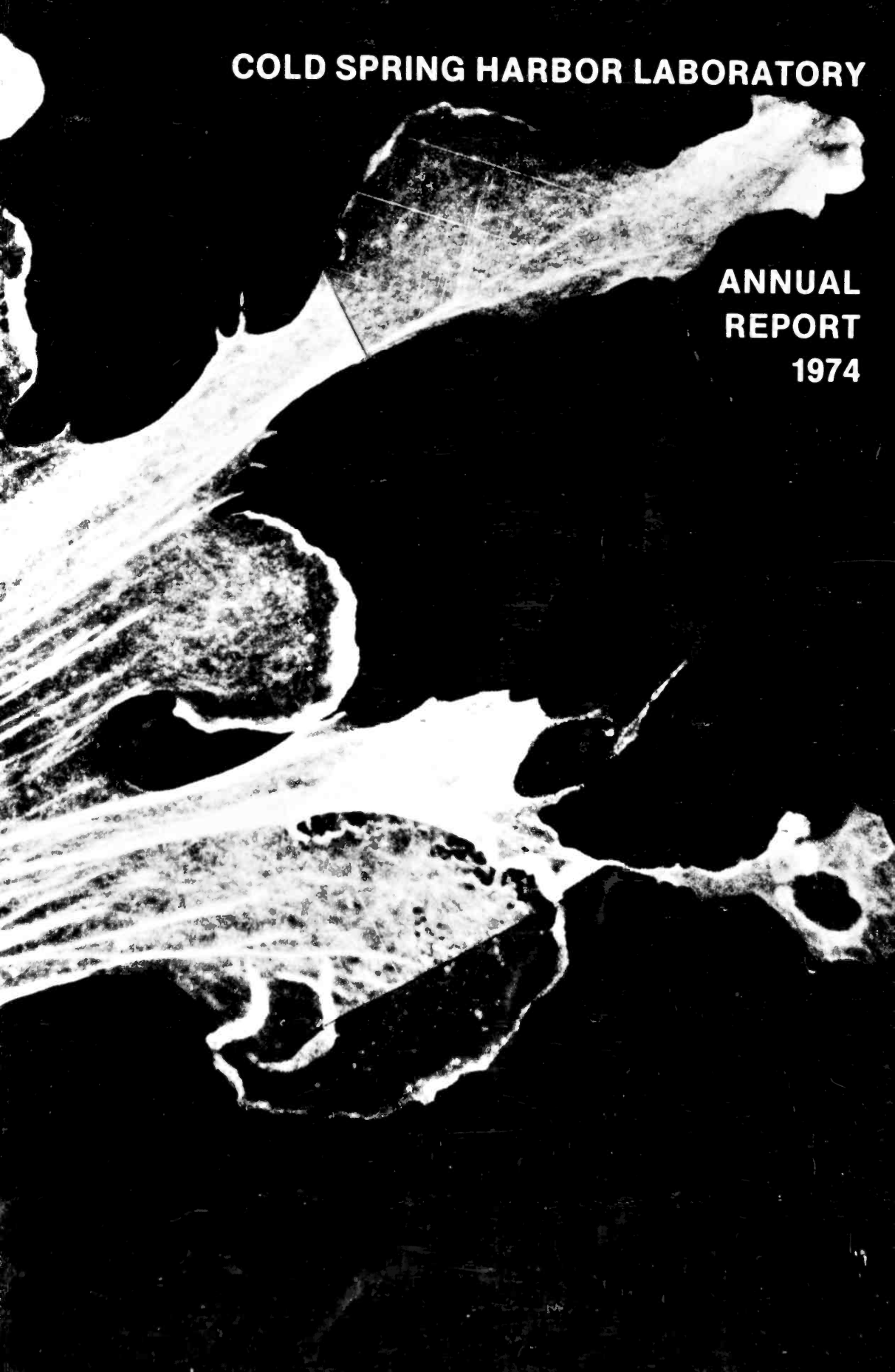
COLD SPRING HARBOR LABORATORY



ANNUAL REPORT 1974

Cover: A single cell from a mouse embryo, moving about on a glass slide. The cell was fixed and then stained with antibody to actomyosin, a contractile protein complex of muscle cells. The antibody was visualized by fluorescence, and its pattern revealed that the embryo cell contained actomyosin in sheaths, even though it was not a muscle cell. (*Photo by B. Pollack, K. Weber, G. Felsten*)

ANNUAL REPORT 1974

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

Until very recently it has been easy to be a biologist and not worry whether the ethics of our profession was above reproach. While it is taken for granted that corruption of some sort often accompanies the attainment of successful careers in business and politics, this charge could not be leveled against the world of the American biologist. Salaries were too low to generate envy, we gave the appearance of being solely concerned with the truth, and what we knew had so little of practical consequence that no one would ever be silly enough to want to buy us out.

This, of course, was once also true of our physicists. But after the bomb appeared, their world became irrevocably altered, and we owe the transit of many clever physicists into biology to their desire to remain pure, unbedeviled by the world of security clearances, million-dollar contracts, and the need for entrepreneurial skill in order to stay competitive. A clever mind was fine, but unless it had an entree into the world of the megabuck, it might not be financeable. And loners who loved to do things at their own pace, unruffled by the need for immediate progress, became a luxury that only the most wealthy institutions could afford to subsidize.

Now, however, biology is beginning to look like the physics that so frightened us two decades ago. No longer do we consider $100,000 a large sum of money, but even in terms of the old dollar, many of us have to think in terms of million-dollar sums if we wish to stay in tune with the times. A prime reason for this escalation, of course, has been our vast successes of the past twenty-five years. Molecular biology is no longer a simple subject mastered after a few weeks of expert tutoring, and today its inherent complexity almost automatically generates an apparent need for more and still more sophisticated equipment. Almost inevitably, we seem to be moving into a situation where large collective teams, not the individual, are the manner of the day, and where the success of a younger biologist depends not only on his brains, but more and more on the skill of his boss to hustle up vast piles of research money.

The question must thus be asked whether an ever-increasing expenditure of both human and financial resources will be a dominant motif for the biology of the next several decades. Must biology necessarily move that way leading to the creation of still larger and larger labs and its subsequent localization to fewer and fewer sites of creative research? Or might some unexpected new ideas make the next ten to twenty years manageable within the boundaries of our current resources and do away with the need for the type of expansion that would by its very nature make our lives less unique and research more impersonal?

The trouble, however, with this now very old-fashioned approach is that it fails to appreciate the extent to which biology now runs fast, not only because it is inherently exciting, but also because it offers the promise to improve on our lives. Our main support comes not from University, Foundation, or private monies, but from Federal funds, solicited for the most part not by the scientists themselves, but by spokesmen for the various health lobbies (the National Foundation, American Heart Association, American Cancer Society, National Cystic Fibrosis Research Foundation, etc.). So while perhaps a majority in Congress think biology is a fine enterprise and should always be encouraged, I doubt that even one of our five hundred and thirty-six Congressmen would be pushing over a billion research dollars a year into the NIH unless they thought they were buying some very real benefits for our nation's health.

No one knows, however, the rate at which these dividends will materialize, and so it is much to the point that few individuals are likely to be excited by the thought that, for example, schizophrenia might be controlled at the time of our Tricentennial. In contrast, if we had good assurances that it could be beaten over the next decade, all of us would make the financial sacrifices to permit virtually unlimited Federal money to flow out for its cure. Thus, when promoting any form of health-related research, it usually pays to talk of potential benefits within a five- to ten-year framework. Even if you fear much longer will be required, you know that your arguments will be judged in competition with other programs, many of whose promoters will dangle the prospects of a quick war climaxed by total victory.

Thus, almost inevitably, the selling of a potential great leap forward immediately gives its progenitors queasy feelings. They usually know that the money initially appropriated, while a fine yearly amount if a 20- to 30-year interval is considered, is grossly inadequate for a war programmed to last at most a decade. So almost as soon as their plan is in motion, they must ask for still more money, suspecting that victory demands some entirely new outlook, and hoping that the more minds that can be quickly enlisted, the better the chance for the unexpected.

This is, I fear, all too much the situation in the current, highly touted "War on Cancer." While all the initial protagonists (the Yarborough Committee) for the Cancer War honestly pointed out that the war was likely to be very long, there was no hesitation in proclaiming that, given a major infusion of new cancer monies, major battlefield successes would soon substantially affect the grim statistics still existing for all the major cancers. Unfortunately, these latter claims may all too soon come back to haunt the National Cancer Institute (NCI), since, despite infinite hoopla, the statistics may not be radically improving for any major cancer (e.g., colon or lung), and we still remain woefully in the dark about the nature of cancer cells. Even today, three years after the official start of the war and the subsequent creation of some sixteen new clinically based research centers, there may be no reason to project over the next decade a substantial decline in cancer mortality beyond that which had occurred prior to 1960 through improvements in the use of surgical and radiation therapies. Although recently real cures have been obtained for several types of deseminated cancer by highly skilled employment of chemotherapy, curable deseminated cancers are still the great exception (1-2% of total cancers), and there are no solid leads upon which to attack the predominant killers.

Naturally, as human beings, we all hope for striking clinical advances in the near future. But as scientists per se, all that we can truthfully say is that at long last cancer research can be very good science indeed, and we are now moving much faster than we could have anticipated only several years ago. For example, no one would have then predicted that today we would understand so much about the relation between viruses and cancer. But the more we learn, the more complex the problem becomes. And given the hindsight afforded by the pace at which we are coming to understand the much simpler bacterial cell, it would be most surprising if less than 25-50 years of high level research will be required to map out the essential differences between human cancer cells and their normal counterparts. So, in all honesty, we cannot dangle the assurance that our work of the next decade or two will have many immediate clinical consequences.

We would be naive, however, to believe that such low-keyed statements coming after almost a decade of well-orchestrated press releases will cut much ice with the general public. The chances are thus high that soon the NCI budget will no longer be regarded as a

sacred cow, but as an item which has to face the scrutiny of serious criticism, this time emanating not from an inept administration that loathes science or from misguided scientists long out of touch with the real world, but from the front rank of the biological and medical communities long annoyed by the unique privileges given to the cancer research world, often to the apparent great detriment of other scientific (medical) endeavors.

It is unlikely, however, that the first such attacks will carry much punch. But unless the creation of the new clinical centers causes the mortality curves to bend downward, the NCI most probably will lose its special clout, and the President's Panel, the special entree of the cancer world into the White House bureaucracy, may vanish without any lasting trace. A return of the NCI into the ordinary world is unlikely, however, to have any real benefits for the rest of biology (medicine) — just the opposite, at least in the beginning. Because of its immense size, the NCI carries along a vital part of pure biological research, and if the axes fall, the clinically dominated cancer advisory bodies are apt to keep most of the remaining funds for themselves, leaving pure science much poorer than when the NCI was riding high. While attempts might be made to transfer some of the (new) cancer money into other areas, there is little reason to expect that the other branches of the NIH would gain additional backing as a result of loss of faith in the NCI. So while most non-NCI-supported scientists have no love for the sloppy use of its vast powers and almost instinctively want it cut back, on second thought they should shudder at the consequences of a drastic fall in its credibility.

We thus may be facing the unfortunate situation in which the immediate future of all too much biological research may seem to depend upon the ability of the NCI to live true to its most optimistic hopes and come up with real breakthroughs, both in research and, much more importantly, in the clinic. And as they may have to come soon or its critics will have their day, the NCI may believe it has no alternative but to put on a still brighter face, update its list of potentially hopeful situations (e.g., chemotherapy in the treatment of breast cancer), and indiscriminately escalate still more its monetary requests for new clinically oriented cancer centers. Here they are likely to argue that large numbers of lives can be saved, but only if more opportunities exist to refine the newer methods of treatment.

Such a plan of action, however, will be very risky since the NCI's objectives, of necessity, will be even more short term in nature, both in the research laboratory and in the clinic. And correspondingly, there will be even less chance of real success. Moreover, the still essentially slow rate at which fundamental new clinical clues about cancer appear means that even more of these centers will be duplicating each other's research efforts.

Of course if one seriously thought that mere increase in manpower would generate cancer cures, massive duplications of efforts would be the moral approach, despite the real cost of the anxieties generated by the resulting competitive races. But if instead what is needed is a unique brain tomorrow, not masses of somewhat lost bodies today, we may be taking the risk that by trying too often to run in front of our ideas we shall so poison the atmosphere of the first act that no one of decency will want to see the play through to the end. Now, even in the best of cancer oriented labs, I frequently sense a mood of semi-hysteria, with many of the younger, still unknown scientists feeling they have to produce big or be forever lost. So it is hard for them to avoid jumping in on the bright idea of the moment, even though cold reflection may tell them that enough minds already are committed to its resolution.

This is not to say, however, that all the better Labs doing research related to cancer have more money than they have ideas. Many of us could do more imaginative research if we worried less about next year's financing and more about some current observation which does not fit into the dogma of the moment. But even so, we should realize that most biological (clinical) problems have a momenta of their own, which can only be influenced within limits by sudden massive infusions of Federal funds. Instead, rapid movements forward occur only rarely, and then generally because of observations that never would have been supported by "closely targeted" funds. Thus, almost paradoxically, we may move faster toward curing a particular human affliction by largely ignoring its existence and emphasizing pure science instead, rather than beating our brains against stone walls merely because they are in the way.

Yet at this moment in the Cancer War, the targeted approach is gaining more and more favor, often based on the specious argument that twenty-five years of a more pure approach has gotten us almost nowhere (e.g., with breast cancer). So I fear that over the next several years, hundreds of millions of dollars will be wasted in crash assaults against specific cancers, like those of the breast, colon and pancreas, despite the fact that the recipients of all this cash have no intellectual bases to march forward from. Unfortunately, such scientifically sterile forays are almost unavoidable consequences of battles that seem to go nowhere and where any action, no matter how half-brained, seems better than no action. Yet someone is bound to add up the cost and wonder very aloud to the people, who can count, whether such money might have been better spent to house and feed some of our poor rather than to maintain a cancer research community whose noble purposes appear to put it above the need for self-examination.

I thus suspect that a major problem now facing cancer research, if not the world of pure biology, is how to be realistic about what we can do for society without seeming to reject the hands that may want to overfeed us. Certainly we must always join in when we can give much needed expert advice or carry out key experiments. But at all times we must remain honest and not further the myth that given enough money, biologists (clinicians) will quickly find the knowledge to do away with much of the world's agony. By so talking, we create the danger that we may be taken too seriously and be given money for tasks that we are not yet ready to carry out.

But given this proviso, we should of course continue to respond to challenges as difficult as the conquest of cancer. Life would be very pointless if we did not. But in doing so, we must always be candid about the pace at which we can reasonably expect to move. For if we promise in excess of our ideas, we may have no recourse but to mask our doubts by requesting still larger sums of money and then hoping that luck is on our side, and when success still persists in being around the corner, to cry for still more of the National cash. This does not mean that great masses of money will not have to be spent before cancer is cracked. I believe they will, and more than Congress now thinks. But we must also realize that thoughtlessly dispensing very large sums may be totally without any positive effect. For unless we are careful, the current War on Cancer will increasingly resemble our Vietnamese debacle—"Just a few hundred million more into this year's budget, and the tables will finally turn." And American biology will have all the inherent credibility now afforded to Presidential Press Secretaries.

Highlights of the Year

An Unusually Productive Year of Research

This past year has witnessed a drastic upsurge in the pace of our year-round research on cancer-related topics. Given the security provided by our umbrella-type center grant from the NCI, we could afford to think about a variety of different problems and come forth with many interesting results. Somewhat arbitrarily, we can divide our work into that which emphasizes the molecular biology of tumor viruses and that concerned with the unique nature of the cancer cell itself.

On the tumor virus side, we have been shifting more and more away from SV40 and toward the various adenoviruses, having as our general objective a complete understanding of how these viruses replicate and as a more specific objective moving in on what gene(s) of these viruses convert normal cells into their cancerous, "transformed" equivalents. Now it appears that only the extreme left end (~2000-3000 base pairs) of a given adenovirus chromosome (~35,000 base pairs) carries the "cancer genes" (oncogenes), with hints existing that perhaps only one gene is involved — this being one which functions early in the viral multiplication cycle. So using a variety of different techniques, we are working very hard to identify the product of this gene, hoping somehow to pinpoint its normal function during the viral multiplication cycle.

Most of our study of the cancer cell itself still involves cells made cancerous by introduction of the SV40 chromosome into one of the host chromosomes. Here all the viral genome generally is present, but for still unexplained reasons, only one gene seems to

function. It codes for a protein of still unknown function, which is called the "T" (tumor antigen). Work with mutant viruses reveals that this protein has to be present continuously to maintain the cancerous phenotype, and it is tempting to ascribe it a key role in the synthesis of both SV40 DNA and RNA. In any case, given sufficient levels of active T antigen, the transformed cells exhibit a variety of properties not found in their normal counterparts. They not only grow to higher densities on flat surfaces, but also grow well when suspended in agar, a capability that normal cells lack. Also a very characteristic property of SV40-transformed cells is their preferential release of the "cell factor," a proteolytic enzyme first seriously studied by Reich's group at The Rockefeller University, with whom our staff has been actively collaborating. As the amount of growth in agar is strongly correlated not only with the amount of proteolysis but also with the ability to form tumors, it is very tempting to believe that the increased synthesis of the cell factor by cancer cells is the consequence, either direct or indirect, of T antigen functioning.

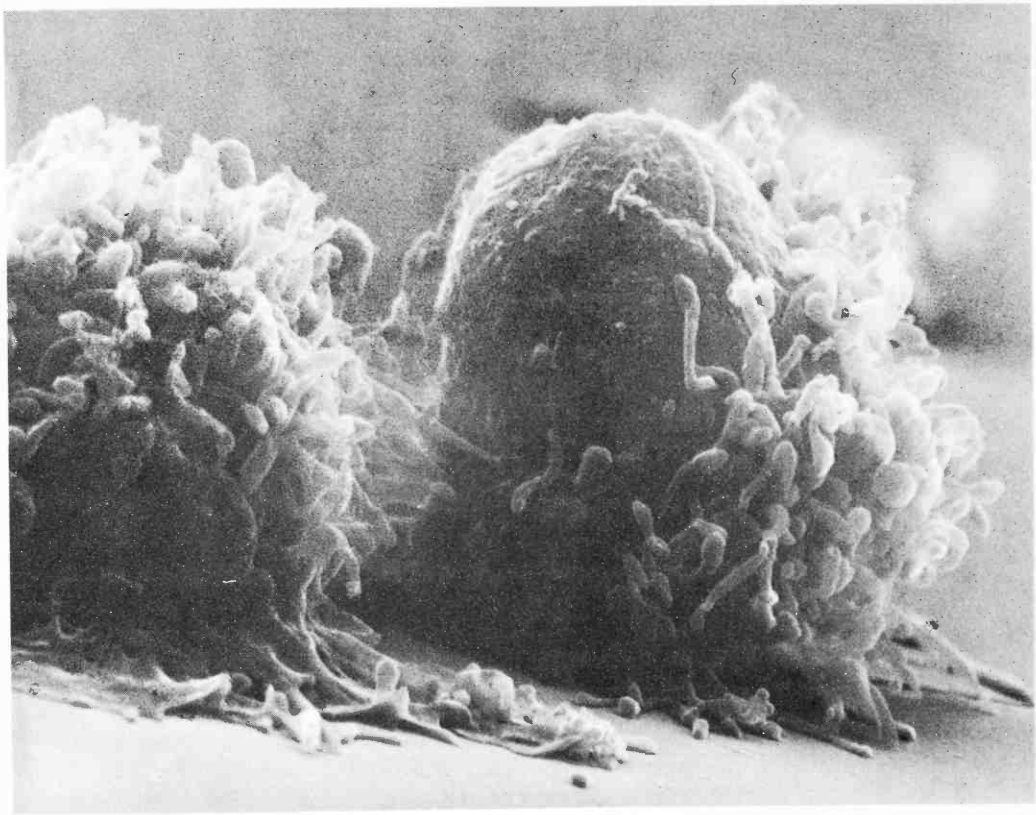
How proteolysis, per se, might lead to tumorigenicity is still very open, with our only hint being observations, both here and elsewhere, that cancerous cells have a less well organized system of intracellular muscles than their normal equivalents. Greatly aiding our experiments has been the development here of in situ immunological procedures that have for the first time convincingly demonstrated organized actin-myosin-tropomyosin complexes running through long sections of cells growing in culture. While many scientists believed that such structures existed, no one previously had a procedure for unambiguously visualizing them. Now, however, the use of antibodies directed against the various muscle proteins should open up a very exciting new phase of cell biology. Here the main objective will be to understand how these intracellular muscle fibers give to cells the capacity for directed movement. Hopefully somewhere in this analysis we shall understand whether the loss of an organized muscular apparatus is a direct cause of the cancerous state or an indirect expression of a still undiscovered mechanism through which cells lose the ability to regulate their proliferation.

Support from the Robertson Research Fund

That we have been able to grow so rapidly over the past two years to become again a major site for imaginative research owes much to the existence of the Robertson Research Fund, monies set aside two years ago by the Robertson family of Lloyd Harbor for the promotion of research at Cold Spring Harbor. Initially, most of these funds went toward the purchase of equipment for either new or completely renovated laboratory facilities. As a result of this fund, vital portions of Demerec, James and Davenport labs are now superbly equipped. For example, the new Phillips 201 electron microscope now operating in Davenport was so obtained, as was all the equipment for the space recently renovated in James for work with high levels of radioactive isotopes. Now with all our labs almost fully equipped, we contemplate using more and more of these funds to bring more scientists to Cold Spring Harbor for visits of several months to several years. Already this was possible on a modest scale this past year with, for example, the several month stays of Jim Williams from Glasgow and Phil Gallimore from Birmingham. And equally important has been our ability to continue through Robertson support our long-standing program of summer research for undergraduates, as well as to cover the expenses of our new summer course on the neurobiology of the leech (see below).

Return of an Animal Facility

During the period when microbial work dominated research at Cold Spring Harbor, our very outdated facilities in the Animal House (now McClintock Laboratory) for housing small laboratory mammals (e.g., mice, rabbits, hamsters and rats) were phased out. Since then we have had to rely on the State University at Stony Brook for help whenever we wanted to make antiserum or have a reliable supply of small rodents for preparation of extracts for mammalian cell-free protein synthesis. Over the past few years, this deficiency has proved to be an increasing hardship, despite very generous assistance from the Stony Brook staff. So when the Supreme Court ruled that the impounding of General Medical Support Money by the Nixon administration was illegal, we suddenly received unexpected



Spreading of 3T3 mouse fibroblasts in the presence of 10 hg/ml of the drug cytochalasin B. One effect of cytochalasin B is the enucleation of mammalian cells. The nucleus of the cell on the right is just moving out of the cell surface which is covered with microvilli and small blebs. Tilt angle: 80°; horizontal magnification: 5200X. (*Photo by Gunter Albrecht-Buehler*)

funds sufficient to initiate work on a modest animal facility to be located in the old sheep shed situated next to McClintock. With design help from Rex Risser and Jim Lewis, work began in the spring, and by early November, the cold, damp smell that previously had permeated the building disappeared and a remarkably cheerful, very functional "Mouse House" became ours. Ably in charge of its operation is Mr. Robert DeCarlo, formerly of Stony Brook, and we anticipate its wide-ranging use through the coming years.

A Scanning Electron Microscope Facility in McClintock Laboratory

Thanks to assistance from the Human Cell Program of the National Science Foundation, we now have in operation a new and very high resolution scanning electron microscope VTC 1000 manufactured by the Advanced Metals Research Corporation. Because it was the first production instrumentation of its kind, it gave us more than the normal amount of teething difficulties when Bob Goldman and Bob Pollack first took it over. But with the complete cooperation of the manufacturer and through the timely arrival of the physicist turned cell biologist, Gunter Albrecht-Buehler, our instrument is now highly functional and in extensive use for examination of the surfaces of a variety of cell types.

A Mammoth, Yet Very Satisfying, Symposium on Tumor Viruses

In June we were host to some 350 visitors for our annual Symposium which this year focused on the molecular aspects of tumor viruses. Some 115 papers were on the formal program, and knowing that we would want to listen to many others who had unexpected new data, we feared that the pace would be impossible and many participants would leave the Symposium saying never again. But, in fact, it was a remarkably exciting meeting, the

various chairmen kept the formal talks to properly short intervals, and by serving food in Jones as well as Blackford, the lunch and dinner lines never became impossible. So we rate it one of the best of our Symposia and suspect that the resulting two-volume set of the proceedings will be very widely read. While we still have some reservations about resorting to two volumes, each some 700 pages long, a single volume of 135 articles would have had the bulk of the Manhattan telephone directory.

Again following a long tradition, many of our neighbors gave dinner parties for the speakers of the meeting, and we wish to thank in particular Mr. and Mrs. Ward C. Campbell, Mr. and Mrs. Arthur Crocker, Mr. and Mrs. Norris W. Darrell, Jr., Mrs. William A. Flanigan, Jr., Mr. and Mrs. Clarence E. Galston, Mr. and Mrs. Angus McIntyre, Mr. and Mrs. Robert H.P. Olney, Mr. and Mrs. Samuel D. Parkinson, Mr. and Mrs. James J. Pirtle, Mr. and Mrs. Edward Pulling, Mr. and Mrs. Franz Schneider, Mrs. Alex White, and Mr. and Mrs. William Woodcock. As usual, the various speakers at first seemed troubled by the prospect of even briefly interrupting discussion of their experiments, but upon return to the Lab, were full of praise for the remarkable hospitality of our neighbors.

A New Neurobiology Course on the Leech

The number of our summer courses advanced to ten this past year with the addition of an advanced experimental course on the leech. The relatively simple circuitry of the leech nervous system offers the potentiality of eventual deep understanding, and increasing numbers of neurobiologists are considering using it as their new animal for investigation. So our new course, ably taught by John Nicholls, Jan Jansen and Ann Stuart, was heavily oversubscribed, making it very difficult to choose the final list of ten students. As the course progressed, we saw that our high hopes were more than justified, with some students seeming never to leave their setups until much after midnight.

Also essentially new this past summer was our course on bacterial genetics, which became upgraded to the needs of students already highly versed in its general aspects. Thus the ten students chosen were in a position to profit from its special emphasis on specialized transducing phages and their use for the development of in vitro systems for studying gene function. Taught by Benoit de Crombrugghe, Max E. Gottesman and Jeffrey Miller, it was very successful, and a similar course will be given this coming summer.

The remaining courses followed the same general pattern as in previous years, but since many of the instructors were new, we had no fear that the material presented would be in any bad sense dully repetitious of previous offerings. Taking all courses together, we had approximately the same number of applicants (~330) as in previous years, and so our instructors continued to have wide leeway in choosing the students to be accepted. As a consequence of less money being available for training students, we received more requests for scholarship aid. Thus we were very relieved when the release of impounded training grant funds allowed the awarding of a training grant specifically designed to back our neurobiology program. This, together with much needed help from the Crass Foundation, allowed us to provide substantial financial help to over half of our neurobiology students.

A Very Full Program of Summer Meetings

Following the completion of the summer courses, we again were hosts to a rapid succession of summer meetings that brought some 500 additional visitors for periods of a week or more. The nine days allotted to phage represented the largest period yet devoted to it, and we were particularly pleased with the large contingent who came for the morphogenesis section. The DNA replication meeting, while bringing back many familiar names, was notable for the large number of new faces, thus arguing well for the intellectual health of this field over the next decade. The summer concluded with the second in our Conferences on Cell Proliferation which this year focused on proteolytic enzymes and their role in a variety of cellular control processes. Organized by Ed Reich, Dan Rifkin and Elliott Shaw, this was indeed a high quality meeting which should have long-term beneficial effects in delineating the extent to which protease action is involved in generating signals to commence cell division. Proceedings of this meeting are in press, and we expect the resulting some 800-page volume to be available in mid-summer.

Further Growth in Our Publishing Program

During the past year we have published three books, in addition to a very large Symposium volume on chromosomes. Particularly notable was the appearance of the first volume in our Cell Proliferation Series. Edited by Bayard Clarkson and Renato Baserga and entitled "Control of Proliferation in Animal Cells," it runs over 1000 pages in length and so represents a relatively large capital investment. We have been pleased with its broad acceptance so far and suspect that with time it will break even despite its relatively low price of $30. Like other publishing houses, we have experienced massive cost increases, yet we are hesitant to price our books accordingly because so many of our readers are short of cash and can only buy if the price is reasonable. But how long we can continue to set prices so much lower than our commercial equivalents is hard to predict. If the current recession markedly chokes off sales to private individuals, thereby leaving only the library sales, we will have no choice but to act like everyone else. In the meantime, we take great pleasure in the continued excellence of our publications, and, in particular, are most pleased with our handsome volume on "Ribosomes" which represents the fourth volume in our Molecular Biology Monograph Series. Edited by Masayasu Nomura, Alfred Tissières and Peter Lengyel, and growing out of a meeting held here in September '73, we find it a remarkably well put together book which hopefully will find wide usefulness throughout the worlds of molecular biology and biochemistry.

The Size of Our Scientific Staff Remains Approximately Constant

By now we have grown accustomed to the fact that our relatively constant size implies steady chances in our scientific staff. Nonetheless, we can only feel a sharp sense of loss when such productive scientists as Bernard Allet, Hajo Delius, Francoise Falcoz-Kelly, Martha Howe and Phil Sharp move on to other positions. Happily all have moved to positions where they can continue very high level science — Bernard back to Geneva, Hajo to Munich, Francoise to the Institute Pasteur, Martha to Madison, and Phil to MIT. We shall also miss Jane Flint who was a postdoc with Phil Sharp and went with him to his MIT lab, Jeremy Bruenn who moved on to Buffalo from Ray Gesteland's lab, and Peter Greenaway who returned to England to work in the Government Laboratory at Porton. Also greatly missed is Art Vogel who worked with Bob Pollack while completing the Ph.D. component of his M.D.-Ph.D. program at N.Y.U. School of Medicine.

On the credit side, James lab is now much stronger due to the arrival of Mike Matthews from England, via a stay in Tompkin's San Francisco lab, and to the promotion to the scientific staff of Mike Botchan who first came here as a postdoc after doing his thesis at Berkeley. Another new addition to our staff is Jim Lewis who initially came as an advanced postdoc with Ray Gesteland.

We Continue Our Program of Extensive Capital Improvements

The apparently never-ending task of upgrading our physical facilities continued in full urge over the past year. Of particular importance has been the collaboration between our uilding staff headed by Jack Richards and the architectural firm of Charles Moore ssociates of Essex, Connecticut, which has resulted in the complete renovation of Airslie" our lovely, ancient, almost manorlike home that dates back to 1806 when it was uilt by Major William Jones. Later acquired by Mr. Henry DeForest, it was passed on to ne Laboratory upon his death, and since 1942 it has served as the Director's residence. ver the past three decades, however, its physical condition steadily deteriorated, and the uestion arose if it could, in fact, still be saved. Fortunately, the answer was very much es, and thanks to the combination of a superb architect and very competent craftsman, ir Lab now possesses a home which will bring it many compliments.

With the completion of Airslie in late October, work commenced on our next two major ojects, the first, the upgrading of Jones lab into a year-round facility for neurobiology, d the second, the construction of a completely new system for treatment of waste water. both these endeavors, we again have relied on the architectural assistance of Charles oore Associates who propose to retain the splendid "end-of-the-century" feeling of Jones ile making a strong architectural statement through the ultra-modern research modules

in which most of the experimental work will occur. As for the waste-water treatment plant, which we had no choice but to site between Hooper House and Davenport, our objective is to keep it as invisible as possible, and so most of it will be underground with the protruding portion camouflaged as a Victorian-like gazebo.

Still Greater Uncertainties about Future Federal Support for Biological Research

While for the short term we remain in a good position with regard to Federal funds, we must be cautious about our chances for equivalent aid over the next several years. While much depends of course on the quality of our science, we must also take into account the fact that medical research, especially of the pure variety, is not high priority with the Ford administration, who have publicly communicated their desire to drastically cut it back in favor of increased expenditures for research which may improve our long-term supply of energy. While it seems likely that Congress will not go along with most of the proposed slashes, no one is sure what is safe, and, for example, we do not know whether we shall receive the General Medical Research Grant of some $100,000 that ordinarily would have been sent to us in early January of 1975. And if their proposal holds to still further favor contracts over grants, we will have no choice but to seek this sort of money even though we consider it a retrogressive step for the long-term future of American biology. So we have to count on the good sense of Congress more than ever and hope that they will not be so overwhelmed by the deepening economic recession that biological research gets accidently lost as they try to find ways out of the National morass.

So we clearly must make contingency plans and consider awful cutbacks here if they become necessary. On the other hand, I feel an immediate retrenchment under the guise of caution would also be very unwise. This Lab cannot accept being dull since we make sense only if we offer something out of the ordinary. We may have to do this with less waste, but move forward we must.

A Very Happy 50th Anniversary Party for the Long Island Biological Association

A major reason why we can still look ahead to a positive future is the continuance of such strong and loyal support from our neighbors. Over the past 50 years their support has been expressed through the activities of the Long Island Biological Association (LIBA), an organization specifically organized to support our endeavors. Expressions of LIBA's activities are visible everywhere, in buildings like Nichols, Davenport, James, Urey, Cole and Page which were built with funds provided by LIBA and in large parcels of land given to us as needed buffers from the outside world. And before Federal funds became generally available for biological research, it was LIBA money that directly covered much of our research costs. So it was clear to all that we must celebrate the 50th anniversary of LIBA as a very special event whose style should be in keeping with the high hopes expressed by the founders when they banded together to make this Lab a very special place. Happily the party, which was superbly organized by Mr. Edward Pulling, our LIBA chairman, lived up to our expectations, in part because we were able to have Jacques Monod, Director of the Institute Pasteur, fly here to give the main address, and in part because of the contagious enthusiasm expressed by our neighbors as they wandered about our grounds and saw what we were up to.

Continued Strong Support from Our Board of Trustees

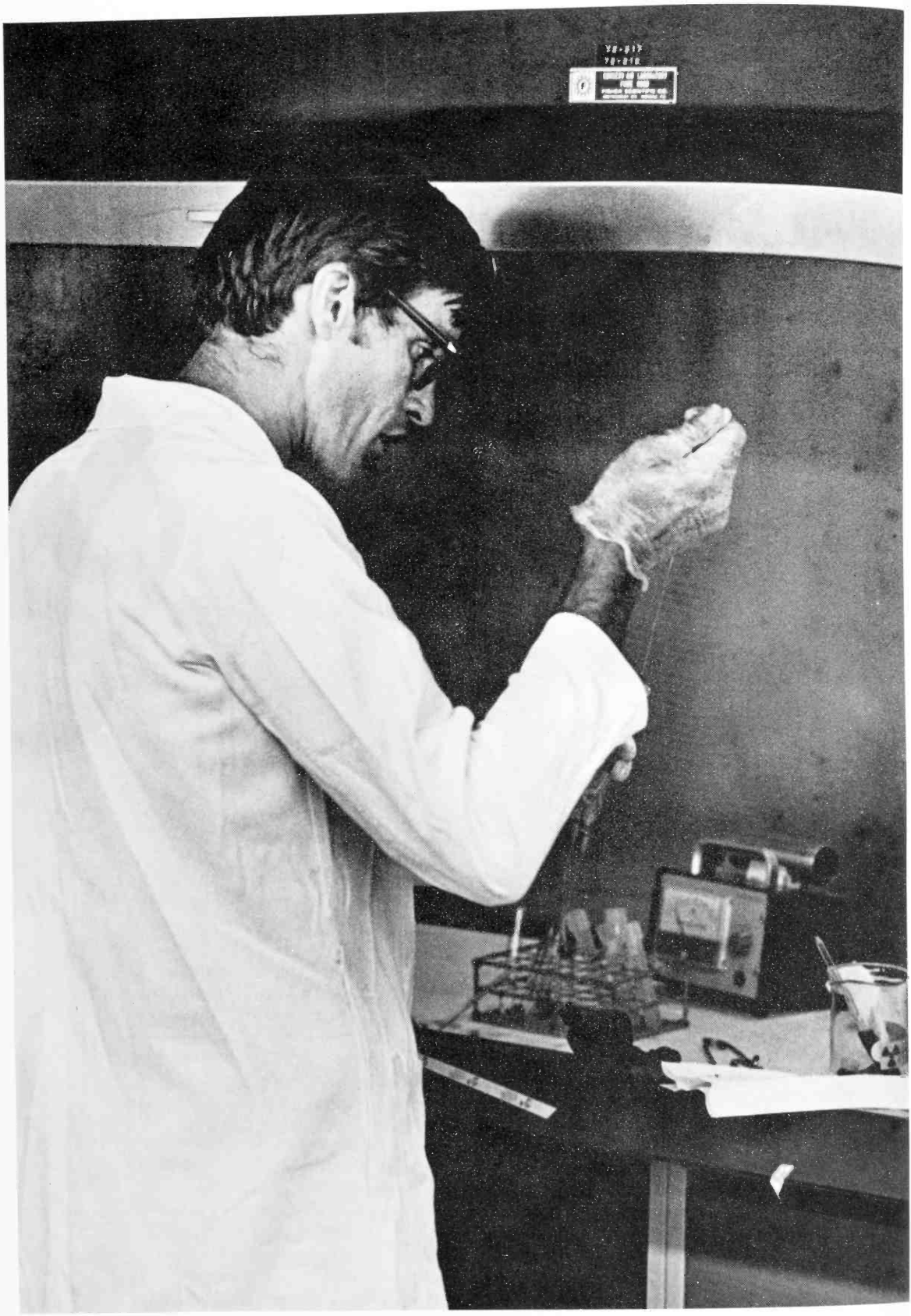
One of our great strengths comes from having a Board of Trustees of diverse outlooks, with members both from our local community and from academic institutes. Thus we have been able to function quite effectively without need of still additional advisory bodies to make up for talents usually not present on a conventional Board of Trustees. My only regret about its organization has been that the by-law limiting office to two three-year terms means that all too frequently we lose members we have come to regard as indispensable. In this regard, I must note the departure of Bob Olney, whose second term ended in October. In a great variety of ways, most recently as Chairman of our Board, Bob has always been of inestimable help, and we shall be a poorer body without him. Fortunately in Dr. Harry

Eagle of the Albert Einstein School of Medicine we have a most able replacement as Chairman. Harry became a member of our Board at the time of our 1962 reorganization, and after the obligatory mandated hiatus, he rejoined the Board a year ago. He brings to us the benefits of a remarkable career that combines major contributions to cell biology and microbiology with a skill in administration unrivaled on the academic scene.

Our Objectives Must Always Remain Very High

Now it is hard to believe that I have been the Director for seven years. Possibly because there has always been so much to do there has been no chance to feel bored and restless, hoping that something novel would emerge to liven the scene. Fortunately, this situation still seems as true as ever, with the prospect that given our horrid National economic picture, we will have to work very hard just to stay even, much less to become more exciting and productive. So I must note how fortunate we have been in being able to assemble such an outstanding group of younger scientists to work here. Without them we would be essentially nothing, and if we are to survive well in the future, we must always be an institution that cherishes their existence and supports them with all our worth. As long as this remains our main objective, we shall continue to have a very bright future.

February 12, 1975 *J.D. Watson*



Ray Gesteland at work in Demerec Laboratory (*Photo by Ross Meurer*)

MOLECULAR BIOLOGY OF TUMOR VIRUS

J. SAMBROOK
J. Arrand
D. Bachman
G. Ballantyne
M. Botchan
R. Cohen
E. Collins
E. Daniell
P. Edson
S. Flint
R. Greene
T. Grodzicker
A. Johnston
P. Jungreis
W. Keller
F. Kelly
R. Lancaster
A. Lewis
M. Lurye
M. Mathews
B. Miller
C. Mulder
B. Ozanne
M. Rust
W. Sann
P. Sharp
J. Stravato
N. Sullivan
I. Wendel

During the last year, work in James Laboratory has concentrated on six major topics—the identification of the adenovirus viral nucleic acid sequences that are involved in transformation, the transcription of adenovirus 2, the characterization and physical mapping of host-range and temperature-sensitive mutants of adenoviruses, the isolation of variants of transformed cells that carry specific genetic markers, the organization of mammalian chromosomes, and the purification and mechanism of action of a protein that unwinds turns from superhelical DNA.

Invaluable for most of these studies has been the collection of restriction enzymes that, in large part, has been isolated at Cold Spring Harbor. During the year we have collaborated with Hajo Delius and members or Rich Roberts' laboratory in a major effort to map the sites at which some of these enzymes cleave the DNAs of various adenovirus serotypes. In addition to the map of the *Eco*RI fragments of adenovirus 2 which was presented in the 1973 Annual Report, we now have available maps of the positions of cleavage of DNAs of adenovirus serotypes 3, 5, 7, 12 and Ad2$^+$ND1 by *Eco*RI and of the DNAs of serotypes 2, 5, 12 and Ad2$^+$ND1 by *Sma*I. We have also nearly completed indentification of the sites in adenovirus 2, 5 and Ad2$^+$ND1 DNAs that are attacked by *Hin*dIII. Thus we now have available overlapping sets of fragments of DNA derived from defined segments of viral genomes, which can be used as probes to study the integration and expression of viral sequences in lytically infected and transformed cells.

Viral DNA Sequences in the Genome of Transformed Cells

Adenovirus Sequences in the DNA of Transformed Cells

We have analyzed the adenovirus 2 sequences integrated in transformed rat cells using the method of Gelb et al. (*J. Mol. Biol. 57:*129-145 [1971]) to determine the kinetics of reassociation of adenovirus 2 DNA and the DNA of each of the *Eco*RI and *Hpa*I fragments in the presence of transformed cell DNA. In all, we have examined a total of ten cell lines, whose history and biological properties are known. Originally, these cells were transformed by adenovirus 2 in Birmingham, England; they were brought to our laboratory by R. Phillip Gallimore who worked here as a visiting scientist during Feburary, 1974. From the kinetics of hybridization of each of the ^{32}P-labeled *Hpa*I and *Eco*RI fragments in the presence of transformed and control DNAs, we conclude that no line of rat cells transformed by adenovirus 2 contains a complete set of viral genes. Because the deletions are of unequal lengths and occur at different positions in the viral DNA, we cannot say yet whether there is a segment of the viral genome whose absence from the transformed cells is mandatory. However, common to all ten of the cell lines is the segment of viral DNA that extends from the left-hand end to a point that lies about 14% along the genome, and in six of the cell lines, these are the only viral sequences that are present. This portion of the genome must therefore code for any viral functions that may be required for the maintenance of the transformed state.

In general, cell lines that were established from cultures infected with high multiplicities of adenovirus 2 contain a greater proportion of the viral genome than those derived from cells infected with low multiplicities of virus. So far, however, we have no reason for believing that any viral sequences other than those of the extreme left-hand end of the genome play a major role in maintaining the phenotype of transformed cells. This conclusion is in good agreement with results obtained in collaboration with Alex van der Eb and Frank Graham, who assayed the ability of isolated *Eco*RI and *Hin*dIII fragments of adenovirus types 2 and 5 to transform baby rat kidney cells. Using the calcium

phosphate infection method developed by Graham and van der Eb, it was found that the *Eco*RI-A and *Hin*dIII-G fragments of adenovirus 2 and 5 DNAs transformed cells with an efficiency approximately equal to that of intact viral DNA. None of the other *Eco*RI or *Hin*dIII fragments of the two viral DNAs carried any transforming capacity; similarly, all of the isolated *Sma*I fragments and a mixture containing all of the *Hpa*I fragments were inactive. From these results, we again conclude that the sequences of adenoviruses 2 and 5 DNAs that are important for transformation are located at the extreme left-hand ends of the viral genomes, entirely within the *Hin*dIII-G fragment, which consists of about 7.5% of the total viral DNA. Both *Sma*I and *Hpa*I cleave the DNAs of adenoviruses 2 and 5 within the *Hin*dIII-G fragment (at positions 3 and 4%, respectively, from the left-hand end); therefore, it is perhaps not surprising that no fragment generated by these enzymes has the ability to transform cells. We have also examined the viral DNA sequences in two lines of transformed cells that were established after infection of secondary cultures of hamster embryo fibroblasts with the *ts*14 mutant of adenovirus type 5 (Williams, *Nature 243*:162-164 [1973]) and which were brought to the laboratory by Dr. J. Williams during his tenure as a visiting scientist in February-March, 1974. The results of experiments in which the kinetics of renaturation of each of the specific *Eco*RI and *Hpa*I fragments of adenovirus 5 DNA were measured in the presence of DNA extracted from transformed and control cells showed that the transformed cells contained viral DNA sequences homologous to three of the seven *Hpa*I fragments, to part of another *Hpa*I fragment, and to part of one of the *Eco*RI fragments. From the position of these fragments on the adenovirus 5 map, we conclude that the two cell lines contain segments of DNA extending in from the left-hand end to points about 35 and 40% along the viral genome, respectively, and carry no other viral DNA fragments.

Taken together, these results very strongly suggest that the information in the left-hand ends of the genomes of adenoviruses 2 and 5 is necessary for the establishment and maintenance of the transformed state. It also seems likely that a similar situation will be found with other adenovirus serotypes. Preliminary results, obtained both by infection of cells with isolated fragments and by analysis of viral DNA sequences in cells transformed by intact virions, suggest that the cell-transforming activity of adenovirus type 12 is carried by a segment which maps at the left-hand end of the viral genome and which consists, at most, of 16% of the viral DNA.

SV40 Sequences in the Genome of Transformed Cells

By contrast to adenovirus-transformed cells, which contain partial copies of the viral genome, most lines of cells transformed by SV40 contain the entire sequences of the viral DNA; infectious virus can be detected in heterokaryons formed between transformants and permissive cells (Watkins and Dulbecco, *Proc. Nat. Acad. Sci. 58*:1396 [1967]), and virus replication occurs in monkey cells which have been exposed to high molecular weight DNA that has been extracted from transformed cells (Boyd and Butel, *J. Virol. 10*:399 [1973]). In the past, it has been assumed that all segments of the viral genome are present in the transformed cells at equal frequencies. However, during the past year, we have shown that this assumption is unjustified. We have used renaturation kinetics to measure the amounts in the SVT2 line of SV40-transformed mouse fibroblasts of each of the four specific fragments which are obtained when SV40 is cleaved sequentially with *Eco*RI and *Hpa*I. We found that the transformed cells contain sequences homologous to all four segments of the viral genome. However, each of the segments was present in SVT2 cells at different frequencies: the best interpretation of our data is that a contiguous region of the SV40 genome (roughly corresponding to the early genes) is represented about 6 times per diploid quantity of cell DNA and that the remainder of the viral genome is present at a frequency of 0.8 copies per diploid quantity of cell DNA.

We can think of two possible ways by which biased representation of DNA tumor virus genomes can occur in transformed cells — either the cells are transformed by virus particles which carry defective or altered genomes, or the final complement of viral nucleic acid is determined by a process of selective amplification and/or loss of nonessential or deleterious viral sequences, which occurs during or after the transformation event. We are now trying to distinguish between these models by studying the structure of the genomes

of defective particles of adenoviruses and by determining the permutation of the viral DNA sequences in transformed cells. For the latter approach, we are measuring the distribution of viral sequences among the different sizes of fragments obtained by cleavage of transformed cell DNA by restriction enzymes. By using a procedure in which high molecular weight transformed cell DNA is cleaved with various restriction enzymes fractionated by electrophoresis through agarose gels, denatured in situ, and transferred directly to a cellulose nitrate filter for hybridization to virus-specific nucleic acid labeled at high specific activity with ^{125}I, we shall be able to compare the arrangement of the integrated viral sequences in many different transformed cell lines.

Characterization of DNA from Virus Rescued from Transformed Cells

To characterize further the sequences of viral DNA that are present in transformed cells, we have begun a study of the types of SV40 DNA molecules that are produced after cell fusion of transformed mouse cells and permissive monkey cells. Our interest in this work is motivated in part by the findings, described above, that transformed cells can contain combinations of partial copies of viral sequences plus complete genomes. Therefore, novel forms of viral DNA may be produced by excision and subsequent replication of defective molecules. Further, in order to draw linear maps of the integrated viral DNA and its neighboring cellular DNA sequences, we must be certain that the restriction sites produced by lytic infection that have been mapped in viral DNA are present in the integrated genome.

Five subclones of the SV40-transformed line SVT2 were picked and independently fused with permissive monkey CV-1 cells. Cell fusion was accomplished by the use of UV-inactivated Sendai virus. Each of these subclones was presumed to be identical since the parent line was itself of recent clonal origin. The presence of virus was assayed in the fusion lysates both by plaque formation and induction of T antigen in monkey cells. All five subclones and the parental cell line produced virus that was able to induce T antigen in these permissive cells. Plaque-forming virus, however, could be detected only in the lysates of one subclone and the parental cells. Nevertheless, after 3 serial passages enough viral DNA could be extracted from all of the lysates to allow biochemical analysis of both plaque-forming and non-plaque-forming rescued viruses. We found three different types of viral DNA molecules had evolved, although each fusion lysate contained only one type. The viral DNA rescued from three clones consisted of molecules whose *Hin*dII and III, *Eco*RI and *Hpa*I restriction patterns were identical to those of small-plaque SV40 DNA. The parent clone and one subclone produced viral DNA that showed alterations in two discrete regions of the genome: *Hin*dII and III fragment C migrated as if it had suffered a deletion of 150 nucleotide pairs, and *Hin*dII and III fragment F migrated as if it had suffered a deletion of 30 base pairs. The deletion in fragment F was determined by double enzyme digestion experiments to lie clockwise of the *Eco*RI site. The third type of viral DNA produced by another subclone of SVT2 cells again had deletions in *Hin*dII and III fragments C and F; however, in this case, fragment C migrated with an apparent deletion of 60 base pairs and fragment F with a deletion of 30 base pairs.

We do not believe that these three different types of sequences preexisted in the transformed cells, but rather arose during the rescue and subsequent passages. The reasons are as follows: (a) The initial fusion lysates were defective, in that plaque-forming titers were either absent or negligible, while the lysates induced T antigen efficiently; (b) hybridization experiments indicate that these cells contain enough information for only one complete virus and several partial copies.

Interestingly enough, infectious viral DNA rescued from three other transformed cell lines, namely, MSV-SV101, LS SV101 and SV3T3 cl 9, also showed deletions in *Hin*dII and III fragments C and F. No simple statement can be made concerning the efficiency with which these resuced virus stocks transform — while some seem to transform with a higher frequency than does plaque-purified SV40, others transform with reduced efficiency.

It is clear that by restricting our analysis to the viral DNA in infectious particles, we may be missing a significant fraction of the SV40 DNA molecules that are produced in heterokaryons. The "helper lawn" technique developed for isolating defective SV40 genomes (Brockman, Lee and Nathans, *Cold Spring Harbor Symp. Quant. Biol. 39:*119 1975]) should provide a useful tool in examining partial viral genomes in fusion lysates.

Transcription of Adenovirus 2

During Lytic Infection

In order to map the species of RNA that are transcribed from adenovirus 2 DNA during lytic infection, we have used the six fragments of the viral genome that are generated by cleavage of the intact DNA by endonuclease *Eco*RI and the seven that are obtained by cleavage with *Hpa*I. To map minute quantities of viral RNA sequences by hybridization to ^{32}P-labeled DNA, it is necessary to avoid DNA:DNA hybridization during long periods of incubation. It was therefore necessary to separate the two strands of each fragment of adenovirus 2 DNA and to anneal each strand to saturating amounts of RNA extracted from lytically infected cells. The conventional method of separating the strands of duplex DNA has been the equilibrium sedimentation of a mixture of denatured DNA and poly(U:G) in a cesium chloride gradient (Szybalski et al., *Methods in Enzymology*, XXI D [ed. L. Grossmann and K. Moldave] p. 383. Academic Press [1971]). Landgraf-Leurs and Green (*J. Mol. Biol. 60:*185 [1971]) and later Tibbetts et al. (*J. Virol. 13:*370 [1974]) have used this method to separate the intact strands of adenovirus 2 DNA; unfortunately, however, the amount of separation of the two strands is highly dependent upon the particular lot of poly(U:G) used. Hayward (*Virology 49:*342 [1972]) described the separation of T7 and λDNA strands by electrophoresis in 0.6% agarose gels, and we have adapted his method to separate the strands of the specific fragments of adenovirus 2 DNA. Using ^{32}P-labeled fragment strands in solution hybridization experiments, the fraction of each strand complementary to RNA extracted from infected or transformed cells was assayed by chromatography on hydroxylapatite. In this manner, a tentative map of the viral RNA sequences extracted from the cytoplasm of cells both "early" and "late" during lytic infection was constructed (see Fig. 1). To determine the polarity of the viral DNA strands, an experiment similar to that described previously for SV40 DNA was carried out (Sambrook et al., *Proc. Nat. Acad. Sci. 70:*3711 [1973]). Linear duplex adenovirus 2 was digested with exonuclease III to remove about 100 bases from the ends of two strands of the genome. The resulting molecules, with their internal 3′-OH groups and protruding 5′-phosphoryl tails, were used as primer templates for DNA synthesis with RNA-dependent DNA polymerase. After incorporation of [^{32}P]dTMP at the 3′ ends of the molecules, the DNA was cleaved by *Hpa*I and the resulting fragments were separated by gel electrophoresis. *Hpa*I fragments E and G, which contain the left and right termini of the viral DNA and were the only fragments that contained label, were recovered from the gel and hybridized in the presence of the separated strands of adenovirus 2 which had been prepared by cesium chloride-poly(U:G) centrifugation. The ^{32}P-label in *Hpa*I fragment G annealed specifically to the "light" strand of adenovirus 2 DNA, whereas the ^{32}P-label of *Hpa*I fragment E hybridized only to the "heavy" strand. In the map shown in Figure 1, therefore, the 5′ end of the *r* strand of viral DNA lies to the right, while the 5′ end of the *l* strand lies to the left.

Because "early" cytoplasmic RNA anneals to four distinct regions of the viral genome, adenovirus 2 probably codes for at least four early functions. From the proportion of the

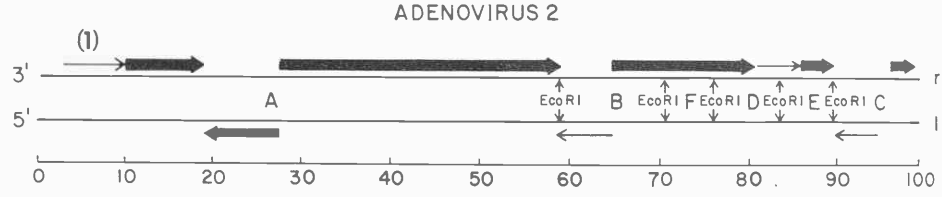


Figure 1

The arrows show the position and direction of transcription for both "early" (⟶) and exclusively "late" (⟶) cytoplasmic viral RNA sequences. (1) denotes the "early" RNA sequences found in all viral transformed cells. The nomenclature adopted by the group working on bacteriophage has been used to name the two strands of Ad2. The *r* and *l* strand are transcribed to the right and left, respectively, on the conventional map. The *r* and *l* strand correspond to the Ad2 DNA strand with the lower and higher affinity for poly(U:G), respectively. Therefore, the *r* and *l* strand sediment to equilibrium in a CsCl gradient containing poly(U:G) at lower and higher densities, respectively.

viral DNA that hybridizes with early RNA, it seems that about 22% of the viral genome codes for early functions. Summation experiments have shown that all RNA sequences found in the cytoplasm of cells early during infection are also present in the cells' cytoplasm at late times.

At late times after infection, the equivalent of between 95 and 100% of one strand of adenovirus 2 DNA is transcribed into stable cytoplasmic species of RNA. A sum of the saturation hybridization values shows that about 20% of the RNA is transcribed from one viral DNA strand (the l strand) and 75-80% is transcribed from the other.

Both early and late during the lytic cycle, viral RNA sequences are found in the nucleus that are not found in the cytoplasm. While early cytoplasmic RNA is complementary to 22% of one strand equivalent of viral DNA, early RNA extracted from total cells is complementary to the equivalent of at least one entire strand. Late during the lytic cycle, the nucleus of adenovirus 2-infected human cells contains RNA complementary to essentially all of the sequences of both strands of the viral DNA. The model that accounts for these data most satisfactorily is that proposed by Aloni (*Nature New Biol. 243*:2 [1973]) for transcription of SV40 DNA: both strands of the duplex genome are copied into long RNA transcripts in the nucleus late during infection, and a subset of these RNA sequences, equivalent to one strand of the genome, survives to become stable cytoplasmic RNA.

In Transformed Cells

In general, the complexity of viral RNA sequences in transformed rat cells reflects the complexity of the viral DNA sequences present in the chromosomes. In the simplest cases, transformed cells that contain only 14% of the viral genome, it is found that the virus-specific RNA which is present is complementary to about half of these viral DNA sequences. Therefore, 7-8% of the *r* strand of adenovirus 2 is complementary to RNA in the cytoplasm of this class of transformed cells. Summation experiments show that the segment of the viral DNA that is expressed is also present in cytoplasmic RNA early during lytic infection. Because the only set of RNA sequences common to every adenovirus transformant is transcribed from the early region that lies between 3 and 10% from the left-hand end of the adenovirus 2 genome, we believe that the putative viral function responsible for maintenance of transformation must be an early gene product — a result that has already been predicted by others on the basis of genetic experiments.

Finally, the two other classes of adenovirus 2-transformed rat cells, which carry complicated sets of viral DNA sequences, contain viral RNA sequences complementary to 2 or 3 of the regions of the genome transcribed into "early" cytoplasmic RNA. All of them, however, contain a transcript derived from the left-hand end of the *r* strand of viral DNA.

Variants of Transformed Cells that Carry Specific Genetic Markers

Revertants of 3T3 Cells Transformed by Murine Sarcoma Virus

In collaboration with Arthur Vogel, we have isolated from populations of MSV-transformed nonproducer 3T3 cells (line KA31) classes of variants that are unable to grow in dense culture, are unable to synthesize DNA when suspended in medium containing methyl-cellulose, or are resistant to the toxic effects of concanavalin A. The revertants, all of which are indistinguishable from 3T3 cells in their growth characteristics, can be retransformed by MSV but not by SV40.

From the following results, it appears that the variants result from a defect in the genome of the resident murine sarcoma virus, which can be complemented by a gene function specified by murine leukemia virus: (1) After infection with MLV, the concanavalin A-resistant variants become transformed and release MSV. (2) Treatment of the revertants by 5-iododeoxyuridine results in the induction of endogenous MLV, retransformation of some of the cells, and rescue of MSV. While MSV rescued from the parental KA31 cells transforms 3T3 cells with one-hit kinetics, that rescued from the concanavalin A-resistant variants manifests two-hit kinetics. This result suggests that the genetic material of both MLV and MSV must be present for transformation to occur. The nature of the defect in the MSV genome of the revertants is under investigation.

Chromosomal Markers and SV40 DNA Sequences in Transformed Cells Resistant to Cytochalasin B

The karyotypes of cells of an SV40-transformed line of Balb/c mouse fibroblasts (SVT2 cells), determined by the acetic-saline-Giemsa banding technique, are remarkably homogeneous, with the majority of cells containing two copies of most chromosomes. The number of chromosomes per cell ranges from 35-45, with a mean value of 40: two X chromosomes are present, and chromosomes 1, 3, 14 and 19 have given rise to biarmed markers; chromosome 3 is often present three times. In all probability, the biarmed marker chromosomes were created by reversible centric fusion of telocentrics because the total number of chromosomes per cell increases as the number of biarmed markers decreases. The near diploid nature of SVT2 cells sets them apart from most other established mouse cell lines, which have a subtetraploid number of chromosomes.

We reported last year that the drug cytochalasin B is more toxic for SV40-transformed mouse cells than for normal mouse cells. We have used this toxic effect to select sublines of SVT2 cells that are resistant to cytochalasin B. Two of the sublines were found no longer to express SV40-specific T antigen and both had lost one copy of chromosome B and one X chromosome. By renaturation kinetics, it was shown that the cytochalasin B-resistant sublines contain less SV40 DNA than the parental cells. Instead of 6 copies of the "early" region and 1 of the "late" region (see above), the resistant cells were found to contain 2-3 copies of the "early" region and 1 of the late. One explanation for these results is that the SV40 DNA sequences that were lost were responsible for T antigen synthesis and were located either on chromosome X or chromosome 3, or both.

Virus Genetics

Characterization of Host-range and Temperature-sensitive Mutants

We have continued to isolate and characterize both absolute-defective and temperature-sensitive (*ts*) mutants of the nondefective adenovirus 2-SV40 hybrid virus $Ad2^+ND1$. $Ad2^+ND1$ grows efficiently in both monkey and human cells, and this extended host-range property is presumably due to the insertion of SV40 sequences into the Ad2 genome. We reported last year on the isolation of host-range mutants and the detailed characterization of one of them (H39), which has the properties expected of a virus that has lost the enhancement function provided by SV40. The host-range mutants grow and plaque poorly in monkey cells, although they still grow as well as the $Ad2^+ND1$ parent in human cells. It is clear that the patterns of protein synthesis of $Ad2^+ND1$ and H39 in monkey cells are different. H39 is defective in the synthesis of several late proteins and, in this respect, behaves like Ad2 in these restrictive conditions. In human cells, $Ad2^+ND1$ and H39 are similar, with the exception that H39-infected human cells do not contain a 30,000 MW protein present in $Ad2^+ND1$-infected cells but absent in Ad2-infected cells. Although the behavior of H39 differs greatly from $Ad2^+ND1$, we could detect no differences in DNA isolated from the two viruses, either by analysis with restriction endonucleases or by heteroduplex mapping. Thus the mutation in H39 did not appear to be a large deletion or the total loss of SV40 genetic material. It now seems clear that H39 is a point mutant since we have been able to isolate mutagen-induced revertants that grow equally well in monkey cells and human cells. Thus H39 could contain a mutation of the missense or nonsense type. We have also carried out complementation studies of H39 with two other host-range mutants and found that they all fail to complement one another. The simplest interpretation is that all of the host-range mutants contain defects that affect a single function, namely, the enhancement function provided by SV40. Since we can obtain mutants with point mutations leading to an absolutely defective phenotype, this provides a workable system for isolating and analyzing nonsense mutants.

We have also isolated several temperature-sensitive (*ts*) mutants of $Ad2^+ND1$. These mutants fail to grow at 38.5°C in both human and monkey cells, although they have normal growth properties at 32.5°C. This is the phenotype expected if the mutations are located in the adeno portion of the genome. These mutants are located in several complementation groups and presumably occur in different genes. We are presently investigating the nature of the functional lesion in these mutants.

Since mutations leading to a *ts* phenotype can occur in many regions of the genome, one wants to be able to locate the sites of the mutations on the genome and their organization relative to one another. We have developed a new way to align the genetic and physical maps of adenoviruses by locating the positions of *ts* mutants on the physical map. The principle of the method, which can be applied to other systems, is as follows: Recombinants are formed between *ts* mutants of different adenovirus serotypes whose DNAs differ in their pattern of cleavage with restricting endonucleases. The genomes of such recombinants contain DNA sequences derived from each of the parental serotypes; they can be dissected with various restriction enzymes; and the resulting DNA fragments, after separation by gel electrophoresis, can be compared with those obtained from the parental genomes. In this way, it is possible to deduce the positions of the cross-over events that generated the recombinants, to assign map positions to mutants on the parental genome, and to align the physical and genetic maps of these viruses.

We have isolated recombinants from crosses between four Ad2$^+$ND1 *ts* mutants and six Ad5 *ts* mutants. The Ad5 mutants were isolated by Jim Williams (Williams et al., *J. Gen. Virol. 11*:95 [1971]) who brought them to the laboratory during his visit here. The fragments produced by *Eco*RI, *Hpa*I and *Hin*dIII digestion of thirty-nine recombinants have been analyzed. The recombinants all have recombinant genomes, i.e., they have some cleavage sites corresponding to Ad5 sites and some corresponding to those of Ad2$^+$ND1. For each recombinant, the position of the junctions between the Ad5 sequences and the Ad2$^+$ND1 sequences has been mapped. So far, we have positioned the sites of three Ad2$^+$ND1 mutants and five Ad5 mutants on the corresponding physical maps. The assignments of map positions deduced by restriction enzyme analysis are consistent with each other and with the genetic map. The recombinants must contain both Ad5 genes and Ad2$^+$ND1 genes and therefore synthesize both Ad5 and Ad2$^+$ND1 proteins: we have found that some recombinant viruses are neutralized by both Ad5- and Ad2-specific antisera and therefore specify antigens of both serotypes. As more specific antibodies are used as reagents, we expect to be able to map corresponding proteins more precisely. We are also extending intertypic crosses to adenoviruses which belong to distantly related subgroups and which therefore show large differences in their biological properties.

Sequence Organization of Mammalian Chromosomes

The genomes of most multicellular organisms contain large amounts of highly reiterated DNA sequences (which may account for as much as 50% of the organism's DNA), are usually sequestered in long tandem arrays, and are genetically inert. An understanding of the organization of DNA within these tandem repeats might shed some light on the functions and evolution of reiterated sequences. The development of electrophoretic techniques for the separation of different sizes of DNA molecules coupled with the discovery of a large battery of restriction enzymes has allowed for some progress in this respect.

When unfractionated bovine (*Bos taurus*) DNA is cleaved with *Eco*RI, five distinct bands are seen against a background of heterogeneous DNA after separation of the hydrolyzed DNA by agarose gel electrophoresis. One band, which accounts for 6-7% of the total DNA and has a size of 1400 base pairs, has been shown by several criteria (renaturation kinetics, buoyant density, pyrimidine tract analysis) to consist of a single unit of one of the highly reiterated simple sequence DNAs of the bovine genome. This DNA (bovine satellite I) can be isolated in its unhydrolyzed form (molecules at least 10^5 base pairs in length) by virtue of its buoyant density in CsCl (1.716 g/cc) as opposed to a mean density of 1.700 g/cc for the bulk of bovine DNA. When the purified satellite DNA is cleaved to completion with *Eco*RI, the product molecules have a uniform length of 1400 base pairs and coelectrophorese with one of the bands seen after digestion of unfractionated DNA with *Eco*RI; a series of bands is seen after gel electrophoresis, each band having a size which is that expected of DNA that in length was a multiple of 1400 base pairs. This result means that the sequence that is attacked by RI — $\begin{matrix}5'-\text{GAATTC}3'\\3'-\text{CTTAAG}5'\end{matrix}$ — occurs every 1400 base pairs along this DNA. When other enzymes were tested to see whether their restriction sites occurred in bovine repetitive DNA, it was found that *Hin*dII cleaved the purified bovine satellite I DNA to yield fragments 1190 and 210 base pairs in length, respectively. By treating the DNA simultaneously with *Hin*dII and *Eco*RI, the

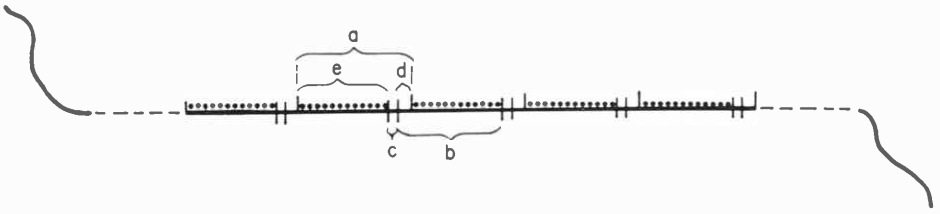


Figure 2

A restriction map of satellite I DNA. Assuming that this satellite is approximately equally distributed on all 58 autosomes, there are 2000-3000 repeats of unit *a* on each chromosome. This represents the spacing of the *Eco*RI restriction sequences. Because more than 95% of the satellite is cleaved to this molecular weight (*a*), there can be no interspersion of other sequences not in this repeat unit at the level of once every 20 repeat lengths. The two *Hin*dII restriction sequences are spaced by a distance *c* from each other and a distance *d* from the *Eco*RI sequence. When the satellite I DNA is cut with *Eco*RI, the *Hin*dII restriction enzyme fragments, *e*, *c* and *d* are generated. When the satellite DNA is cut with the *Hin*dII enzyme alone, fragments *b* and *c* are generated. The dots schematically represent a short repeat unit that is distributed throughout the repeat unit *a*.

cleavage sites could be mapped with respect to one another. Because equimolar amounts of DNA were associated with each length of fragment in the products of the double digestion, there must exist in bovine satellite I DNA a repeat unit of 1400 base pairs that is arranged as shown in Figure 2.

Further physical studies on this DNA indicate that the 1400-base pair repeats are themselves composed of hierarchies of smaller repeats both pyrimidine tract analysis of different restriction fragments of the DNA (carried out in collaboration with Rich Roberts) and reassociation kinetics suggest that there is a simple sequence that is repeated many times within the 1400-base pair unit.

Taken together, these studies indicate that bovine satellite I DNA has undergone several stages of evolution. The first involves a build up of the small units that form the highest level of organization of this DNA; the second is the propagation of this unit into a tandem array, consisting of 2000-3000 repeats on each autosomal chromosome. A model for the evolution of this DNA has been proposed that accounts for the observed final organization: the model includes the excision and cyclization of 1400-base pair DNA segments (or a multiple of 1400) with subsequent "rolling-circle" amplification. The resulting long, linear molecules are postulated as to recombine into the autosomes. Mutational events, such as insertions, deletions and unequal recombinations, could then create new kinds of repeating units that might become amplified in future replication events.

A Protein that Unwinds Superhelical Turns from DNA

During SV40 DNA synthesis, the parental template strands seem to be unnicked and they retain a superhelical configuration. In order to avoid topological restraints, a mechanism must exist by which a swivel is introduced into at least one of the template strands. We have purified from KB cells a protein that may carry out this function.

During electrophoresis through 1.4% agarose gels (Hayward and Smith, *J. Mol. Biol. 63:*383 [1972]), double-stranded circular DNA with no superhelical turns migrates more slowly than supercoiled DNA. Employing this difference as an assay, an activity has been purified from human tissue culture cells (KB-3) which will remove superhelical turns in DNA. The properties of this activity are in many respects similar to those of the ω-protein of *E. coli* (Wang, *J. Mol. Biol. 55:*523 [1971]) and a protein found in nuclei of secondary mouse embryo cells (Champoux and Dulbecco, *Proc. Nat. Acad. Sci. 69:*143 [1973]).

The protein irreversibly removes superhelical turns without introducing permanent nicks into closed circular DNA. Analysis by gel electrophoresis of reactions that have not reached completion resolves a series of DNA bands whose mobilities are intermediate to those of the fully supercoiled and completely relaxed forms. In the case of SV40 form I DNA extracted from virions, the maximal number of DNA intermediates detectable is

nineteen. DNA extracted from the intermediate bands is higher in buoyant density than form I DNA and lower than completely relaxed circles, when sedimented to equilibrium in CsCl-propidium-diiodide gradients; increasing buoyant density correlates with decreasing electrophoretic mobility. Assuming that each intermediate DNA band differs from its nearest neighbors by one superhelical turn, we conclude that form I SV40 DNA contains a total of approximately 22 superhelical turns. This number is in good agreement with those previously established by more indirect methods (Bauer and Vinograd, *J. Mol. Biol. 33:*141 [1968]).

This demonstration that form I SV40 DNA contains twenty-two superhelical turns also provides confirmation that the DNA helix is unwound by 12° by the intercalation of one molecule of ethidium bromide (Fuller and Waring, *Ber. Bunsengesellschaft 68:*805 [1964]).

We are presently testing whether this unwinding activity can provide the necessary swivel for SV40 DNA synthesis.

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MAMMALIAN CELL GENETICS

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This year, as last, we have had full advantage of the Cold Spring Harbor tradition of easy collaboration with friends here and at other institutions. In particular, our lab has been enlivened by a long-lasting and productive collaboration with Klaus Weber and Mary Osborn's Biochemistry Lab in Demerec. We are grateful to Bob Goldman and Chen-Ming Chang, on leave from Carnegie-Mellon Institute, for introducing us to the finer points of microscopy. Our scanning electron microscope, in McClintock Laboratory, is working beautifully in the hands of Dr. Gunther Albrecht-Buhler. Brad Ozanne and Art Vogel have kept up their joint work on revertants. At the same time, two separate outside collaborations worked extremely well, and we have found ourselves immersed in new studies with Dan Rifkin of Rockefeller University and Seung Shin and Vicki Freedman of Albert Einstein Medical Center.

The work of our laboratory has been directed toward understanding the differences between normal cells and their malignant descendants. It has always seemed to us that a sensible approach to this general question would be to experimentally define normality with regard to in vitro regulation of growth and at the same time to define the effect on growth control of a single oncogenic agent. Our ongoing work to date in defining these events falls into two complementary categories: (1) genetic selection in vitro of sets of cell lines with precisely defined variations in growth control, and (2) analysis of nonselective biochemical and biological correlates of growth control in these sets of cell lines. These categories are complementary because the design of in vitro selection protocols can be based on the results of the biochemical analyses.

Transformation

SV40 Transformation of the Mouse Cell Line 3T3

Mouse cells transformed by simian virus 40 show many alterations in their growth properties in vitro. In order to investigate the coordinate nature of these changes, we have analyzed the growth properties of 40 randomly selected colonies arising after SV40 infection of 3T3 cells. Clones of cells, established from these colonies, were characterized as

to saturation density and doubling time in 10 and 1% calf serum, growth in methyl cellulose suspension, colony formation on monolayers of normal cells, and presence of viral antigens. This analysis revealed that only five of the clones were indistinguishable from 3T3 cells; the remaining 35 clones differed from 3T3 cells in that they grew as rapidly in 1% calf serum as standard SV40-transformed cells. Of these 35 clones, ten corresponded to standard transformants previously described. Another ten showed other growth properties intermediate between 3T3 cells and standard transformants. These intermediate clones had lower levels of viral T antigen than standard transformants and showed considerable heterogeneity in staining from cell to cell. The remaining 15 clones were T antigen negative and had saturation densities slightly higher than that of 3T3 cells. These changes in cellular behavior are stable on recloning.

SV40 Transformation of Rat Embryo Primary Cells

We have monitored the transformation of primary rat cells into established cell lines by simian virus 40, using the restrictive assays of colony formation in sparse cultures, dense colony formation on a confluent cell sheet and colony formation in semisolid medium. Primary adult and embryonic cell cultures are considerably less susceptible to infection and subsequent transformation than the established mouse 3T3 cell line. Embryonic cells show stage-dependent susceptibility to transformation, with a maximum susceptibility achieved at the 16th day of gestation. Adult tissues are more resistant. Susceptibility to in vitro transformation is also increased by passage in vitro.

We have isolated several lines of SV40-transformed rat embryo cells. All these lines are fully transformed with regard to saturation density and serum sensitivity, but they differ greatly in their anchorage dependence, as assayed by efficiency of plating in methyl cellulose suspension. This set of lines reveals a consistent relation of plasminogen activator production to plating efficiency in methyl cellulose. T antigen-positive transformed lines which synthesize activator grow in methyl cellulose suspension, whereas T antigen-positive transformed lines which do not synthesize activator fail to form colonies in suspension. Normal rat embryo cells produce very little plasminogen activator and do not grow in methyl cellulose. Sera which permit high levels of plasmin formation and activity support growth in semisolid medium better than sera whose plasminogen is activated poorly and/or contain inhibitors to plasmin.

Effect of Transformation on the Localization of Intracellular Actin

Living mouse 3T3 cells display a complex array of fibrous structures, visible with phase contrast, Nomarski and polarized light optics. When cells are fixed and stained for indirect immunofluorescence with actin antibody, the same fibers show intense fluorescence, indicating that they contain actin. Electron microscopy reveals that these fibrous structures consist of submembraneous bundles of microfilaments located primarily on the attached side of the cells.

Encouraged by the convenience of immunofluorescence in rapidly screening a large number of cells, we have used the antibody to examine normal rat embryo (RE) cells, the growth-controlled mouse cell lines 3T3 and Balb/3T3, a variety of virus-transformed clones of these lines and revertants derived from the transformants. As judged by immunofluorescence studies, the number and size of thick actin-containing fibers seems to be diminished in transformed clones and is partially recovered in some revertant clones.

Reversion

New Revertants

Revertants of Kirsten sarcoma virus-transformed nonproducer Balb/3T3 cells (KA31 cells) were isolated after exposing the transformed cells to 5-fluorodeoxyuridine at high cell density or when suspended in methyl cellulose. Revertants were also isolated by treating KA31 cells with the lectin, concanavalin A, which is manyfold more toxic to transformed cells than for normal cells. The revertants resemble Balb/3T3 cells in their morphology and growth characteristics in that they have a low saturation density, fail to grow in 1% calf serum or when suspended in methyl cellulose, and cease to synthesize DNA after reaching

their saturation density. Infection by murine leukemia virus rescues Kirsten sarcoma virus from only the concanavalin A-selected variants, although all the revertants are susceptible to infection by leukemia virus. The concanavalin A revertants also become transformed after infection with murine leukemia virus. All the revertants can be transformed by Kirsten sarcoma virus, but not by simian virus 40.

Intracellular Cyclic AMP Studies

Intracellular concentrations of cyclic AMP increase when cells are deprived of serum. Studies with the mouse fibroblast line 3T3, an SV40-transformed subline of 3T3, and six different revertant lines derived from this clone show that a marked increase in cyclic AMP occurs only when the serum concentration is reduced below the minimum necessary for growth of a given line. Conversely, density-dependent inhibition of growth is not accompanied by an increase in cyclic AMP concentration in any line.

Mechanisms of Growth Control

The ability to synthesize DNA and enter mitosis was studied in Balb/c and Swiss 3T3 cells, SV40 and MSV-transformed 3T3 cells and revertants of these transformed cells in cultures of different serum concentrations and cell densities. Three ways were found by which cells were able to maintain a constant cell number in nonpermissive growth conditions: cessation of DNA synthesis, synthesis of DNA coupled with failure to enter mitosis, and the slow traverse of the cell cycle coupled with cell shedding.

Growth control of the revertant of an MSV-transformed Balb/3T3 cell most closely resembled that of Balb or Swiss 3T3. This line did not grow in 1% serum and did not synthesize DNA in either nonpermissive condition.

Serum-sensitive revertants of SV40-transformed 3T3 cells are also unable to grow in 1% serum and also do not grow beyond confluence in 10% serum, but these cells differ from 3T3 in the manner in which this growth arrest is accomplished. In 1% serum, revertants synthesize DNA, but do not enter mitosis. At confluence in 10% serum, they slowly traverse the cell cycle, with dividing cells replacing cells that are shed into the medium.

Susceptibility to Retransformation

The susceptibility of two classes of revertants of SV40-transformed 3T3 cells to retransformation by SV40 or murine sarcoma virus was studied. Both serum-sensitive and density-sensitive revertants are not retransformable by SV40.

Murine sarcoma virus can transform both types of revertants. The MSV-transformed revertants grow to high cell densities and form colonies when suspended in semisolid methyl cellulose medium, but are unable to grow in 1% calf serum. The MSV-transformed revertants produce infectious MSV and MLV and possess the same number of chromosomes as the untransformed revertants.

DNA Content

Revertants of SV40-transformed 3T3 cells have been examined for chromosome number and amount of DNA per cell. All classes of revertants have more DNA and chromosomes per cell than 3T3 or SV40-transformed 3T3 cells.

Enucleated Cells

Many normal cells are restricted in movement over each other's surfaces. We have quantitatively examined this restriction in the established mouse cell line 3T3, in a polyoma virus-transformed subline Py3T3, and in enucleated cytoplasms prepared from these lines by cytochalasin B treatment.

Overlapping of trypsinized, replated cells was quantitated by phase contrast and scanning electron microscopy. Cells of the normal mouse line 3T3 did not overlap one another. Cells of Py3T3 overlapped one another extensively. Trypsinized, replated 3T3 enucleates did not overlap one another, whereas replated enucleates of Py3T3 displayed many overlaps.

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PROTEIN SYNTHESIS

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Translation of Adenovirus Messengers

In the past year, we have been examining the mRNA in adenovirus-infected cells, using cell-free protein synthesis of specific proteins as an assay. Through this approach, we are constructing a map of the adenovirus genome and identifying those mRNA's expressed in transformed cells. This complements the transcription mapping being carried out by the tumor virus group here.

The total crude RNA fraction extracted from adeno-infected cells at late times can be used directly as mRNA in a fractionated cell-free system similar to that described by Schreier and Staehelin. (*J. Mol. Biol. 73:*329 [1973]). Examination of the polypeptide products reveals a complex pattern that contains at least ten recognizable viral proteins, five of which have been identified by comparative fingerprint analysis. Eron and Westphal (*J. Biol. Chem. 249:*6331 [1974]) at NIH have recently obtained similar results, but using S-30 extracts from mouse ascites cells.

With this functional assay for specific messengers, we can now ask a number of questions about the properties of the mRNA. We first looked at the size distribution of each species. The bulk cytoplasmic RNA was first dissociated in formamide and then fractionated on sucrose density gradients. Each fraction was used as a source of mRNA for cell-free protein synthesis. The messages for hexon, fiber, hexon-associated protein (IX), and protein 115K were about the size predicted from the size of the protein. Exceptions were particularly obvious in a few cases. The 100K and 27K proteins were translated from the same size class of mRNA, which was much larger than needed to encode both proteins. Also, the core precursor message was five times larger than expected. We don't think that these large mRNA's make large precursor proteins, which are then cleaved into the final polypeptide, since extensive efforts to find the precursors among in vivo or in vitro synthesized proteins have failed. Therefore, we think that these large mRNA's either have long untranslated regions or are polycistronic, carrying information for synthesis of more than one protein. Polycistrony of this sort is so far unknown in higher cells, and so we are looking in detail at this possibility.

In the last months we have been developing a general method for routine preparative hybridization of mRNA that can be recovered in a biologically active state. The bulk cytoplasmic RNA is hybridized with fragmented adenovirus DNA under conditions where hybridization is virtually complete after 10 minutes at 68°C. The hybrids are separated from free RNA by chromatography on hydroxylapatite. The eluted hybrids are denatured and the RNA is separated from the small DNA pieces by sizing. We can recover about 25% of the input mRNA activity as judged by its cell-free translation. Late RNA from infected cells when annealed to adeno DNA yields protein products essentially the same as the RNA before fractionation, whereas RNA from mock-infected cells annealed to adeno DNA gives no translation products above the background.

This method allows us to select messenger RNA species complementary to any specific DNA piece, then translate this RNA and identify the protein it encodes. We are exploiting this now to identify early and late adeno-specific messengers and those viral messengers found in transformed cells. By using specific DNA fragments cleaved from adeno DNA by restriction endonucleases, we can construct a biochemical map of adenovirus. At the moment we are doing this for the RI- and *Hpa*I-produced fragments. Since the physical map of the restriction fragments is known, we can thus place the gene corresponding to each assayable mRNA on the physical map.

Preliminary data show that messenger RNA encoding the penton base (III), the core precursor (P-VII) and protein V hybridize only to the *Eco*RI-A fragment. Messages for hexon (II) and pVI hybridize to both A and B. Message for 100K is from B, F and D; and fiber (IV) and IVa_2 messages hybridize to E and C. More experiments are needed to firm this up and to refine the mapping by use of other restriction enzymes (*Hin*III, *Hpa*I, etc.), and to assign the strand from which each message is transcribed, using separated strands for hybridization.

Hybridization and translation of early adeno RNA reveals messages for three proteins: 71K, 15K, and 11.5K. These have yet to be mapped on restriction fragments.

The translatable adeno mRNA from the cytoplasm has poly(A) on it, since adsorption to and elution from oligo(dT)-cellulose does not substantially alter the translation pattern.

With Uno Lindberg, who spent two months in this laboratory, we started to investigate the mRNP particles in adeno-infected cells. Preliminary experiments suggest that these can be translated in vitro to yield adeno proteins. More work is needed to see if there are any quantitative differences between translation of the particles and free mRNA. Also we wish to know if the use of particles abolishes the need for any of the factors needed for cell-free translation.

Mapping of Promoters and Bacteriophage Chromosomes with Restriction Enzymes

We showed that coupled transcription and translation of λ DNA can be accomplished in cell-free extracts supplemented with RNA polymerase and appropriate DNA. This synthesis of protein is repressed if purified λ repressor is added to the extracts, as long as the DNA template is homoimmune. Specific peptide products could be ascribed to specific early regions of the DNA by use of appropriate mutants, but conclusive identification of the N or *tof* proteins was not possible.

We turned our attention to using specific endonucleases for a more detailed dissection of the λ chromosome so that regions of particular interest could be studied directly. The restriction enzyme RI makes five cuts in λ DNA, which have been mapped by comparing fragments digested from various well-characterized deletion and substitution mutants using polyacrylamide gradient gels developed here. One RI fragment contains the whole immunity region, and when it was used for coupled cell-free synthesis of proteins, the early region could be studied more directly.

This led to a more thorough study of fragments produced by many other enzymes. It now seems clear that, at least with a phage λ where a storehouse of mutants is available to simplify mapping, almost any region of the genome can be specifically purified. We and Ptashne's group (Maniatis, Ptashne and Maurer, *Cold Spring Harbor Symp. Quant. Biol. 38:*857 [1973]) tried to identify fragments produced by various enzymes that contained promoter for *E. coli* RNA polymerase. This led to the discovery that *Hemophilus influenzae* enzyme *Hin*II cleaves within the two early λ promoters p_L and p_R. The specific palindrome recognized by *Hin*II, GTCGAC, is within the region of binding of RNA polymerase, since binding of polymerase to λ DNA prevents cleavage by *Hin*II at p_L or p_R, but does not prevent the many other cleavages elsewhere in the λ genome. That this sequence is really part of the promoter is shown by the fact that the mutant *sex*, which has an altered p_L, no longer has the *Hin*II cleavage site at p_L. Here it appears that sequence is not necessary for activity of the p_L early λ promoter. Ptashne's group has now gone on to use small fragments from these regions to determine the nucleotide sequence of p_L.

Hin cleavage sequences are also found in T7 promoters and in promoters for *E. coli* polymerase found in SV40 and adeno-2 viruses. It is clear, however, that there are promoters for *E. coli* polymerase that do not have one of these sequences, and that the sequences alone are not sufficient for promotion.

Detailed maps are now available for cleavages in λ DNA produced by *Hpa*I, *Hin*III, R•RI, *Hae*III and *Hpa*II. This has been extended to phage DNA from Mu and from *plac* λ derivatives, so that the recently produced λ -Mu hybrids can be analyzed. This is being carried on by A. Bukhari here at Cold Spring Harbor.

A New Mutant Promoter for the Lac Operon

In an attempt to find mutants of *E. coli* with more stable mRNA, an unusual mutant in the lactose operon was discovered. The selection was for cells that would express the *lac* operon

for a long period after a brief induction. Instead of having a grossly altered mRNA half-life, the strain turned out to have a new, highly efficient promoter for the Z gene that maps within the *i* gene. On induction, it produces 25 times more *lac* mRNA than wild type. There are many peculiar phenotypic properties of this strain, and it is unclear how many are due to the unbalanced mRNA synthesis.

Translation of RNA Phage RNA

Careful examination of the cell-free translation products from R17 (or MS2 or f2) RNA showed in addition to the major coat protein and synthetase products, a protein slightly larger than synthetase (66K vs. 62K). It represents only 5-7% of the product found in the synthetase and appears to come from the synthetase gene since an amber mutant or addition of excess coat protein abolishes its synthesis. We used various suppressors to see if this was a read-through protein similar to that found from the $Q\beta$ coat protein. The amount of the 66K protein is not convincingly increased by any suppressor, but strikingly, a protein very slightly larger and barely separable from synthetase is made in amber-suppressing extracts. The synthesis of this protein is also enhanced by addition of antiserum to release factor R-1, which is specific for UAG and UAA. The implication is that UAG is the normal first termination site at the end of the synthetase gene, and that if this is suppressed, a few amino acids more are added to the polypeptide up to the next termination triplet. This is at variance with the experiments of Capecchi and Klein (*Nature 226:*1029 [1970]) who concluded that UAA was the terminator based on the need for both R-1 and R-2 for release. So far, however, we have no simple explanation for the longer read-through of 66K.

Protein Splicing

Recently it has been demonstrated by B. Apte and D. Zipser that in merodiploids of *E. coli* containing two different nonsense mutations in the *lac* Z gene (one on the chromosome, one on the episome), not only can β-galactosidase activity be detected (long thought to be due to intracistronic complementation), but also, in many pairs, one continuous polypeptide chain is synthesized, identical in size to wild-type β-galactosidase. During the past year, we have been collaborating with Apte and Zipser to try to determine the mechanism by which this process, dubbed protein splicing, occurs. To do this, we are making use of the DNA-directed, cell-free protein-synthesizing system originally developed by Zubay and his colleagues (Zubay, Chambers and Cheong, *The Lactose Operon* [ed. J. Beckwith and D. Zipser] p. 375. Cold Spring Harbor Laboratory [1970]). Using DNA extracted from various λ *plac* Z$^-$ transducing phages to program the system, we have been able to detect the synthesis of several nonsense fragments and one, or perhaps two, restart fragments. Although nonsense fragments are synthesized in good yield and are apparently quite stable in the in vitro system, the restart fragments that the system produces are found in very poor yield when compared to the amount synthesized in vivo. In fact, the two restarts synthesized with the highest efficiency in vivo cannot be detected at all among the products of the in vitro system.

Our current work includes altering the components in the in vitro system so that we can optimize the synthesis of restart fragments. Also, we are preparing extracts that contain restarts made in vivo and asking whether in vitro-synthesized nonsense fragments can be "spliced" to these in vivo fragments. So far, no cell-free splicing has been demonstrated.

Eukaryotic Suppression

Marianna Wolfner, a participant in our undergraduate research program, attempted to develop a cell-free protein-synthesizing system from yeast that would translate natural messages. She found that these extracts were inactive, but that addition of mammalian ribosomes and initiation factors gave reasonable synthesis, using either crude yeast RNA or adeno RNA as message. We now know that only the S-100 fraction from yeast is sufficient, and that globin mRNA and bacteriophage RNA's from $Q\beta$ and R17 also are active templates, each yielding the expected products. We are now using amber phage RNA mutants to see if we can detect suppression in extracts prepared from various yeast suppressor mutants. This would allow characterization of these suppressors and provide a first step in development of mammalian suppressors. This work is greatly aided by the presence of D. Botstein, J. Roth and G. Fink, who are here on sabbatical leave.

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MOLECULAR GENETICS

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In the past year, the molecular genetics section has concentrated its efforts on two broad fronts: (1) the processing of mutant β-galactosidase peptides and (2) the mechanism of phage Mu integration.

The Degradation of Reinitiation Polypeptides

Previous work in our laboratory and by others has shown that polypeptides initiated at the normal start of β-galactosidase, but prematurely terminated by nonsense mutations, are very rapidly and specifically degraded. A mutation isolated by Ahmad Bukhari in this laboratory several years ago greatly slows this degradation. However, this mutation, now called *deg*-T, was shown by Bal Apte not to affect the rapid degradation of the subsequently initiated peptides. Thus it was reasonable to hypothesize that there are different systems responsible for the degradation of termination and initiation fragments. To test this hypothesis, Apte and Harker Rhodes, a student in our undergraduate research program, set up a selection based on the procedure used by Bukhari to get the *deg*-T mutant. However, they made modifications that would bias the selection toward mutants that stabilize restarts. Their selection techniques were successful and produced a mutant that prevents the degradation of reinitiation fragments but has no effect on the degradation of termination polypeptides. The mutant is called *deg*-R. This result clearly establishes that termination and reinitiation fragments are degraded by distinct systems.

Splicing In Vivo

Last year, we discovered that the enzymatically active product made by some complementing β-galactosidase mutants was not a simple association of two mutant polypeptides, but resulted from these polypeptides being spliced together to form a single protein indistinguishable from the wild-type protomer. We have continued to study splicing this year. A pair of nonoverlapping deletions, which come in from opposite ends of the *z* gene and give rise to a spliced product, have been found. This result rules out any possibility that the information for the spliced product could be derived from a single functioning operon. Many new pairs of mutants that splice have been identified. Some of these lie very close to either the C- or the N-terminal end of the β-galactosidase molecule. These new splicing pairs confirm our original hypothesis that splicing can occur at many places within the protein, rather than being limited to a few highly specific sites.

In Vitro Splicing

Elucidating the mechanism of polypeptide splicing is one of our main goals. We feel that this will ultimately require the ability to carry out the splicing reaction in vitro. We have been putting a great deal of effort into this project and have been collaborating extensively with Jim Manley in Ray Gesteland's laboratory. Using an in vitro protein-synthesizing system which responds to added DNA, we have tried to make both the termination and the initiation fragments that must go into the splicing reaction. The required genes are placed on phage DNA. Various mixtures of genes known to splice in vivo are added to the system, and then acrylamide gels are run to see if any spliced product has been made. The results of these experiments are very clear. Termination fragment is made in the expected yield, but no initiation or spliced product is made. Indeed, no significant amount of any initiation fragment needed for splicing has yet been made in vitro. Clearly, this problem must be solved before splicing can be expected.

In an attempt to sidestep this problem, we have tried to add extracts of strains that make the appropriate restart proteins. These experiments led to the finding that restart proteins made in vivo very rapidly lose their complementing activity under conditions similar to those used in the in vitro protein-synthesizing system. This finding has raised the possibility that the restart degradation system is active in vitro and is preventing the build-up of sufficient restart materials to allow splicing.

Degradation and Polarity

In the course of a study of splicing between very early mutants in the *z* gene, Bal Apte found that the quantity of spliced product observed greatly exceeded the amount expected from the polarity of the mutant. That is, if it is assumed that the fraction of restart protein made equals the fraction of other distal products made, the maximal amount of spliced product can be determined. It is this amount that is exceeded. This means that either the above assumption is wrong, or splicing is in some way able to reverse polarity. Further study showed that both splicing and complementation reverse polarity.

Consider, for example, the following experiment: A diploid was made between a deletion coming into the *lac* operon from the *i* gene side and ending in the *z* gene and another, nonoverlapping deletion coming into the operon from the other side and also ending in the *z* gene. The deletion coming from the *i* side was highly polar and made only 2% acetylase by itself, whereas the other deletion took out the *a* gene and thus could make no acetylase at all. The diploid between these two deletions complemented, but did not splice, and made 60% of the wild-type acetylase. Many similar experiments between pairs of splicing or complementing nonsense mutants showed great increase in acetylase activity. Noncomplementing pairs showed no effect on acetylase. Clearly, something about complementation reverses polarity. Our working hypothesis is that complementation and/or splicing reverse polarity by preventing the degradation of the restart fragment. Just what processes mediate this reversal are not yet clear. However if the hypothesis is correct, it implies that any other factor that decreases the degradation of restart would also reverse polarity. In particular, the mutation that stabilizes restarts against degradation, described above, would also be expected to reverse polarity. This has in fact been shown to be the case. For example, the very polar ochre mutant *z*⁻ 118, which produces no detectable acetylase alone, combined with the *deg*R mutant produces 70% acetylase.

Mu Integration Associated with Deletion

About 25% of all Mu-induced *z*⁻ mutants have been shown to be associated with deletions at the site of Mu insertion. These are generally quite large deletions, removing all or most of the lactose operon. One hypothesis about the origin of these deletions is that they arise when a pair of Mu's are integrated close to each other and then drop out the intervening chromosome by recombination. This process would require an intact *rec* system, and this hypothesis is now being tested.

Integration-Excision Genetics

The frequency of reversion of a Mu-induced bacterial mutation is too low to be readily observable. However, it is possible to isolate a mutation in prophage Mu that raises this

reversion frequency to an easily detectable level. These reversions lose the DNA of Mu and re-form the original gene. Bukhari has called this mutation X. It completely inactivates all the known killing functions of Mu. No amber X's have been found. X does not complement with gene B, but B ambers do not have an X phenotype. This rather puzzling result suggests that X is a control site mutation of some kind. An important finding is that amber mutations in the A gene of Mu prevent the excision of Mu X mutants. This suggests that the A gene of Mu may be involved in Mu excision.

Transfection

Regine Kahmann has found conditions which allow Mu DNA to infect bacteria directly. This transfection procedure should make it possible to study the effect of direct physical alteration of Mu DNA on its subsequent ability to replicate.

Restriction Analysis of Mu and λ-Mu Hybrids

In cooperation with Bernard Allet, we have carried out an extensive analysis of the fragments produced by restriction enzymes acting on the DNA of Mu and λ-Mu hybrids. This has enabled us to correlate the physical and genetic maps of Mu. Some other results of this work include the finding that the C-gene end of Mu is heterogeneous and may contain some bacterial DNA* as does the S end. The amount of heterogeneity at this end, however, is very small — of the order of 100 base pairs. In addition, the gene that is responsible for the G-loop inversion has been localized to a region very near the G loop itself. The immunity gene has been accurately mapped at the C end, and an additional function affecting integration has been found between the C end and the repressor structural gene.

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ELECTRON MICROSCOPY

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Molecular Weight Determinations of Viral RNA

In collaboration with P. Duesberg and W. Mangel (Berkeley), we have studied the structure and molecular weight of tobacco mosaic virus (TMV) and Rous sarcoma virus (RSV) RNA. The RNA was stretched out for length measurements by the attachment of 32-protein.

TMV RNA and 30-40S RNA derived from 60-70S RSV RNA by heating are linear

structures with molecular weights of 2.2 and 2.8 x 10^6 daltons, respectively, in confirmation of previous measurements.

The 60-70S RSV RNA exhibits a network structure consisting of several RNA strands linked together at various points. No distinct pattern of linkage points could be established, and no extensive double-helical regions could be demonstrated. The majority of these complexes contain a total length of RNA corresponding to 6.1 x 10^6 daltons, i.e., about twice the amount of RNA determined for 30-40S RSV RNA. These results confirm the subunit hypothesis for the 60-70S RNA complex and imply that it contains at least two 30-40S RNA molecules.

Partial Denaturation Mapping of Adeno DNAs

The method of alkaline partial denaturation of DNA was modified to allow denaturation at low pH and low temperature without loss in the quality of differentiation between high and low GC-containing DNA. Also, a method to obtain complementary denaturation maps with preferential denaturation of GC-rich regions has been developed.

Partial denaturation mapping was used to determine the positions of restriction enzyme-produced fragments of adeno DNAs by fitting the partial denaturation maps of fragments to the denaturation maps of intact DNA.

In addition to the sequences of R•*Eco*RI fragments of various adeno DNAs already described, the arrangement of the four fragments produced by R•*Sa* restriction enzyme from adeno 12 DNA was determined to be C, D, B, A. Also, the positions of smaller fragments produced by R•*Sa* from the A fragment of adeno 2 DNA cut by R•*Eco*RI have been determined with this procedure.

Bacteriophage Mu1 DNA

The size and arrangement of R•*Eco*RI fragments of Mu DNA were determined by partial denaturation mapping, in collaboration with E. Bade. The order of the fragments is C, A, B, with sizes of 3.2, 11.4 and 9.2 x 10^6 daltons, respectively. Fragment B contains the G-loop and the split-end region of the Mu DNA, as was determined by denaturation and renaturation of Mu DNA followed by cutting the heteroduplexes with the restriction enzyme and measurement of the fragments containing the split-end part. Variations in size of fragment B can be attributed to the length variations of the split-end part.

Together with A. Bukhari and B. Allet, we studied the structure of various *plac*-Mu hybrid DNAs. After strand separation by agarose gel electrophoresis and mixed renaturation with either *plac* or Mu DNA, the structure of the resulting heteroduplex molecules was analyzed to determine origin and position of the Mu DNA stretches inserted into the *plac* genome.

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The main aim of this laboratory is to investigate the use of restriction endonucleases as tools in the field of nucleic acid sequence analysis.

New Endonucleases

An active search for new specific endonucleases is under way, and 17 such enzymes have been isolated and characterized in this laboratory. Of these, 11 show new and different specificities, bringing the total number of different specific endonucleases now known to 25. Of particular interest for DNA sequence analysis are the endonucleases from *Haemophilus haemolyticus* (*Hha*I), *Arthrobacter luteus* (*Alu*I) and *Moraxella bovis* (*Mbo*I and *Mbo*II), which make many cuts on most DNAs and which, we believe, recognize tetranucleotide palindromes. An enzyme (*Sal*I) from *Streptomyces albus* G is also of great interest because of the relatively small number of breaks introduced. This enzyme introduces two breaks on bacteriophage λ DNA and three breaks on adenovirus-2 DNA. DNAs from SV40, polyoma and bacteriophages ϕX174, fl and fd are not cleaved by this enzyme.

Mapping of Ad2 $^{+}$ND1

Complete maps of the adenovirus-2 genome have been produced using the specific endonucleases *Hpa*I and *Sal*I, and these have been related to the *Eco*RI map. In addition, fine-structure mapping, using all available specific endonucleases, of the left-hand 4% of the genome (the *Hpa*I "e" fragment) is under way. These data will serve as the prelude for detailed sequence analysis of the region.

In connection with a project to define, by sequence analysis, the site of integration of SV40 sequences in the nondefective hybrid virus Ad2^{+} ND1, we have begun preparing a fine-structure map of the regions involved. These include the junctions of adenovirus-specific and SV40-specific sequences in the hybrid virus and also the corresponding region present in adenovirus 2 but deleted in Ad2^{+} ND1. Small fragments of Ad2^{+}ND1 containing the junctions are being characterized, and sequence analysis is under way.

End Labeling

In collaboration with W. Keller, we have developed a procedure for terminal labeling of DNA in vitro, using *Escherichia coli* exonuclease III and avian myeloblastosis virus DNA polymerase. This involves partial degradation of each strand at its 3′ end, followed by repair in the presence of [α-^{32}P]- deoxynucleoside triphosphates. The resulting DNA may then be cleaved by a specific endonuclease, giving terminal fragments in which one strand is labeled with ^{32}P and the other strand is unlabeled. These fragments may then be used to great advantage for direct DNA sequence analysis. Internal regions of the DNA may be labeled by using appropriate fragments as substrates for the labeling procedure, which is thus of general use.

Inverted Terminal Repetition of Adenovirus-2 DNA

Using end-labeled Ad2 DNA, it has been possible to estimate the length of the inverted terminal repetition as between 100 and 140 nucleotides. Two endonucleases (*Hha*I and *Hph*I) cleave within the inverted terminal repetition and give identical fragments from both ends. In the case of *Hph*I, the terminal fragment is 100 nucleotides in length. Two further endonucleases (*Hae*II and *Hpa*II) give dissimilar fragments from the two ends, the smallest of which is about 140 nucleotides long and thus serves to define the upper limit for the repetition. Sequence analysis of this fragment together with the corresponding fragment from the other end is in progress in order to define exactly the length of the inverted terminal repetition.

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PROTEIN NUCLEIC ACID CHEMISTRY

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Studies on Bacterial Restriction Endonucleases

Restriction enzymes are highly specific nucleases which degrade native, unmodified, double-stranded DNA. These enzymes may be divided into two classes depending on whether they require S-adenosylmethionine, adenosine triphosphate and magnesium ions for their activity (class I) or only magnesium ions (class II). Restriction enzymes have been isolated from a variety of bacterial strains; each strain may contain several different restriction enzymes. Therefore, these enzymes provide interesting model systems for studying protein:DNA interactions and protein evolution in bacteria.

During the past year we have conducted a preliminary characterization of the class II restriction enzymes from *Haemophilus influenzae* and *Haemophilus parainfluenzae*. The restriction enzymes were purified from these bacterial strains in relatively large amounts by a combination of ion exchange and exclusion chromatography. The heterogeneity of each enzyme preparation was determined by polyacrylamide gel electrophoresis, and preliminary data on the molecular weight of each enzyme were obtained. One enzyme from *H. parainfluenzae* was purified to homogeneity and its amino acid composition was determined.

Each restriction enzyme preparation was used as an antigen for antibody production in rabbits. These antibodies were purified to free them from contaminating nucleases and then used in immunodiffusion studies to investigate the cross-reactions (antigenic similarities) between the *H. influenzae* and *H. parainfluenzae* restriction enzymes and ten other restriction enzymes. Cross-reaction, as indicated by precipitin band formation, was observed with only few enzymes. However, the restriction enzyme preparations used as differentiation between specific cross-reactions due to antigenic similarities between restriction enzymes and a nonspecific cross-reaction due to antigenic similarities between restriction enzymes and a nonspecific cross-reaction due to the presence of other contaminating antigens was therefore not possible. An enzyme inhibition test was devised to overcome this problem, and it was shown that the purified antibodies inhibited only those restriction enzymes which had identical nucleotide cleavage site sequences to the restriction enzymes used initially to prepare the antibodies. Enzyme inhibition was used as a criterion of cross-reaction and antigenic similarity. It was concluded that restriction enzymes that have identical cleavage site sequences are antigenically similar.

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SV40 A-Gene Function and the Maintenance of Transformation

Transformants have been isolated following infection of rat embryo cells at 33°C with either wild-type SV40 or with the temperature-sensitive gene A mutants. Examination of properties usually associated with transformation, such as growth in low serum, growth rate, saturation density and morphology, show that these properties are temperature independent in the case of the wild-type transformants, but are temperature sensitive in the case of the *ts*A transformants. In the most thoroughly characterized *ts*A transformants, the expression of the SV40-specific T antigen also appears temperature sensitive. These results suggest very strongly that in SV40 transformation, an active A function is required for the maintenance of the transformed phenotype. In the lytic cycle, the A function is involved in the initiation of viral DNA synthesis. Thus transformation by SV40 may be the direct consequence of the introduction of the SV40 replicon and the presence of its DNA initiator function, which causes the cell to express a transformed phenotype. This finding is important because it indicates for the first time that in DNA tumor viruses a single gene product may govern the transformed state (Osborn and Weber 1975a,b).

Similar results have been independently obtained in the laboratories of J. Butel, R. Martin and P. Tegtmeyer.

Studies on the purification of SV40-induced T antigen have continued (Osborn and Weber 1975b). We are currently studying its DNA binding properties in collaboration with A. Levine at Princeton University.

Localization of Major Structural Proteins in Cells

We have approached this problem by the preparation of antibodies specific for actin, myosin and tubulin (Lazarides and Weber 1974; Weber and Groeschel-Stewart 1974; Weber et al., in prep.). These antibodies were used in indirect immunofluorescence microscopy to directly visualize the structure containing the particular antigen. In the case of myosin and tubulin, the original antigen used was the highly purified native protein (Weber and Groeschel-Stewart 1974; Weber et al., in prep.). In the case of actin, we have approached the problem in a different way. Actin was purified from SV40-transformed mouse 3T3 cells using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as the major purification step. The protein corresponding to actin was recovered as SDS denatured protein and used as antigen. The antibody obtained showed immunological cross-reactivity with native actin (Lazarides and Weber 1974). This result seems to indicate a rather general procedure to obtain antibodies against interesting cellular proteins. The cumbersome problem of purifying each particular protein to homogeneity using standard biochemical purification procedures may be circumvented by the use of SDS gel electrophoresis as a major purification step, thereby allowing the simultaneous isolation of several proteins in a state suitable for immunization.

Actin purified from mouse fibroblasts by SDS gel electrophoresis was used as antigen to obtain an antibody in rabbits. The elicited antibody was shown to be specific for actin, as judged by immunodiffusion and complement fixation against partially purified mouse fibroblast actin and highly purified chicken muscle actin. The antibody was used in indirect immunofluorescence to demonstrate by fluorescence light microscopy the distribution and pattern of actin-containing filaments in a variety of cell types. Actin filaments were shown to span the cell length or to concentrate in "focal points" in patterns characteristic for each individual cell. The fibers often run parallel to each other and are concentrated toward the adhesive side of the cell (Lazarides and Weber 1974). These actin-containing fibers have

PROTEINS IN SV40 INFECTED AND TRANSFORMED CELLS

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been correlated with the microfilament system using a variety of microscopic techniques, including electron microscopy (Goldman et al. 1975).

Myosin in human, rat, mouse and chicken fibroblasts was localized by indirect immunofluorescence microscopy using antibodies prepared in rabbits against highly purified chicken gizzard myosin. Filaments containing myosin span the interior of the cells and are often parallel to each other. The majority of the fibers are concentrated toward the adhesive side of the cell. Most of the myosin-containing filaments show "interruptions" or "striations." From a comparison of these fibers in fluorescence and phase microscopy and from previous results on actin-containing fibers, we conclude that at least some of the cytoplasmic myosin can be found in the actin-containing fibers, which themselves have been shown to be very similar or identical to the microfilament bundles. The occurrence of both myosin and actin in the microfilament bundles provides a basis for the motility and contractility of the cell (Weber and Groeschel-Stewart 1974).

Cytoplasmic microtubules in mammalian cells can be directly visualized by immunofluorescence microscopy. Antibody against tubulin from sea urchin sperm outer doublets decorates a fine network of cytoplasmic fibers with uniform thickness in a variety of cells from tissues of human, monkey, rat, mouse and chicken. These fibers are lost either in medium containing colchicine or after subjecting the cells to low temperature. The same treatments do not destroy the microfilamentous structures, which can be visualized using antibodies against actin and myosin. In living cells, microtubular antibody gives strong nuclear and perinuclear fluorescence. When enucleated cells are replated, most of this fluorescence is lost, and microtubules are seen to run freely throughout the cytoplasm with a preferred convergence to the center of the enucleates (Weber, Pollack and Bibring, in prep.).

We have used the actin antibody to study the expression of actin-containing fibers in a variety of SV40-transformed mouse and rat cells as well as in some revertants of these transformants and in the corresponding nontransformed cell lines (Weber et al. 1975). In general, we have noticed that the pattern of thick actin-containing fibers, typical for the majority of the nontransformed cells, is greatly diminished in the corresponding SV40 transformants. Some, but not all, revertants recover from the drastic loss of thick actin cables observed upon transformation. SDS gel analysis of these different cell lines shows that the relative amount of actin stays constant. Therefore, the change in the expression of actin fibers observed in transformed cells must be due to a change in the organization and assembly of the actin, rather than a decreased level of actin in transformed cells (Weber et al. 1975).

It has also been shown that antibody against physarum actin cross-reacts with actin in mammalian cells (Weber, in prep.). This result reinforces our knowledge of the conservative character of actin in nature.

In addition, work of Lazarides and Lindberg (1975), who visited Dr. Gesteland's Laboratory, has shown that the widely occurring specific inhibitor of DNase I previously characterized by Lindberg is identical with actin. This finding is based on biochemical and immunological evidence and opens the question of a physiologically important interaction between DNase I and actin.

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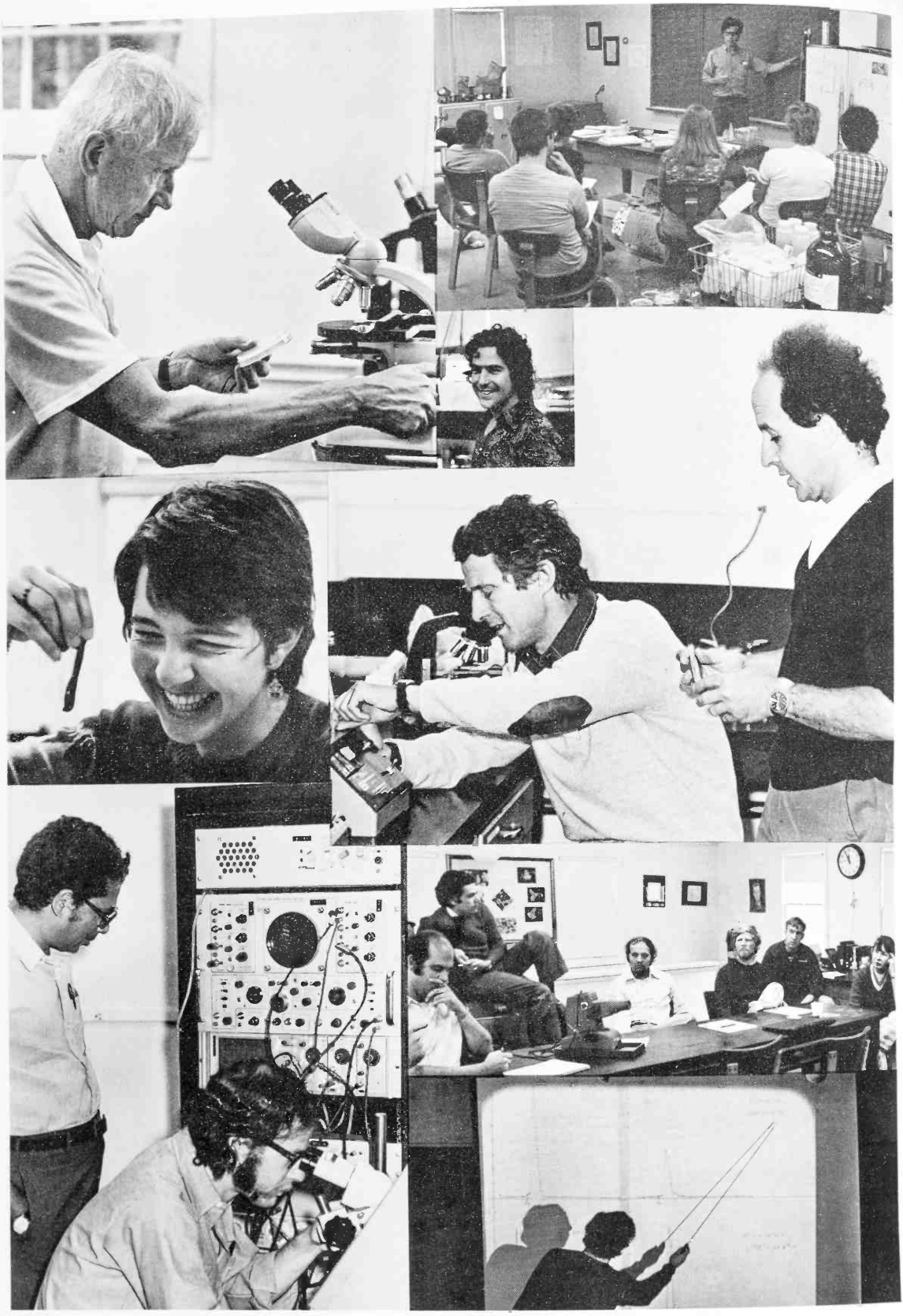
Weber, K. and U. Groeschel-Stewart. Antibody to myosin: The specific visualization of myosin containing filaments in non-muscle cells. *Proc. Nat. Acad. Sci.* (in press).
Weber, K., E. Lazarides, R.D. Goldman, A. Vogel and R. Pollack. 1975. Localization and distribution of actin fibers in normal, transformed and revertant cells. *Cold Spring Harbor Symp. Quant. Biol.* 39 (in press).



Photos by Ross Meurer

POST GRADUATE TRAINING PROGRAMS

SUMMER 1974

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

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

ANIMAL CELL CULTURE WORKSHOP – June 10 - June 30

The course consisted of a series of laboratory exercises and lectures, with experiments ranging from an introduction to the basic techniques of propagation and study of animal cells in culture to more sophisticated experiments on somatic cell genetics and cell biology. Although the emphasis was on the use of continuous cell lines, the establishment of primary cultures was also performed. Other experiments included determination of parameters of cell transformation in vitro, cell transformation by tumor viruses, induction of and selection of HGPRT$^-$ mutations, cell fusion and hybridization. Cell synchronization, the induction of differentiated functions in various cell lines, and the synthesis of ribosomal RNA in a temperature-sensitive cell mutant were also studied. In addition to the laboratory exercises, there were discussion groups and a number of lectures by instructors and invited guests, covering the most recent developments in the field.

INSTRUCTORS

Basilico, Claudio, Ph.D., New York University Medical School, New York, New York
Chasin, Lawrence, Ph.D., Columbia University, New York, New York

ASSISTANTS

Melnick, David, B.A., Columbia University, New York, New York
Deutsch, Eva, M.D., New York University School of Medicine, New York, New York

PARTICIPANTS

Chow, Louise T., Ph.D., University of California Medical Center, San Francisco, California
Fey, Georg, Dr., Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland
Gidari, Anthony S., M.D., Ph.D., Downstate Medical Center, Brooklyn, New York
Griepp, Eva B., M.D., Stanford University School of Medicine, Stanford, California
Harrison, Robert W., M.D., Vanderbilt University, Nashville, Tennessee
Jacquemin-Sablon, Alain G., Ph.D., Institut Gustave-Roussy, Villejuif, France
Jeng, Ingming, Ph.D., Washington University Medical School, St. Louis, Missouri
Johnson, Paul H., Ph.D., California Institute of Technology, Pasadena, California
Lane, M. Daniel, Ph.D., Johns Hopkins University Medical School, Baltimore, Maryland
Liwerant, Irene, M.Sc., Institut Pasteur, Paris, France
McCarthy, Micheline B., A.M., Harvard University, Cambridge, Massachusetts
Nakanishi, Shigetada, M.D., National Institutes of Health, Bethesda, Maryland
Payne, Michael R., B.A., Brandeis University, Waltham, Massachusetts
Pietropaolo, Concetta, Ph.D., Columbia University, New York, New York
Pullman, Maynard E., Ph.D., Public Health Research Institute of the City of New York, New York
Quinlan, Dennis C., Ph.D., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts
Talavera, Antonio A., New York University Medical Center, New York, New York
Yep, Dennis, A.B., Cornell University, Ithaca, New York
Zain-Ul-Abedin, M., Ph.D., University of Karachi, Karachi, West Pakistan
Zetter, Bruce R., B.A., University of Rhode Island, Kingston, Rhode Island

DeMars, R., University of Wisconsin. *The induction and use of mutations in cultured human fibroblasts.*

Perry, R., Institute of Cancer Research. *Biosynthesis of ribosomes in eukaryotic cells.*

Schildkraut, K., Albert Einstein College of Medicine. *The temporal replication of some DNA cistrons in synchronized mammalian cells and its possible relation to control mechanisms.*

Siniscalco, M., Sloan-Kettering Institute. *Alternative to sex in mammalian cells.*

Huberman, J., Massachusetts Institute of Technology. *Discontinuous DNA synthesis in eukaryotes.*

Thompson, B., National Institute of Cancer, National Institutes of Health. *Expression of steroid responses: Relation to steroid receptors in cells and cell hybrids.*

Meiss, H.K., New York University Medical Center. *Isolation and characterization of temperature-sensitive mutants of somatic cells.*

Adesnik, M., New York University Medical Center. *Erythroid differentiation in Friend leukemia cells.*

Attardi, G., California Institute of Technology. *Molecular approaches to the dissection of the mitochondrial genome in mammalian cells.*

Robbins, P., Massachusetts Institute of Technology. *Membrane biochemistry of normal and transformed cells.*

Stoker, M., Imperial Cancer Research Fund. *Role of the microenvironment in the growth of fibroblasts.*

Scharff, M., Albert Einstein College of Medicine. *The isolation and characterization of variants of immunoglobulin producing cells.*

Coon, H., National Cancer Institute, National Institutes of Health. *Study of neurons in culture by cell hybridization.*

Khoury, G., National Institutes of Health. *The patterns and controls of transcription in DNA animal virus systems.*

Bancroft, F.C., Columbia University. *Studies of growth hormone and its mRNA in cultured cells.*

MOLECULAR CYTOGENETICS WORKSHOP – June 10 - June 30

The molecular cytogenetics program consisted of lectures, discussions and laboratory exercises on eukaryotic chromosomes, with particular emphasis on procedures which can relate the structural and molecular aspects of chromosomes. These procedures included the electron microscopic visualization of DNA transcription and of the structures formed when strands from two slightly different DNA molecules are annealed together; the chromosomal localization by autoradiography of Drosophila DNA molecules which had been amplified in bacteria; the function and structure of polytene and other chromosomes; and problems associated with repeated-sequence DNA, which may be analyzed by restriction enzymes, DNA sequencing and renaturation kinetics.

The aim of this workshop was to more fully acquaint molecular biologists and biochemists with the higher organization of chromosomes.

INSTRUCTORS

Pardue, Mary Lou, Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts
Walker, Peter, Ph.D., University of Edinburgh, Edinburgh, Scotland

ASSISTANTS

Mounts, Phoebe, Ph.D., University of Edinburgh, Edinburgh, Scotland

PARTICIPANTS

Anderson, Carl W., Ph.D., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Artavanis, Spryros S., B. Sc., MRC Laboratory of Molecular Biology, England
Broker, Thomas, Ph.D., California Institute of Technology, Pasadena, California
Castro-Sierra, Eduardo, M.D., Centro de Invest. y Estudios Avanzados, Mexico
Grodzicker, Terri, Ph.D., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Glätzer, Carl H., Ph.D., University of Dusseldorf, Dusseldorf, West Germany
Hand, Roger, M.D., McGill University, Montreal, Canada
Peden, Keith W.C., M. Sc., University of Edinburgh, Edinburgh, Scotland

Sanders, Marilyn M., Ph.D., Rutgers Medical School, Piscataway, New Jersey
Vosberg, Peter, M.D., California Institute of Technology, Pasadena, California

SEMINARS

Judd, B., University of Texas. *The relationship between genes and chromomeres in* Drosophila.

Walker, P., University of Edinburgh. *Sequence divergence and DNA function.*

Pardue, M.L., Massachusetts Institute of Technology. *In situ hybridization studies of eukaryotic chromosomes.*

Bakken, A., University of Washington. *Eukaryotic ribosomal RNA genes — Their transcription and amplification.*

Davis, R., Stanford University School of Medicine. *Biochemical constitution of lambda transducing phage containing eukaryotic DNA.*

Ashburner, M., Cambridge University. *Control of gene activity in polytene chromosomes by hormones.*

Miller, O.J., Columbia University. *Mammalian chromosome banding with anti-nucleoside antibodies.*

Georgiev, G.P., Institute of Molecular Biology, USSR. *Studies on chromosome organization.*

PRINCIPLES OF NEUROBIOLOGY WORKSHOP – June 10 - June 30

Designed as an introductory course in neurobiology for research workers with no previous experience in the field, the program was comprised of lectures, discussion groups, experimental demonstrations and seminars by guest lecturers.

The initial lectures focused on some of the basic phenomena in neurophysiology, including the electrical properties of nerve membranes, synaptic structure and function, ionic pumps, and sensory transduction and adaptation. The visual system was then presented as a model of a sensory system, and the processing of information in the central nervous system was illustrated. Other topics included the structural development and regeneration of the central nervous system and the biochemistry of the chemical synapse.

INSTRUCTORS

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

PARTICIPANTS

Baker, Michael E., Ph.D., The Salk Institute, San Diego, California
Boyd, Donald B., M.Sc., Columbia University Graduate School of Arts and Sciences, New York, New York
Carlson, Steven S., B.S., University of California, Berkeley, California
Gopalakrishnan, T.V., Ph.D., National Institutes of Health, Bethesda, Maryland
Harris, William A., B.A., California Institute of Technology, Pasadena, California
Horvitz, H. Robert, M.A., Harvard Biological Laboratories, Cambridge, Massachusetts
Jan, Lily K.C.Y., M.S., California Institute of Technology, Pasadena, California
Jan, Yuh Nung, M. Sc., California Institute of Technology, Pasadena, California
Layer, Paul Gottlob, M.S., Universität Konstanz, Konstanz, West Germany
Lipes, Richard G., Ph.D., California Institute of Technology, Pasadena, California
Lipson, Edward D., Ph.D., California Institute of Technology, Pasadena, California
Merriam, John R., Ph.D., University of California, Los Angeles, California
Morrison, Marcelle R., Ph.D., University of Cincinnati College of Medicine, Cincinnati, Ohio
Norman, Jon A., M.A., University of Calgary, Alberta, Canada
Rall, William F., B.A., University of Tennessee, Oak Ridge, Tennessee
Shaw, Gordon L., Ph.D., University of California, Irvine, California
Teichberg, Vivian I., Ph.D., The Weizmann Institute of Science, Rehovot, Israel
Weber, Jeffrey S., B.A., New York University Medical Center, New York, New York

SEMINARS

Nicholls, J., Stanford University Medical School. *Introductory lecture.*
— *Properties of excitable membranes (I), (II), (III).*

Kelly, J., Harvard University Medical School. *Cellular Neuroanatomy.*
— *Developmental neuroanatomy.*
— *Organization of the brain.*

Kuno, M., University of North Carolina. *Sensory transduction.*
— *Spinal cord physiology (I), (II).*

Hubel, D., Harvard Medical School. *C.N.S. physiology — The visual system (I), (II), (III), (IV), (V), (VI).*
Kelly, R., University of California, San Francisco. *Introduction to cellular neurobiology.*
Finkelstein, A., Albert Einstein College of Medicine. *Artifical membranes.*
— *Electrical properties of membranes.*
Hagiwara, S., University of California, Los Angeles. *Voltage-dependent calcium channels.*
Dennis, M., University of California, San Francisco. *Introduction to developmental neurobiology.*
Rakic, P., Harvard Medical School. *Development in the C.N.S. (I), (II).*
Fischbach, G., Harvard Medical School. *Development in culture (I), (II).*
Rosenthal, J., Yale University Medical School. *Regulation of the acetylcholin receptor.*
Frank, E., University of Oslo. *Development of the neuro-muscular junction.*
Shatz, C., Harvard Medical School. *Introduction to integrative neurobiology.*
Yoon, M., Dalhousie University. *Retino-tectal specificity.*
Wiesel, T., Harvard Medical School. *Influence of experience on C.N.S. function.*
Schiller, P., Massachusetts Institute of Technology. *Oculomotor systems.*
Julesz, B., Bell Laboratories. *Depth perception.*
Geschwind, N., Boston City Hospital. *Higher functions.*

NERVOUS SYSTEM OF THE LEECH WORKSHOP – June 10 - June 30

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

The initial work was devoted mainly to recognizing the individual cells, learning how to record from them, 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.

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

INSTRUCTORS

Nicholls, John, Ph.D., Stanford University Medical School, Palo Alto, California
Frank, Eric, Ph.D., University of Oslo, Oslo, Norway
Jansen, Jan, Ph.D., University of Oslo, Oslo, Norway
Stuart, Ann, Ph.D., Harvard Medical School, Boston, Massachusetts

PARTICIPANTS

Bittner, George, Ph.D., University of Texas, Austin, Texas
Chen, Victor, Michigan State University, East Lansing, Michigan
Henry, Richard W., Ph.D., Bucknell University, Lewisburg, Pennsylvania
Kelly, Amy S., Ph.D., University of California, Berkeley, California
Njå, Arild, M.D., University of Oslo, Oslo, Norway
Ross, William N., Ph.D., Yale Medical School, New Haven, Connecticut
Salzberg, Brian M., Ph.D., Yale University, New Haven, Connecticut
Ware, Steven, B.A., University of Texas, Austin, Texas
Zipser, Birgit, Ph.D., Rose F. Kennedy Center, Bronx, New York

SEMINARS

Nicholls, J., Stanford University Medical School. *Introduction to leech C.N.S.*
— *Receptive fields of sensory cells.*
— *Changes in synaptic connections after lesions and in cultured ganglia.*
— *Glial cells and K accumulation.*
Sawyer, R., University of South Carolina. *Biology of leeches.*
— *Development of leeches.*
Jansen, J., University of Oslo. *Hyperpolarization of sensory cells following impulses.*
— *The S interneuron.*

— *Regeneration in leech C.N.S.*
Stuart, A., Harvard Medical School. *Motor cells.*
Muller, K.J., Harvard Medical School. *Supernumerally sensory and motor cells.*
— *Synaptic connections of sensory and motor cells.*
Purves, D., Washington University Medical School. *Fine structure of leech C.N.S.*
McMahan, U.C., Harvard Medical School. *Peroxidase injection of individual cells.*
Stent, G., University of California, Berkeley. *Analysis of swimming (I), (II).*
Kristan, W., University of California, Berkeley. *Analysis of swimming (II).*
Cohen, L., Yale Medical School. *Optical recognition of signalling in leech C.N.S.*
Coggeshall, R., Marine Biology Laboratory, Galveston, Texas. *Morphology and chemistry of retzius cells.*

ANIMAL VIRUS WORKSHOP – July 3 - July 23

The emphasis in this workshop was on lectures, discussions and laboratory exercises, including preparation of primary cell cultures; growth of continuous tissue culture cell lines; growth of virus stocks and their titration by plaque assay and hemagglutination; purification and electron microscopic examination of virions; electrophoretic analysis of viral nucleic acids and proteins; determination of virus-specific macromolecules in infected cells by cell fractionation techniques; assay of virus-induced and virion-associated enzymes; effects of inhibitors on virus replication; translation of viral RNA by cell-free extracts; and virus-mediated oncogenic transformation and cell fusion.

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

INSTRUCTORS

Shatkin, Aaron J., Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey
Levine, Arnold, Ph.D., Princeton University, Princeton, New Jersey

ASSISTANTS

Laipis, Philip, Ph.D., Princeton University, Princeton, New Jersey
Saborio, Jose, M.D., University of Uppsala, Uppsala, Sweden

PARTICIPANTS

Axelrod, Daniel, B.S., University of California, Berkeley, California
Bourguignon, Gerard J., Ph.D., Yale University, New Haven, Connecticut
Cherney, Claudia S., B.A., University of Rochester, Rochester, New York
Chousterman, Suzanne, Ph.D., Institute Recherches Scientifiques sur le Cancer, Villejuif, France
Day, Rufus S., Ph.D., National Institutes of Health, Bethesda, Maryland
Denhardt, David T., Ph.D., McGill University, Montreal, Canada
Gelmann, Edward P., B.S., Stanford University Medical Center, Stanford, California
Humbert, Jerome, B.Sc., Swiss Institute for Cancer Research, Lausanne, Switzerland
Kraiselburd, Edmundo N., Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey
Lebowitz, Jacob, Ph.D., Syracuse University, Syracuse, New York
Liggins, George L., M.P.H., University of Virginia School of Medicine, Charlottesville, Virginia
Linne, Tommy, B.Sc., Wallenberg Laboratory, University of Uppsala, Uppsala, Sweden
Moyer, Richard W., Ph.D., Columbia University, New York, New York
Prusiner, Stanley B., M.D., University of California School of Medicine, San Francisco, California
Rundell, Kathleen, Ph.D., University of California, Berkeley, California
Stevens, Nancy, M.S., Ciba-Geigy Corporation, Summit, New Jersey
Takeshita, Masaru, Ph. D., Albert Einstein College of Medicine, Bronx, New York
Van Roy, Frans M., Lic. Biol. Sci., State University of Ghent, Ghent, Belgium
Watanabe, Hiroko, M.D., Ph.D., Laval University, Ste. Foy, Canada
Yang, David C.H., Ph.D., The Rockefeller University, New York, New York

SEMINARS

Pardee, A., Princeton University. *The animal cell and its division cycle.*
Alberts, B., Princeton University. *DNA replication.*
Darnell, J., Columbia University. *RNA synthesis.*
Basilico, C., New York University. *Animal cell hybrids and* ts *mutants.*
Lodish, H., Massachusetts Institute of Technology. *Protein synthesis.*
Levine, A., Princeton University, and A. Shatkin, Roche Institute. *Survey of animal viruses.*
Maizel, J., National Institutes of Health. *Enteroviruses.*
Denhardt, D., McGill University. *ϕX Replication.*
Moyer, R., Columbia University. *T5 Replication.*
Emerson, S., University of Virginia. *VSV.*
Choppin, P., Rockefeller University. *Myxo- and paramyxoviruses.*

Shefton, B., Massachusetts Institute of Technology. *Arboviruses.*
Bishop, J.M., University of California, San Francisco. RNA *tumor viruses.*
Lebowitz, J., Syracuse University. *Superhelical DNA.*
Kraiselburd, E., Roche Institute, *Herpes transformation.*
Levine, A., Princeton University. *Adenoviruses.*
Sambrook, J., Cold Spring Harbor Laboratory. *Structure and transcription of adeno and SV40 DNA.*
Martin, R., National Institutes of Health. *Papovaviruses.*
Linne, T., Uppsala University. *Adeno proteins.*
Day, R., National Institutes of Health, *Reactivation of adenoviruses.*
Saborio, J., Uppsala University. *Translation of adenoviruses RNA.*
Mulder, C., Cold Spring Harbor Laboratory. *Restriction enzymes.*
Lewis, A., National Institutes of Health. *Adeno-SV40 hybrids.*
Levine, Arthur, National Institutes of Health. *Transcription and structure of adeno-SV40 hybrid virus DNA.*
Prusiner, S., University of California, San Francisco. *Slow viruses.*
Keller, W., Cold Spring Harbor Laboratory. *RNase H and unwinding protein.*
Kates, J., State University of New York, Stonybrook. *Poxviruses.*
Roizman, B., University of Chicago. *Herpesviruses.*
Ward, D., Yale University. *Papovaviruses.*
Rowe, W., National Institutes of Health. *Perspectives in animal virology.*

ADVANCED BACTERIAL GENETICS WORKSHOP – July 3 - July 23

The workshop covered recent techniques which enable the bacterial geneticist to set up experiments for cell-free systems. New methods for isolating *ts* lethal mutants and general strategies for finding any desired mutation were covered, as were methods for isolating specialized transducing phage carrying any host chromosomal gene.

INSTRUCTORS

Miller, Jeffrey, Ph.D., University of Geneva, Geneva, Switzerland
Gottesman, Max, Ph.D., National Institutes of Health, Bethesda, Maryland
de Crombrugghe, Benoit, Ph.D., National Institutes of Health, Bethesda, Maryland

ASSISTANTS

Palmer, Beth, University of Illinois, Urbana, Illinois
Pampeno, Chris, B.A., Hunter College, New York, New York

PARTICIPANTS

Bassford, Philip J., Jr., B.S., University of Virginia, Charlottesville, Virginia
Buchanan, Christine E., Ph.D., Harvard University, Cambridge, Massachusetts
Coulondre, Christine, L'ecole de Medecine, Geneva, Switzerland
Hammer-Jespersen, Karin, Cand. Scient., University Institute of Biological Chemistry B, Copenhagen, Denmark
Isaksson, Leif A., Ph.D., Wallenberg Lab, University of Uppsala, Uppsala, Sweden
Johnson, James R., Ph.D., Veterans Administration Hospital, Durham, North Carolina
Karam, Jim D., Ph.D., University of South Carolina, Charleston, South Carolina
Saint Girons, Isabelle, D.E.A. de Biochimie, Institut Pasteur, Paris, France
Speyer, Joseph F., Ph.D., University of Connecticut, Storrs, Connecticut
Springer, Mathias, Dr. 3e Cycle, Institut de Biologie Physico-chimique, Paris, France

SEMINARS

de Crombrugghe, B., National Cancer Institute, National Institutes of Health. *Regulation of transcription of the galactose operon of* E. coli.

EXPERIMENTAL TECHNIQUES IN NEUROBIOLOGY – July 3 - July 29

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

Emphasis was placed on the following experimental techniques: intracellular

recordings of controlled membrane potentials: intracellular injection of ions; ionophoretic application of drugs; and physiological and electrical stimulation of selected activities. These techniques were used in explaining problems of cellular neurophysiology such as resting potential, action potential, the sodium potassium pump, and ionic and pharmacological bases of synaptic transmission.

INSTRUCTORS

Kehoe, JacSue, Ph.D., Ecole Normale Superieure, Paris, France
Kunze, Diana, Ph.D., University of Texas Medical School, Galveston, Texas
Stefani, Enrico, M.D., University of Buenos Aires, Buenos Aires, Argentina
Chiarandini, Dante, M.D., University of Buenos Aires, Buenos Aires, Argentina

PARTICIPANTS

Baden-Kristensen, Keld, M.Sc., Massachusetts Institute of Technology, Cambridge, Massachusetts
Beach, Robert L., M.S., University of Connecticut, Storrs, Connecticut
Grimm-Jorgensen, Yvonne, B.S., University of Connecticut, Storrs, Connecticut
Harris, William A., B.A., California Institute of Technology, Pasadena, California
Horvitz, Robert H., M.A., Harvard University, Cambridge, Massachusetts
Jan, Lily K.C.Y., M.S., California Institute of Technology, Pasadena, California
Jan, Yuh Nung, M.S., California Institute of Technology, Pasadena, California
Klein, William L., Ph.D., National Institutes of Health, Bethesda, Maryland
Zain-Ul-Abedin, M., Ph.D., University of Karachi, Karachi, West Pakistan

SEMINARS

Tsien, R.W., School of Medicine, Yale University. *Surface charge explanations of epinephine's action on a cardiac pacemaker current.*

Schwartz, J., New York University Medical Center. *Synthesis, axonal transport, and release of transmitter substances in single aplysia neurons.*

— *On the formation and transport of newly synthesized membrane protein.*

Nakajima, S., Purdue University. *Introduction to muscle physiology with an emphasis on E-C coupling and electrophysiology of the T system.*

Mauro, A., Rockefeller University. *A voltage-clamp study of the ventral photoreceptor in* Limulus.

PHYCOMYCES WORKSHOP – July 3 - August 31

As in the past three summers, the efforts of most of our workshop participants were directed toward the development of techniques needed to apply the powerful tools of genetics and biochemistry toward the goal of a molecular understanding of the sensory responses of *Phycomyces*.

To this end, *Jurgen Weber* and *Terrance Leighton* were able to report that newly germinated spores, called germlings, contain the input and output parts of the photoresponse machinery. These germlings are most suitable for biochemical analysis, in contrast to sporangiophores, because they can be grown in uniform, large-scale, liquid culture. A significant step forward was made this summer by *Horst Binding* and *Jurgen Weber*, who treated germlings with two crude enzyme preparations to obtain a high yield of round protoplasts. Work with these protoplasts determined that they can regenerate cell wall and develop into normal mycelium, and furthermore, under special conditions, fusion of protoplasts can be induced before regeneration. These discoveries should have far-reaching consequences for the isolation of intact subcellular organelles, for the uptake of DNA or organelles, for the labeling of the plasma membrane, and for the facilitation of complementation tests. The complementation analysis of altered phototropism or *mad* mutants by the effective, but cumbersome, grafting of sporangiophores was continued by *Peter Fisher*. He found one more cistron, bringing the current total to six.

Pat Burke continued her work with auxotrophic mutants begun last year with the isolation of five mutants. Due to some modifications, including the growth of mycelia in microtest trays, Burke was able to lower the amount of work needed to isolate these mutants. And *Walter Schroeder* showed that the ^{3}H-suicide technique, used successfully with bacteria and yeast, can be used with germlings, which should prove useful for isolating specific types of auxotrophic and temperature-sensitive mutants. *Juan Ramon Medina* and *Max Delbruck* showed that the combined action of UV and caffeine, a drug known to inhibit DNA repair, may be a useful treatment to mutagenize spores without the use of nitrosoguanidine.

Several new qualitative observations on β-carotone, whose presence results in the normal yellow color of *Phycomyces*, were reported by *Paco Murillo*. These suggest that at least one of the protein complexes has a regulatory as well as an enzymatic role. His work makes it clear that the use of mutants and drugs which inhibit or stimulate carotenogenesis can be further exploited for the elucidation of this pathway. The groundwork now laid is also encouraging for the formidable task of demonstrating the enzymatic activities in vitro.

Kostia Bergman has discovered that *Phycomyces* produces a β-glucosidase when grown on maltose or cellobiose, but not when grown on glucose. Since this enzyme can split various synthetic substrates to yield soluble or insoluble colored products, it will be possible to screen for structural gene or regulatory gene mutants. Although *Phycomyces* is clearly not the organism of choice for an exhaustive study of gene-protein relationships, it seems clear that the characterization of β-glucosidase mutants will further our aim of developing genetic techniques and understanding the molecular biology of *Phycomyces* growth and development. This summer Bergman concentrated on the enzymology and protein chemistry of β-glucosidase.

Jose Cabezon started the characterization of mutants resistant to 5-fluorouracil, which previous results by Eslava at Caltech had suggested might be dominant. However, results from heterokaryons formed between these mutants and C_9, a red-sensitive strain, suggest that resistance is recessive. Cabezon also demonstrated that the mutants overproduce uracil and accumulate it in the media.

Max Delbruck discussed the results of two groups, one at Caltech and one in Sevilla, who are now actively developing the sexual genetics of *Phycomyces*, and continued his own experiments on the heterosexual heterokaryons which sometimes result from a sexual cross. Study of these strains and their relative occurrence should help elucidate the karyology of the sexual cycle and the chemical basis of sexual differentiation and could lead to the development of a parasexual cycle.

Amid the activity in genetics and biochemistry, exciting new physiological experiments were in progress. *Jean Matricon* and *Jean-Francois Lafay* opened the questions once again on the mechanism of the avoidance response, whereby the sporangiophore bends away from objects close to, but not touching, its growing zone. They examined the explanation promoted for this response, i.e., that the object (barrier) calms random winds, and the sensitive "wind detectors" then signal the sporangiophore to bend away from the side of relative quiet. They rotated a cylindrical barrier, held close to the growing zone, at various speeds. Suprisingly, the sporangiophore avoided this wind-creating barrier. Of course, careful measurements of wind velocity must be made, but it seems that once again this mysterious response is simply unexplainable.

Ulrike Wulff and *Steven Block* did experiments on the effect of polarized light on the phototropism of sporangiophores. Their results strengthened previous conclusions based on completely different experiments that the photoreceptors must be located near the cell wall on some structure which is rotating.

Cold Spring Harbor is an ideal place for the workshop because of the intense scientific communication and other work in progress. This summer we supplied material to the laboratory of Richard Roberts, where an attempt was made to find a restriction endonuclease from *Phycomyces*. The first attempt showed no specific cleavage of phage λ DNA by any protein in the soluble fraction from mycelia. For future efforts, a mixed sporangiophore-mycelium preparation was harvested and stored.

Another important activity at the workshop is communication with people who work on similar problems with other organisms. Of particular interest this summer, *Francine Prevost* described and demonstrated her results with another light-sensitive fungus, *Pilobolus*. This system seems to have distinct advantages over *Phycomyces* for the study of the morphological effects of light.

ORGANIZERS

Bergman, Kostia, Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts
Delbruch, Max, Ph.D., California Institute of Technology, Pasadena, California

ASSISTANTS

Fischer, Renate, M.D., California Institute of Technology, Pasadena, California
Morterero, Almudene, University of Sevilla, Sevilla, Spain
Parker, Vann, Duke University, Durham, North Carolina

PARTICIPANTS

Binding, Horst, Ph.D., Max-Planck-Institut für Züchtungsforschung, Köln, Germany
Block, Steven, B.A., Oxford University, Oxford, England
Burke, Patricia, Ph.D., University of California, Santa Cruz, California
Cabezon, Jose, California Institute of Technology, Pasadena, California
Dennison, David, Ph.D., Dartmouth College, Hanover, New Hampshire
Fischer, Peter, B.A., California Institute of Technology, Pasadena, California
Foster, Ken, Ph.D., University of Colorado, Boulder, Colorado
Freese, Phil, Ph.D., University of Massachusetts Medical School. Worcester, Massachusetts
Gamow, Igor, Ph.D., University of Colorado, Boulder, Colorado
Lafay, Jean Francois, Universite Paris-Sud, Orsay, France
Leighton, Terrance, Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts
Medina, Juan Ramon, B.A., University of Sevilla, Sevilla, Spain
Matricon, Jean, Ph.D., University of Paris, Paris, France
Murillo, Francisco J., B.A., University of Sevilla, Sevilla, Spain
Prevost, Francine, Ph.D., Universite Paris-Sud, Orsay, France
Ortega, Ken, M.A., University of Colorado, Boulder, Colorado
Russo, Vincenzo, Ph.D., Max-Planck-Institut fur Mokekulare Genetik, Berlin, Germany
Schroeder, Walter, Ph.D., California Institute of Technology, Pasadena, California
Weber, Jurgen, Ph.D., California Institute of Technology, Pasadena, California
Wulff, Ulrike, M.A., Institut für Biologie III, Universität, Freiburg, Freiburg, Germany

TUMOR VIRUS WORKSHOP – July 26 - August 14

The tumor virus field was covered in depth, from molecular studies to epidemiologic factors. Both DNA- and RNA-containing tumor viruses and their interaction with cell and animal hosts were discussed. The workshop consisted for the most part of lectures and discussions. There were also occasional experiments to demonstrate transformation, virus rescue from transformed cells, and various aspects of the lytic viral cycle.

INSTRUCTORS

Jaenisch, Rudolf, Ph.D., Salk Institute, La Jolla, California
Todaro, George, M.D., National Institutes of Health, Bethesda, Maryland

PARTICIPANTS

Ambrose, Charles T., M.D., University of Kentucky, Lexington, Kentucky
Barnes, David W., B.A., Vanderbilt University, Nashville, Tennessee
Biron, Karen K., B.S., Rutgers Medical School, Piscataway, New Jersey
Boccara, Martine, Ph.D., Institut Pasteur, Paris, France
Chirikjian, Jack G., Ph.D., Georgetown University School of Medicine, Washington, D.C.
Duguid, John R., B.S., California Institute of Technology, Pasadena, California
Fernandez-Munoz, Rafael, Ph.D., Columbia University, New York, New York
Fleckenstein, Bernhard, M.D., University of Erlangen, Erlangen, Germany
Guyer, Mark S., Ph.D., California Institute of Technology, Pasadena, California
Levin, Daniel H., Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts
Ludwig, Hanns O., Ph.D., Institut für Virologie, Giessen, Germany
Mautner, Vivian, Ph.D., National Institute for Medical Research, Mill Hill, London, England
Oster, Artom W., M.D., National Institutes of Health, Bethesda, Maryland
Parkhurst, James R., Ph.D., University of Wisconsin, Madison, Wisconsin
Pihl, Alexander, M.D., Nork Hydro's Institute for Cancer Research, Oslo, Norway
Roberts, Bryan E., Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts
Schindler, Joel M., B.Sc., Hebrew University, Jerusalem, Israel
Schiegeura, Harold T., Ph.D., Merck Institute for Therapeutic Research, Rahway, New Jersey
Ulrich, Kay, M.D., Fibger Laboratory, Copenhagen, Denmark
Weller, Nancy K., M.A., University of Colorado, Boulder, Colorado

SEMINARS

Pollack, R., Cold Spring Harbor Laboratory. *Growth control.*
Todaro, G., National Institutes of Health. *Transformation, RNA tumor viruses.*
Jaenisch, R., Salk Institute. *Polyoma and SV40 lytic cycle.*
Anderson, C., Cold Spring Harbor Laboratory. *Viral proteins.*
Francke, B., Salk Institute. *SV40, polyoma DNA replication.*
Eckhart, W., Salk Institute. *SV40, polyoma genetics.*
Nathans, D., Johns Hopkins University School of Medicine. *Use of restriction endonucleases to map viral genomes.*
— *Evolutionary variants of SV40.*
Bolognesi. D., Duke University Medical Center. *Biology and antigens of avian tumor viruses.*
August, T., Albert Einstein College of Medicine. *Proteins of mammalian RNA viruses.*
Neiman, P., Providence Hospital. *DNA of avian RNA tumor viruses.*

Lilly, F., Albert Einstein College of Medicine. *Genetic factors in mouse leukemia.*
Benveniste, R., National Institutes of Health. *DNA of mammalian RNA tumor viruses.*
Pagano, J., University of North Carolina School of Medicine. *The Epstein-Barr virus and human cytomegaloviruses.*
Roizman, B., University of Chicago. *Herpesviruses.*
Burger, M., Moffett Laboratories, Princeton University. *Some general aspects of the cell surface in transformation and a critical evaluation of the increased lectin agglutinability.*
Levine, A., Princeton University. *Adenoviruses.*
Crumpacker, C., Boston City Hospital. *Adeno-SV40 hybrids.*
Mulder, C., Cold Spring Harbor Laboratory. *Infectious DNA of adenoviruses.*
Essex, M., Harvard School of Public Health. *Infectious cat leukemia.*
Weiss, R., University of Washington. *Genetics of avian type C viruses.*
— *Pseudotypes.*
Duesberg, P., University of California, Berkeley. *RNA virus structure and function.*
Bentvelzen, P., Radiobiological Institute. *Genetics of mammary tumor viruses.*
Parks, W., National Institutes of Health. *Control of mammary tumor virus expression.*
Coggin, J., University of Tennessee. *Immunology of viral neoplasia.*
Benjamin, T., Harvard Medical School. *Transformation by polyoma virus: Cell mutants.*

MOLECULAR BIOLOGY AND GENETICS OF YEAST – July 26 - August 14

The yeast workshop was held concurrently with the tumor virus workshop, thereby allowing participants in both these courses to exchange information. This interchange highlighted the importance of yeast as a simple model for all eukaryotic cells. Numerous studies on the induction of abnormal cell growth by tumor viruses have implicated defects in early events in the cell cycle as the basis for the abnormal growth. These early events are best studied in a system where the tools of genetics and biochemistry can be used to identify the molecules controlling them.

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

INSTRUCTORS

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

ASSISTANT

Wolf, Dieter, Cornell University, Ithaca, New York

PARTICIPANTS

Clark, Alvin J., Ph.D., University of California, Berkeley, California
Conde, Jaime, Dr. Ingeniero Agronomo, Facultad de Ciencias, Sevilla, Spain
Davidson, Jeffrey N., B.S., Harvard University, Cambridge, Massachusetts
Dickson, Robert C,, Ph.D., University of California, San Diego, California
Galas, David J., Ph.D., University of California, Livermore, California
Gross, Kurt J., Ph.D., New York Blood Center, New York, New York
Grossman, Lawrence I., Ph.D., California Institute of Technology, Pasadena, California
Guerrini, Anna Maria, Dr. in Biology, Instituto Internazionale di Genetica e Biofisica, Napoli, Italy
Lange, Peter, Ph.D., Institut für Biologie II, Universität Tübingen, Tübingen, Germany
Menzel, Rolf, B.A., University of California, Berkeley, California
Palmer, Graham, Ph.D., Rice University, Houston, Texas
Poyton, Robert O., Ph.D., University of Connecticut Health Center, Farmington, Connecticut
Ray, Durwood B., Ph.D., University of Texas, Dallas, Texas
Roon, Robert J., Ph.D., University of Minnesota, Minneapolis, Minnesota
Thorner, Jeremy W., Ph.D., Stanford University, Stanford, California
Zinker, Samuel R., M.D., Ph.D., Albert Einstein College of Medicine, Bronx, New York

Hartwell, L.H., University of Washington, Seattle. *Genetic control of cell division in yeast.*
— *Integration of the cell cycle with the life cycle.*
McLaughlin, C.S., University of California, Irvine. *Mutations and antibiotics that affect protein synthesis (I), (II).*
Marmur, J., Albert Einstein College of Medicine. *DNA in yeast.*
— *Mitochondrial mutants of yeast.*
Roth, R., Illinois Institute of Technology. *Genetic control of replication during meiosis.*
Moens, P.B., York University. *Morphological aspects of sporulation, normal and mutant.*
Mortimer, R.K., University of California, Berkeley. *Genetic mapping in yeast.*
— *Gene conversion.*
Henry, S., Kansas State University. *Genetic control of mating in yeast.*
Sherman, F., University of Rochester. *Deletions in the iso-1-cytochrome c gene.*
—*Nucleotide sequence governing initiation of translation.*
Prakash, L., University of Rochester. *Control and specificities of chemical mutagenesis.*
Lawrence, C., University of Rochester. *Genetic control of UV mutagenesis in yeast.*
Fink, G.R., Cornell University. *The "killer" phenomenon in yeast.*
— *The regulation of histidine biosynthesis in yeast.*

NEUROBIOLOGY OF *DROSOPHILA* WORKSHOP – August 2 - August 22

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

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

INSTRUCTORS

Pak, William L., Ph.D., Purdue University, W. Lafayette, Indiana
Hartl, Daniel, Ph.D., Purdue University, W. Lafayette, Indiana
Götz, Karl, Ph.D., Max-Planck Institute for Biological Cybernetics, Tubingen, Germany

ASSISTANTS

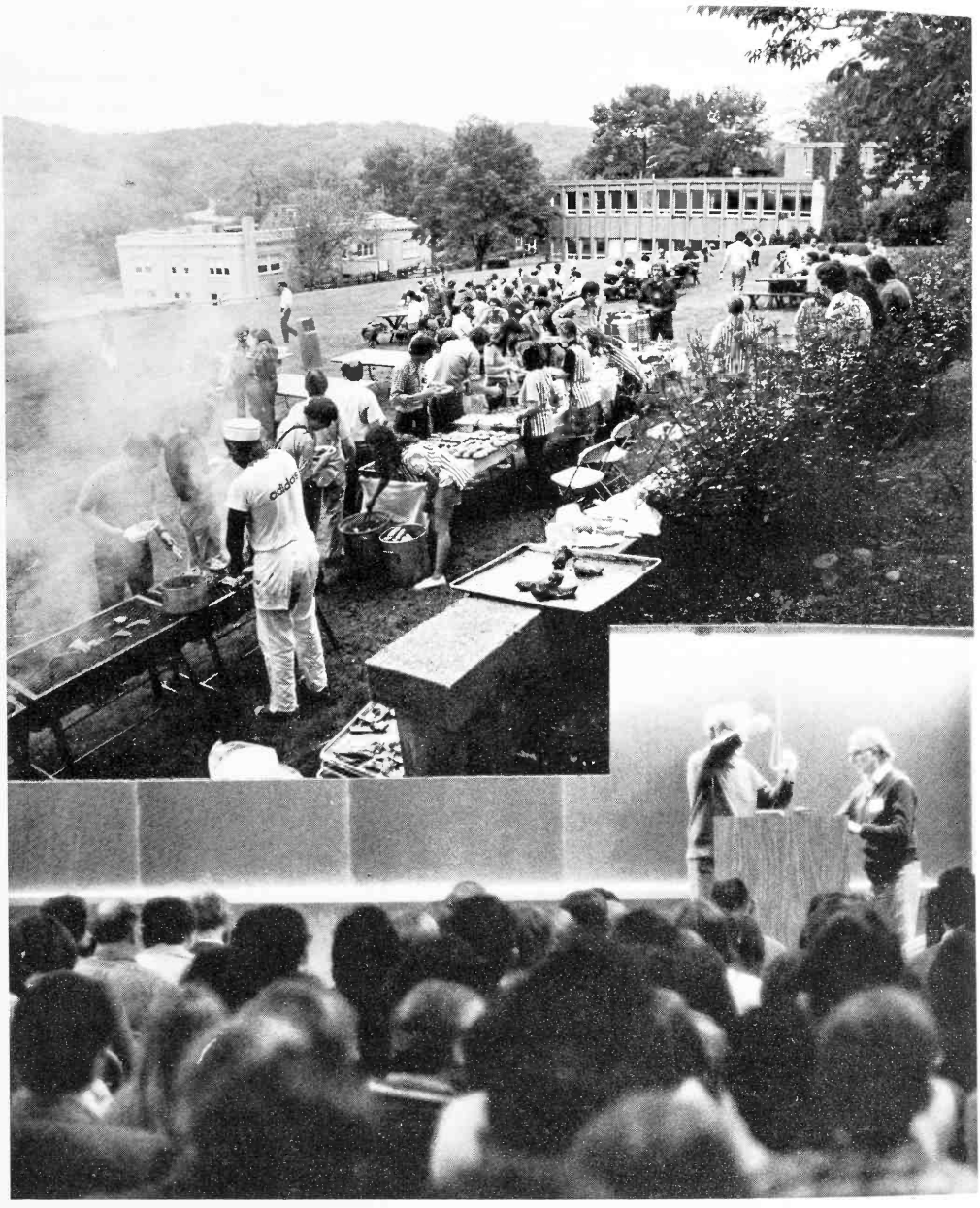
Wu, Chun-Fang, B.S., Purdue University, W. Lafayette, Indiana
Mieling, Beth, B.A., Purdue University, W. Lafayette, Indiana

PARTICIPANTS

Bahn, Erik, Ph.D., University of Copenhagen, Copenhagen, Denmark
Ohelala, Oscar, M.D., Ph.D., Public Health Research Institute, New York, New York
Dudai, Yadin, B.Sc., Weizmann Institute of Science, Rehovot, Israel
Gavrin, Edward S., S.B., Massachusetts Institute of Technology, Cambridge, Massachusetts
Grabowski, Sandra R., Ph.D., Purdue University, W. Lafayette, Indiana
Horvitz, H. Robert, Ph.D., Harvard University, Cambridge, Massachusetts
Kells, Sandra S., B.A., University of Chicago, Chicago, Illinois
Schmidt-Nielsen, Bent K., B.A., Massachusetts Institute of Technology, Cambridge, Massachusetts
Taylor, Duncan P., B.S., Oregon State University, Corvallis, Oregon
von Ehrenstein, Günter, M.D., Ph.D., Max-Planck Institüt für Experimentalle Medizin, Gottingen, West Germany
Wilcox, Michael J., B.S., Purdue University, W. Lafayette, Indiana
Wilson, Thomas G., M.S., Oak Ridge National Laboratory, Oak Ridge, Tennessee

SEMINARS

Kankel, D., Yale University. *Mosaic analysis of behavioral mutants.*
Hall, J., Brandeis University. *Neurogenetics of* Drosophila *courtship.*
Hartl, D., Purdue University. *Spermatogenesis in* Drosophila.
Pak, W.L., Purdue University. *Photoreceptor mutants.*
Seecof, R., City of Hope National Medical Center. Drosophila *neurons, muscle cells and synapses in vitro.*
Suzuki, D., University of British Columbia. *Studies on conditionally expressed locomotor mutations.*
Quinn, C., California Institute of Technology. *Learning in* Drosophila.
Götz, K., Max-Planck Institüt für biologische Kybernetik. *Processing of visual cues in the navigation system of the fruitfly.*
Levine, J., University of California, Berkeley. *Fruitfly flight.*



(*Top*) Symposium barbecue
(*Bottom*) Session being held in Vannevar Bush Lecture Hall
(*Photos by Carl Anderson*)

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Tumor virus research has a uniquely favorable position in contemporary biology. It affords the opportunity to probe both the fundamental biology and chemistry of higher animal cells, as well as the nature and origins of cancer. So it combines exciting science with only a marginal concern for next year's research dollars. Not surprisingly, masses of scientists have moved into this arena, and the pace of good research has increased almost overwhelmingly. Rapid accumulation of new facts, however, should not be confused with deep understanding, and all too many basic principles about how tumor viruses act remain to be worked out.

The moment seemed propitious, therefore, to hold a Symposium aimed at providing a broad overview of the tumor virus field, especially in its molecular aspects. We knew, of course, that for both fairness and completeness we should issue an unprecedented number of invitations. We hoped, nonetheless, that the resulting colloquy would be of such high quality that it would not be overshadowed by the thought that the right to work at one's own pace may almost totally be lost. Fortunately, our optimism appears to have been justified. Virtually all the 346 participants came fearing the worst, but went away with the feeling that this indeed had been a most important occasion. For this success I am most indebted to the many colleagues who helped me put together the final program. In particular, I acknowledge the assistance of Joe Sambrook and David Baltimore, who compiled lists of invitees much longer than a week's time would permit to speak at length. So all too many persons with interesting results could not be on the formal program. Fortunately, many such individuals were in the audience and generously contributed brief resumes of their current work.

That we were able to invite and house more speakers than have come to any previous Symposium we owe to the most generous assistance provided by the National Institutes of Health, The National Science Foundation, and the Atomic Energy Commission. Their continued strong support over so many years has been a major factor in giving us the opportunity to mold our Symposia around the nature of the occasion.

J.D. Watson

THURSDAY, May 30

Chairman: Maurice Green, Institute for Molecular Virology, St. Louis University School of Medicine, St. Louis, Missouri

Opening Remarks: Renato Dulbecco, Imperial Cancer Research Fund Laboratories, London, England

G.G. Wickus, P.E. Branton, C.B. Hirschberg, P. Fuchs, P.M. Blumberg, B.J. Gaffney and P.W. Robbins, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Transformation of the chick fibroblast cell surface by Rous sarcoma virus."

H. Bauer, R. Kurth, L. Rohrschneider, G. Pauli and R.R. Friis, Robert Koch Institut, Abteilung für Virologie, Berlin, W. Germany: "On the role of cell surface changes in RNA tumor virus-transformed cells."

E. Reich, The Rockefeller University, New York: "Fibrinolysis and transformation: Recent studies."

FRIDAY MORNING, May 31

Chairman: Bernhard Hirt, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland.

M. Fried, B. Griffin and E. Lund, Imperial Cancer Research Fund, London, England: "Polyoma wild type, mutant and defective DNA — Physical maps."

C.-J. Lai and D. Nathans, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: "Mapping *ts* mutants and deletion mutants of SV40."

T.E. Shenk, C. Rhodes, P.W.J. Rigby and P. Berg, Department of Biochemistry, Stanford University, California: "S_1 nuclease, a restriction endonuclease for mismatched regions in duplex DNA."

K. Yoshiike, A. Furuno, S. Watanabe, S. Uchida, K. Matsubara and Y. Takagi, National Institute of Health, Tokyo, and Kyushu University School of Medicine, Fukuoka, Japan: "Characterization of defective SV40 DNA: Comparison between large-plaque and small-plaque types."

E. Winocour, N. Frenkel, S. Rozenblatt and S. Lavi, Virology Section, Weizmann Institute of Science, Rehovot, Israel: "Substituted tumor virus DNA."

L.T. Chow, H.W. Boyer,* E.G. Tischer and H.M. Goodman, Department of Biochemistry and Biophysics and *Department of Microbiology, University of California Medical Center, San Francisco: "Electron microscope mapping of the attachment sites on SV40 DNA during lytic infection."

J.E. Mertz, J. Carbon, P. Berg, M. Herzberg and R. Davis, Department of Biochemistry, Stanford University, California: "SV40 mutants containing deletions, duplications and insertions in their DNA."

W.W. Brockman, T.N.H. Lee and D. Nathans, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: "Characterization of cloned evolutionary variants of SV40."

M.A. Martin, G. Khoury and G.C. Fareed,* Laboratory of Biology of Viruses, NIAID, NIH, Bethesda, Maryland; *Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: "Specific reiteration of viral DNA sequences in mammalian cells."

D. Davoli and G.C. Fareed, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: "Formation of reiterated SV40 DNA."

FRIDAY EVENING, May 31

Chairman: Peter Duesberg, Virus Laboratory, University of California, Berkeley, California.

D. Baltimore, I.M. Verma,* A. Panet, R.M. McCaffrey, S. Drost, W. Mason,† P. Besmer and D. Smoler, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; *The Salk Institute, San Diego, California; †Institute for Cancer Research, Philadelphia, Pennsylvania: "Reverse transcriptase: Physiology and genetics."

J.E. Dahlberg, F. Harada and R.C. Sawyer, Department of Physiological Chemistry, University of Wisconsin, Madison: "Structure and properties of an RNA primer for initiation of in vitro Rous sarcoma virus DNA synthesis."

G. Monroy, M. Jacquet, Y. Groner and J. Hurwitz, Department of Developmental Biology and Cancer, Division of Biological Sciences, Albert Einstein College of Medicine, New York: "The in vitro synthesis of avian myeloblastosis viral RNA sequences."

L. Rymo, J.T. Parsons, J.M. Coffin and C. Weissmann, Institute for Molecular Biology I, University of Zurich, Switzerland: "In vitro synthesis of Rous sarcoma virus RNA."

R. Gallo, D. Gillespie and R. Gallagher, National Cancer Institute, NIH, Bethesda, Maryland: "Relatedness of reverse transcriptases (R.T.) and nucleotide sequences from subhuman primate viruses and human leukemic cells."

S. Spiegelman, Columbia University, New York: "Molecular virology of human cancer."

R.E. Thach, D. Robertson, N. Baenziger and D. Dobbertin, Department of Biochemistry,

Washington University School of Medicine, St. Louis, Missouri: "Reverse transcriptase in plasmacytoma cells."

E. Fleissner, H. Ikeda, J-S. Tung, E. Tress, W. Hardy, Jr., L. Stockert, E. Boyse, T. Pincus and P. O'Donnell, Sloan-Kettering Institute, New York: "Characterization of murine leukemia virus-specific proteins."

V.M. Vogt,* R. Eisenmann and H. Diggelman, Swiss Institute for Cancer Research, Lausanne; *Inst. für allgem. Mikrobiologie, Bern, Switzerland: "Synthesis of the structural proteins of avian RNA tumor viruses: The cleavage scheme and the ordering of proteins on the precursor polypeptide."

R. Eisenmann and V.M. Vogt, Swiss Institute for Cancer Research, Lausanne; Inst. für allgem. Mikrobiologie, Bern, Switzerland: "Synthesis of avian RNA tumor virus precursor polypeptide in RSV-transformed heterologous cell lines."

SATURDAY MORNING, June 1

Chairman: Lennart Philipson, The Wallenberg Laboratory, University of Uppsala, Uppsala, Sweden

J.K. McDougall, A.R. Dunn and P.H. Gallimore, Department of Cancer Studies, University of Birmingham, England: "Recent studies on the characteristics of adenovirus-infected and transformed cells."

J. Sambrook, U. Pettersson, B. Ozanne, P.A. Sharp, J. Williams* and P.H. Gallimore,† Cold Spring Harbor Laboratory, New York; *MRC Virology Institute, Glasgow, Scotland; †Department of Cancer Studies, University of Birmingham, England: "Viral DNA sequences in transformed cells."

F.L. Graham, P.J. Abrahams, S.O. Warnaar, C. Mulder,* F.A.J. de Vries, W. Fierst† and A.J. van der Eb, Laboratory for Physiological Chemistry, University of Leiden, The Netherlands; *Cold Spring Harbor Laboratory, New York; †Laboratory for Molecular Biology, University of Ghent, Belgium: "Studies on in vitro transformation with viral DNA and DNA fragments."

A.M. Lewis, Jr., J.H. Breeden, Y.L. Wewerka, L.E. Schnipper and A.S. Levine, National Institutes of Health, Bethesda, Maryland: "Studies of hamster cells transformed by AD2 and nondefective AD2-SV40 hybrids."

V. Defendi and K. Hirai, The Wistar Institute, Philadelphia, Pennsylvania: "Viral and cellular factor(s) which affect the process and extent of integration of the viral genome in SV40 infected and transformed cells."

J.S. Butel, J.S. Brugge and C.A. Noonan, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: "Transformation of primate and rodent cells by temperature-sensitive mutants of SV40."

G. Khoury, P.M. Howley and M.A. Martin, Laboratory of Biology of Viruses, NIAID, NIH, Bethesda, Maryland: "The detection and quantitation of SV40 genetic material."

P.W.J. Rigby, M. Hsu, T.A. Landers, C. Rhodes, T.E. Shenk and P. Berg, Department of Biochemistry, Stanford University Medical Center, California: "Integration of SV40 DNA."

SATURDAY EVENING, June 1

Chairman: George Klein, Karolinska Institute, Stockholm, Sweden

N.M. Wilkie, J.B. Clements, J.C.M. Macnab and J.H. Subak-Sharpe, Institute of Virology, University of Glasgow, Scotland: "Structure and biological properties of herpes simplex virus DNA."

P. Sheldrick and N. Berthelot, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: "Repetitious nucleotide sequences in herpes simplex virus DNA."

R.H. Grafstrom, J.C. Alwine, W.L. Steinhart and C.W. Hill, Department of Biological Chemistry, Hershey Medical Center, Pennsylvania State University, Hershey: "Terminal redundancy of herpes simplex virus type 1 DNA."

B. Roizman, R. Honess, M. Kozak, N. Frenkel and S. Silverstein, Department of Microbiology, University of Chicago, Illinois: "Multilevel regulation of viral macromolecular synthesis in herpesvirus-infected cells."

F. Rapp, Department of Microbiology, Hershey Medical Center, Pennsylvania State University, Hershey: "Demonstration of the oncogenic potential of herpes simplex viruses and cytomegalovirus."

B.R. McAuslan, B. Garfinkle, R. Adler, D. Testa and R. Florkiewicz, Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: "Transformation of XC cells by herpes simplex virus — Fact of fantasy??"

S. Kit, W.-C. Leung, G.N. Jorgensen, D. Trkula and D.R. Dubbs, Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas: "Thymidine kinase isozymes of normal and virus-infected cells."

J.H. Subak-Sharpe, S.M. Brown, J. Hay, J.M. Macnab, H.S. Marsden, D.A. Ritchie and M.C. Timbury, Institute of Virology, University of Glasgow, Scotland: "Genetic and functional studies with temperature-restricted mutants of herpes simplex virus types 1 and 2."

M. Benyesh-Melnick, P.A. Schaffer and R.J. Courtney, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: "Viral gene functions expressed and detected by temperature-sensitive (*ts*) mutants of herpes simplex virus (HSV)."

SUNDAY MORNING, June 2

Chairman: George Todaro, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

H.-J. Kung, J.M. Bailey, N. Davidson, P. Vogt,* M.O. Nicolson* and R.M. McAllister,* Department of Chemistry, California Institute of Technology, Pasadena; *University of Southern California School of Medicine, Los Angeles: "EM studies of tumor virus RNA."

H. Delius, P. Duesberg and W. Mangel, Cold Spring Harbor Laboratory, New York; Department of Molecular Biology, University of California, Berkeley; Imperial Cancer Research Fund, London, England: "Electron microscopic measurements of Rous sarcoma virus RNA."

P.H. Duesberg, P.K. Vogt,* M. Lai* and K. Beemon, Department of Molecular Biology, University of California, Berkeley; *Department of Microbiology, University of Southern California, Los Angeles: "Studies on genetic recombination between avian tumor viruses."

M.P. Stone, R.E. Smith and W.K. Joklik, Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: "RNA subunits of avian RNA tumor virus strains cloned and passaged in chick and duck cells."

J.A. Wyke and J.G. Bell, Imperial Cancer Research Fund Laboratories, London, England: "Genetic recombination among temperature mutants of Rous sarcoma virus."

P. Neiman and H. Graham Purchase, University of Washington, Seattle; USDA Regional Poultry Research Laboratories, East Lansing, Michigan: "Studies of the interrelationship of chicken leukosis virus and host cell genomes by RNA-DNA hybridization."

M.A. Baluda, M. Shoyab, P.D. Markham, R. Evans and W.N. Drohan, Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles: "Characterization of avian myeloblastosis virus genome by molecular hybridization."

E.M. Scolnick and W.P. Parks, National Cancer Institute, Bethesda, Maryland: "Murine sarcoma viruses with mouse and rat genetic information."

J. Zavada, Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia: "VSV pseudotype particles with surface antigen(s) of animal and of presumed human oncornaviruses."

R.A. Weiss, D.E. Boettiger and D.N. Love, Imperial Cancer Research Fund Laboratories,

London, England: "Phenotypic mixing between vesicular stomatitis virus and RNA tumor viruses."

SUNDAY AFTERNOON, June 2

Chairman: Fred Rapp, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania

G. Miller, J.E. Robinson, L. Heston and M. Newmuis, Departments of Pediatrics and Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut: "Immortalizing and non-immortalizing strains of EB virus."

G. Klein, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden: "Studies on the EBV-genome and the EBV-determined nuclear antigen (EBNA) in human malignancies."

H. zur Hausen, H. Wolf and J. Werner, Institut für Klinische Virologie and Robert-Koch-Institut, Berlin, Germany: "EBV-DNA in non-lymphoid cells of nasopharyngeal carcinomas and in malignant lymphoma obtained after inoculation of EBV into cotton-top marmosets."

J.S. Pagano, Departments of Medicine and Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina: "The Epstein-Barr virus and malignancy: Molecular evidence."

M. Nonoyama and A. Tanaka, Departments of Microbiology, Rush-Presbyterian-St. Luke's and University of Illinois Medical Centers, Chicago: "Epstein-Barr virus DNA as a possible plasmid DNA in mammalian cells."

B. Hampar, A. Tanaka, M. Nonoyama and J.G. Derge, National Cancer Institute, NIH, Bethesda, Maryland; Departments of Microbiology, Rush-Presbyterian-St. Luke's and University of Illinois, Chicago; and Flow Laboratories, Inc., Rockville, Maryland: "Cell virus relationships in human lymphoblastoid cells carrying the repressed EB virus genome."

L.N. Payne, P.C. Powell and M. Rennie, Houghton Poultry Research Station, Huntingdon, England: "The response of B and T lymphocytes, and other blood leukocytes, in Marek's disease."

MONDAY MORNING, June 3

Chairman: Malcolm Martin, National Institutes of Health, Bethesda, Maryland

Y. Aloni, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: "Processing and transport of viral RNA in SV40-infected cells."

R. Dhar, K. Subramanian, S. Zain, J. Pan and S.M. Weissman, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: "Nucleotide sequences about the ends of early and late mRNA from SV40."

R. Kamen, D.M. Lindstrom and H. Shure, Imperial Cancer Research Fund Laboratories, London, England: "Transcription of polyoma virus DNA."

L. Philipson, U. Pettersson, U. Lindberg and C. Tibbetts, Department of Microbiology, The Wallenberg Laboratory, Uppsala, Sweden: "Synthesis and processing of adeno-virus RNA in productive infection."

P.A. Sharp, P.H. Gallimore,* and S.J. Flint, Cold Spring Harbor Laboratory, New York; *Department of Cancer Studies, University of Birmingham, England: "Titration of viral RNA sequences in adenovirus 2 lytically infected cells and transformed cell lines."

J.E. Darnell, M. Georgieff, S. Bachenheimer and P. Hoffman, Department of Biological Sciences, Columbia University, New York: "The biosynthesis of mRNA in adenovirus transformed cells."

E.A. Craig, J. Tal, T. Nishimoto, M. McGrogan, S. Zimmer and H.J. Raskas, Departments of Pathology and Microbiology, Washington University School of Medicine, St. Louis, Missouri: "RNA transcription in cultures productively infected with adenovirus 2."

W. Doerfler, H. Burger, J. Ortin, E. Fanning, M. Westphal, B. Weiser and J. Schick, Institute of Genetics, University of Cologne, Germany: "Integration of adenovirus DNA in productive and abortive infection."

MONDAY EVENING, June 3

Chairman: Daniel Nathans, The Johns Hopkins University School of Medicine, Baltimore, Maryland

A.J. Robinson and A.J.D. Bellett, Department of Microbiology, John Curtin School of Medical Research, Canberra, Australia: "A circular DNA-protein complex from adenoviruses and its possible role in DNA replication."

J.A. Rose, K.W. Berry and C.F. Garon, National Institutes of Health, Bethesda, Maryland: "Arrangement of sequences in the inverted terminal repetition of adenovirus 18 DNA."

J.R. Arrand, W. Keller and R.J. Roberts, Cold Spring Harbor Laboratory, New York: "The extent of the inverted terminal repetition in adenovirus 2 DNA."

T.J. Kelly, Jr., A.M. Lewis, Jr., A.S. Levine and S. Siegal, Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland: "Structure of two Ad2-SV40 hybrids which contain the entire SV40 genome."

N.P. Salzman, J. Lebowitz,* M. Chen, A.G. Kasselberg and C.F. Garon, National Institutes of Health, Bethesda, Maryland; *Department of Biology, Syracuse University, New York: "Properties of SV40 DNA I."

W. Fiers, K. Danna, R. Rogiers, A. Vandevoorde, H. van Heuverswyn and G. Volckaert, State University of Ghent, Belgium: "Nucleotide sequence studies on SV40 DNA."

A.J. Levine, P. van der Vliet, B. Rosenwirth, J. Rabek, J. Frenkel and M. Ensinger, Department of Biochemical Sciences, Princeton University, New Jersey: "Adenovirus-infected, cell-specific, DNA-binding proteins."

A. Parodi, P. Rouget, O. Croissant, D. Blangy and F. Cuzin, Institut Pasteur, Paris, France: "Endonucleolytic cleavage of polyoma virus genome: Site specificity of the virion-associated nuclease and of the integrative recombination in a transformed line."

TUESDAY MORNING, June 4

Chairman: Howard Temin, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin

G.J. Todaro, M.M. Lieber, C.J. Sherr and R.E. Benveniste, Viral Leukemia and Lymphoma Branch, NCI, NIH, Bethesda, Maryland: "Endogenous primate and feline type C viruses."

R.C. Nowinski and S.L. Kaehler, McArdle Laboratory, University of Wisconsin, Madison: "Widespread occurrence of antibody to endogenous oncornaviruses in mice."

S.K. Chattopadhyay, D.R. Lowy, N.M. Teich, A.S. Levine and W.P. Rowe, Laboratory of Viral Diseases, NIAID, and NCI, NIH, Bethesda, Maryland: "Qualitative and quantitative studies of AKR-type murine leukemia virus (MLV) sequences in DNAof high-, low-, and non-virus-yielding mouse strains."

M. Strand and J.T. August, Department of Molecular Biology, Albert Einstein College of Medicine, New York: "Structural proteins of RNA tumor viruses as probes for viral gene expression."

F. Lilly, M. Strand and J.T. August, Departments of Genetics and Molecular Biology, Albert Einstein College of Medicine, New York: "Genetic control of the expression of MuLV p30 and gp69, 71 in tissues of normal, young adult mice."

S.A. Aaronson, Viral Carcinogenesis Branch, NIH, Bethesda, Maryland: "Cellular regulation of endogenous mouse type C RNA viruses."

H. Hanafusa, J.H. Chen, W.S. Hayward and T. Hanafusa, The Rockefeller University, New York: "Control of expression of tumor virus genes in uninfected chicken cells."

P. Bentvelzen, Radiobiological Institute TNO, Rijswijk (Z.H.), The Netherlands: "Endogenous murine mammary tumor viruses."

W.P. Parks and E.M. Scolnick, NCI, NIH, Bethesda, Maryland: "Hormonal regulation of in vitro murine mammary tumor virus synthesis."

R.J. Huebner, Viral Carcinogenesis Branch, NCI, NIH, Bethesda, Maryland: "Genetically transmitted (xenotropic and ecotropic) RNA tumor viruses: Endogenous etiological factors in cancers of experimental animals."

TUESDAY EVENING, June 4

Chairman: Lionel Crawford, Imperial Cancer Research Fund Laboratories, London, England

W.C. Russell and D.H. Metz, Division of Virology, National Institute for Medical Research, London, England: " 'Early' events in adenovirus-infected cells."

S.G. Baum, Departments of Medicine and Cell Biology, Albert Einstein College of Medicine, New York: "The effects of latency and multiplicity of infection on the enhancement of adenovirus SV40."

H. Westphal, L. Eron, R. Callahan, S.-P Lai and F.J. Ferdinand, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland: "Cell-free translation of highly purified adenovirus type 2 messenger RNA."

J.B. Lewis, C.W. Anderson, J.F. Atkins and R.F. Gesteland, Cold Spring Harbor Laboratory, New York: "Cell-free translation of adeno and S40 mRNA."

C. Prives, H. Aviv, E. Gilboa, M. Revel and E. Winocour,* Department of Biochemistry and *Section of Virology, The Weizmann Institute of Science, Rehovot, Israel: "Cell-free translation of SV40 messenger RNA coding for the major SV40 capsid protein VP-1."

T. Friedmann and W. Eckhart, Department of Pediatrics, University of California at San Diego School of Medicine and Salk Institute, La Jolla, California: "Mutant virion proteins in polyoma temperature-sensitive mutants."

G. Fey and B. Hirt, Swiss Institute for Experimental Cancer Research, Lausanne: "Two-dimensional fingerprints of tryptic peptides from polyoma virion proteins and mouse histones."

G. Brandner and N. Mueller, Institute of Hygiene, University of Freiburg, Germany: "Cytosine arabinoside and interferon and the regulation of SV40 genome expression in AGMK cells."

WEDNESDAY MORNING, June 5

Chairman: Thomas Benjamin, Harvard Medical School, Boston, Massachusetts

W. Eckhart, The Salk Institute, San Diego, California: "Polyoma gene functions."

P. Tegtmeyer, Departments of Pharmacology and Microbiology, Case Western Reserve University, Cleveland, Ohio: "Protein synthesis in productive infection by temperature-sensitive mutants of SV40."

R.G. Martin, J.Y. Chou, R. Saral and J. Avila, Laboratory of Molecular Biology, NIAMDD, NIH, Bethesda, Maryland: "Characterization of temperature-sensitive mutants of SV40."

H.S. Ginsberg, M.J. Ensinger, R.S. Kauffman, A.J. Mayer and U.I. Lundholm, Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York: "Cell transformation: A study of regulation with adenovirus temperature-sensitive mutants."

J. Williams, H. Young and P. Austin, MRC Virology Unit, Institute of Virology, Glasgow, Scotland: "Genetic analysis of human adenovirus gene functions expressed in permissive and non-permissive cells."

T. Grodzicker, J.F. Williams,* P.A. Sharp and J. Sambrook, Cold Spring Harbor Laboratory, New York, *MRC Virology Unit, University of Glasgow, Scotland: "Physical mapping of cross-over events between adenovirus 5 and adenovirus 2."

H. Shimojo, K. Shiroki and K. Yamaguchi, The Institute of Medical Science, Tokyo, Japan: "Analysis of viral DNA replication in adenovirus 12-infected cells."

J.S. Sussenbach, D.J. Ellens, P. Ch. van der Vliet, M.G. Kuijk, J.M. Vlak, Th.H. Rozijn and H.S. Jansz, State University of Utrecht, Laboratory for Physiological Chemistry, The Netherlands: "The mechanism of replication of adenovirus type 5 DNA."

L.V. Crawford, Imperial Cancer Research Fund Laboratories, London, England: "Polyoma DNA replication."

G. Magnusson, R. Craig, M. Narkhammar, P. Reichard, M. Staub and H. Warner, Medical

Nobel Institute, Department of Biochemistry, Karolinska Institute, Stockholm, Sweden: "Replication of polyoma DNA: Effects of hydroxyurea and arabinosyl cytosine."

WEDNESDAY AFTERNOON, June 5

Chairman: H. Koprowski, The Wistar Institute, Philadelphia, Pennsylvania

M.-E. Mirault, S.I. Reed and G.R. Stark, Department of Biochemistry, Stanford University, California: "X antigen is a protein bound to mRNA in an SV40-transformed hamster cell line."

M. Osborn and K. Weber, Cold Spring Harbor Laboratory, New York: "SV40 T antigen."

D.M. Livingston and I.C. Henderson, The Childrens Cancer Research Foundation, Harvard Medical School, Boston, Massachusetts; Viral Leukemia and Lymphoma Branch, NCI, NIH, Bethesda, Maryland: "Recent studies on the SV40 T antigen."

C.M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: "Assignment of the gene for SV40 T antigen in SV40 and adeno 7-SV40 transformed monkey cells."

J.F. Watkins, Sir William Dunn School of Pathology, Oxford, England: "The SV40 virus rescue problem."

K. Weber, E. Lazarides, R. Goldman* and R. Pollack, Cold Spring Harbor Laboratory, New York; *Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: "Localization and distribution of actin fibers in non-muscle cells."

R.D. Goldman, C. Chang and J.F. Williams,* Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Cold Spring Harbor Laboratory, New York; *MRC Virology Unit, University of Glasgow, Scotland: "Properties and behavior of hamster embryo cells transformed by human adenovirus type 5."

R. Risser, D. Rifkin* and R. Pollack, Cold Spring Harbor Laboratory, New York; *The Rockefeller University, New York: "The stable classes of transformed cells induced by SV40."

R. Weil, C. Salomon, E. May* and P. May,* Department of Molecular Biology, University of Geneva, Geneva, Switzerland; *Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: "A simplifying concept: The virus-specific 'pleiotropic effector.' "

THURSDAY EVENING, June 6

Chairman: Robin Weiss, Imperial Cancer Research Fund Laboratories, London, England

H.E. Varmus, R.R. Guntaka and J.M. Bishop, Department of Microbiology, University of California Medical Center, San Francisco: "Synthesis and integration of the provirus of Rous sarcoma virus in permissive cells."

M. Green, D. Grandgenett, G. Gerard, H.M. Rho, M. Robins and S. Salzberg, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: "Properties of oncornavirus RNA-directed DNA polymerase, its RNA template, and its intracellular products early after infective cellular transformation."

T. Takano and M. Hatanaka, Department of Molecular Biology, NIAMDD, NIH, Bethesda, Maryland and Department of Microbiology, Keio University School of Medicine, Tokyo, Japan; Flow Laboratories, Rockville, Maryland: "DNA-RNA hybrid found in cells infected by murine leukemia virus."

M. Hill, J. Hillova, D. Dantchev, R. Mariage and M.-P. Plichon, Department of Cellular and Molecular Biology and Equipe de Recherche No. 148 du C.N.R.S., Institute of Cancerology and Immunogenetics, Villejuif, France: "Infectious viral DNA in Rous sarcoma virus (RSV)-transformed nonproducer and producer animal cells."

H.M. Temin and G.M. Cooper, McArdle Laboratory, University of Wisconsin, Madison: "Infectious DNA of avian leukosis-sarcoma and reticuloendotheliosis viruses."

J. Svoboda, I. Hlozanek, O. Mach and S. Zadrazil,* Institute of Experimental Biology and Genetics, Czechoslovak Academy of Sciences, Prague, and Institute of Haematology and Blood Transfusion, Prague; *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague: "RSV rescue from mammalian cells."

Summary: David Baltimore, Massachusetts Institute of Technology, Cambridge, Massachusetts

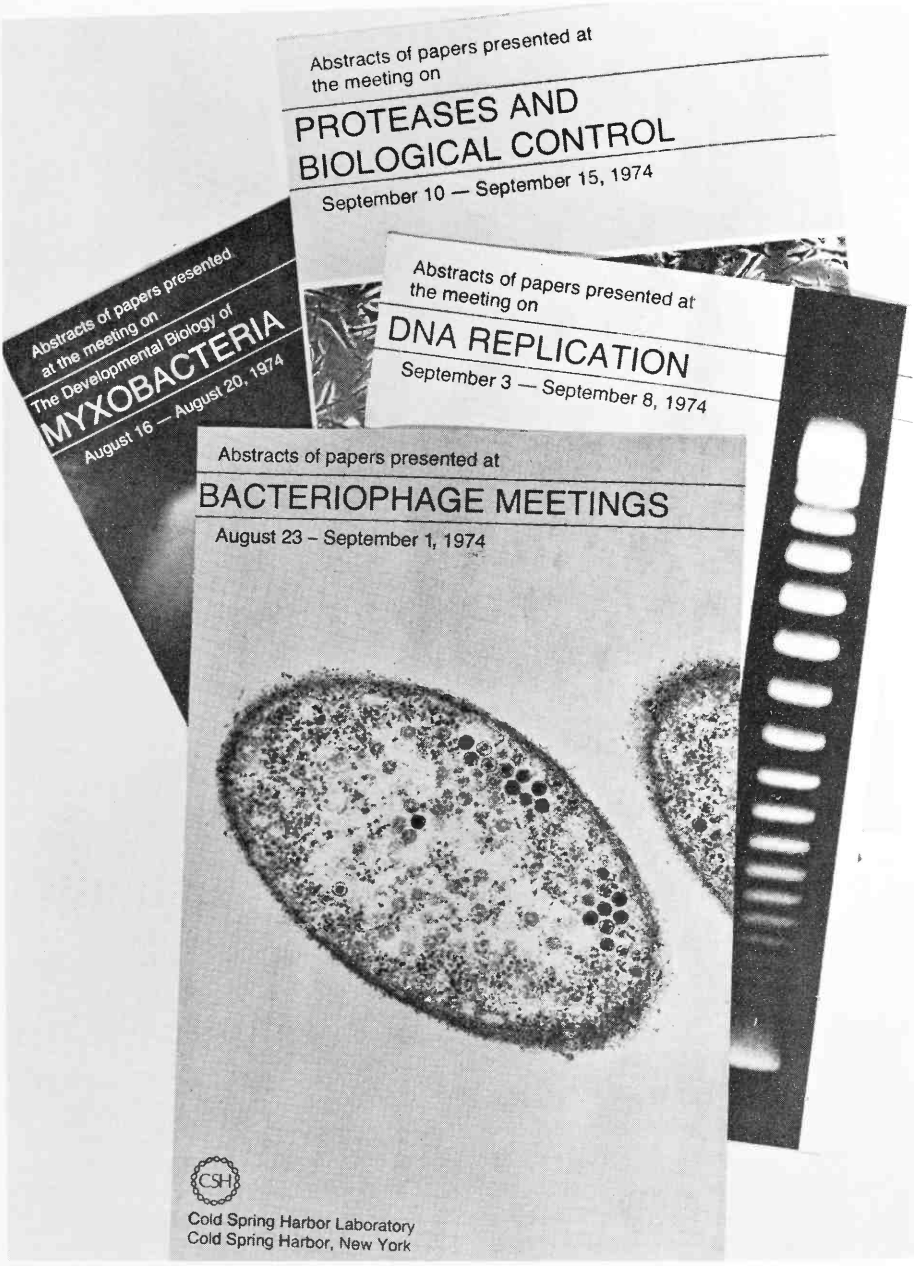


Photo by Bob Yaffee

THE DEVELOPMENTAL BIOLOGY OF MYXOBACTERIA

Arranged by
MARTIN DWORKIN, *University of Minnesota*
DALE KAISER, *Stanford University*

19 Participants

FRIDAY EVENING, August 16

Get-together

SATURDAY MORNING, August 17

H. McCurdy, University of Windsor, Ontario, Canada: "Myxobacteria: General considerations and taxonomy."
K. Gerth and H. Reichenback,* Institut für Biologie II, Fachbereich Mikrobiologie, der Universitat, Freiburg, W. Germany: "Myxospore induction in *Stigmatella aurantiaca.*"

SATURDAY EVENING, August 17

H. Kleinig, Institut für Biologie II, der Universitat, Freiburg, W. Germany: "On the regulation of carotenoid biosynthesis in myxobacteria."
R.P. Burchard, Department of Biological Sciences, University of Maryland, Catonsville, Maryland: "Studies on gliding motility in *Myxococcus xanthus.*"

SUNDAY MORNING, August 18

D. White,* R. Kottel, M. Orlowski, K. Bacon and D. Clutter, Department of Microbiology, Indiana University, Bloomington: "Capsule formation and other physiological changes during myxospore formation."
D. Filer,* S.H. Kindler and E. Rosenberg, Department of Microbiology, Tel Aviv University, Israel: "Increases in the level of enzymes associated with capsule formation during myxospore induction in *Myxococcus xanthus.*"
A. Kimchi and E. Rosenberg,* Department of Microbiology, Tel Aviv University, Israel: "DNA replication during development in *Myxococcus xanthus.*"

SUNDAY EVENING, August 18

J.H. Parish,* N.L. Brown, D.W. Morris and C. Tsopanakis, Department of Biochemistry, University of Leeds, United Kingdom: "DNA and coat proteins from bacteriophage MX-1."
D.R. Zusman* and J.M. Campos, Department of Bacteriology and Immunology, University of California, Berkeley: (1) "Isolation of a transducing phage for *Myxococcus xanthus;*" (2) "Studies of DNA replication in *Myxococcus xanthus.*"

*Indicates speaker.

MONDAY MORNING, August 19

J.H. Parish,* R.P. Burchard and N.L. Brown, Department of Biochemistry, University of Leeds, United Kingdom: "Drug resistance in *Myxococcus.*"

D. Kaiser, Department of Biochemistry, Stanford University, California: "Bacteriophage P1 infection of *M. xanthus.*"

MONDAY AFTERNOON, August 19

D.R. Zusman* and J.M. Campos, Department of Bacteriology and Immunology, University of California, Berkeley: "Regulation of fruiting body formation in *Myxococcus xanthus* by cyclic AMP."

J.W. Wireman* and M. Dworkin, Department of Microbiology, University of Minnesota Medical School, Minneapolis: "Studies of RNA and phosphate metabolism during fruiting body formation in *Myxococcus xanthus.*"

M. Dworkin, Department of Microbiology, University of Minnesota, Minneapolis: "Cell density-dependent germination of myxospores of *Myxococcus xanthus:* A simple cell interaction.

This meeting was sponsored in part by a grant from the Foundation for Microbiology to Dr. Martin Dworkin.

BACTERIOPHAGE MEETINGS

Arranged by
AHMAD BUKHARI, *Cold Spring Harbor Laboratory*
MARTHA HOWE, *Cold Spring Harbor Laboratory*

LYSOGENIC SECTION

104 Participants

FRIDAY EVENING, August 23

D.J. Mackay and V.C. Bode, Biology Division, Kansas State University, Manhattan, Kansas: "The injection of lambda DNA."

D. Court, H. Echols, G. Edlin, L. Green and R. Kudrna, Department of Molecular Biology, University of California, Berkeley; Department of Genetics, University of California, Davis: "Regulation of phage λ development with multiplicity of infection and growth rate of host cells."

A. Folkmanis, A. Chow, H. Echols, C. Garrett and A. Skalka, Department of Molecular Biology, University of California, Berkeley; Roche Institute of Molecular Biology. Nutley, New Jersey: "The need for *cro* gene function in lytic phage development."

M. Chakravorty, S. Taneja and P. Bandyopadhyaya, Molecular Biology Unit, Department of Biochemistry, Banaras Hindu University, Varanasi, India: "Role of m3 gene in the development of phage P22."

J.W. Roberts and C.W. Roberts, Department of Biochemistry, Harvard University, Cambridge, Massachusetts: "Cleavage of lambda repressor in induction."

M.M. Susskind and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Inactivation of λ repressor by P22 antirepressor."

J.R. Scott, Department of Microbiology, Emory University, Atlanta, Georgia: "Superinfection immunity and prophage repression in phage P1."

S. Flashman and M. Ptashne, Biological Laboratories, Harvard University, Cambridge, Massachusetts: "The in vitro analysis of O^c mutations in the operators of phage lambda."

SATURDAY MORNING, August 24

K. Carlson and W. Szybalski, McArdle Laboratory, University of Wisconsin, Madison: "In vitro transcription of the immunity region of coliphage lambda DNA."

R. Dottin, L. Cutler and M. Pearson, Department of Medical Genetics, University of Toronto, Canada: "Effect of *N* protein on the size of λ transcripts made in vitro."

N.C. Franklin, Department of Biological Sciences, Stanford University, California: "Interminable transcription initiated at *p*L of λ in the presence of *N* protein."

P. Tang and A. Guha, Microbiology Department, Erindale College, University of Toronto, Canada: "Non-divergent transcription of 23S and 16S ribosomal RNA species."

S. Adhya, M.E. Gottesman, B. De Crombrugghe and D.L. Court, Laboratory of Molecular Biology, NIH, Bethesda, Maryland: "Regulation in λ's *N*-operon."

D.I. Friedman and R. Ponce-Campos, Department of Microbiology, University of Michigan, Ann Arbor: "Effect of N on transcription initiating from a variety of λ promotors."

P. Ray and M. Pearson, Department of Medical Genetics, University of Toronto, Canada: "Evidence for translational control of the morphogenetic genes of λ ."

N. Sternberg and L. Enquist, NICHD, NIH, Bethesda, Maryland: "*nin, byp, pug,* and *Pas*B."

M. Sunshine,* E.W. Six,* B. Sauer,† R. Calendar,† M. Nomura** and J.B. Egan,†† *University of Iowa, Iowa City; †University of California, Berkeley; **University of Wisconsin, Madison; ††University of Adelaide, Australia: "*groW109,* possibly a ribosomal *gro* mutant for phage P2."

SATURDAY EVENING, August 24

N.E. Kleckner, Massachusetts Institute of Technology, Cambridge: "Gene expression and DNA replication under λ*N*⁻ conditions: O product acts in *cis*."

M. Lieb and M. Danciger, University of Southern California School of Medicine, Los Angeles: "Derepressed λ*N*⁻ lysogens: Prophage replication and λ puffs."

M. Greenstein and A. Skalka, Roche Institute of Molecular Biology, Nutley, New Jersey: "Lambda DNA replication: In vivo studies on the interaction between gamma protein and the host's *rec* BC nuclease."

F. Stahl, J. Crasemann and M. Stahl, Institute of Molecular Biology, University of Oregon, Eugene: "CHI mutations in λ ."

B. Malone, Institute of Molecular Biology, University of Oregon, Eugene: "The role of the CHI mutation in the SPI⁻ phenotype of λ."

R. Weisberg and N. Sternberg, NICHD, NIH, Bethesda, Maryland: "Transduction of *rec*B hosts is promoted by λ *red* function."

S. Hilliker and D. Botstein, Massachusetts Institute of Technology, Cambridge: "Comparison of P22 and λ recombination, replication and control by means of λ *immP22* hybrid phages."

J.R. Christensen, Microbiology Department, University of Rochester, New York: "Which lambda genes are involved in (1) the exclusion of phage T1 and (2) the complementation of T1 gene 4 mutants?"

M. Zabeau, J. Schell and M. Van Montagu, Laboratory of Genetics and Laboratory of Histology and Genetics, State University of Ghent, Belgium: "RAL: A nonessential function involved in the alleviation of host-controlled restriction."

SUNDAY MORNING, August 25

M.M. Howe and D. Zipser, Cold Spring Harbor Laboratory, New York: "Host deletions associated with an integrated Mu prophage."

T.F. Razzaki and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: "Events following prophage Mu induction."

M. Faelen, A. Toussaint and J. De Lafonteyne, Laboratory of Genetics, University of Brussels, Belgium: "Mu duplication after partial thermoinduction of Mu*cts* prophages incapable of autonomous replication."

A.I. Bukhari and P. De Lucia-Curtin, Cold Spring Harbor Laboratory, New York: "Temperature-sensitive mutants of Mu."

P. Van de Putte, C.A. Wijffelman, G.C. Westmaas and M. Gassler, Medical Biological Laboratory TNO, Rijswijk Z.H., and Department of Biochemistry, University of Leiden, The Netherlands: "Transcription and complementation studies of bacteriophage Mu and Mu prophage deletions."

E. Daniell,* D.E. Kohne† and J. Abelson,* *Department of Chemistry, University of California, San Diego, La Jolla; †Scripps Clinic and Research Foundation, La Jolla, California: "Characterization of the inhomogeneous DNA in virions of bacteriophage Mu by DNA reannealing kinetics."

D. Kamp, Institut für Genetik der Universitat Koln, Cologne, W. Germany: "Isolation of plaque-forming λ-Mu hybrid phages."

A.I. Bukhari, B. Allet, D. Kamp and M. Howe, Cold Spring Harbor Laboratory, New York: "Plaque-forming λ-Mu hybrids."

B. Allet and A. Bukhari, Cold Spring Harbor Laboratory, New York: "Identification of λ and Mu DNA in λ-Mu plaque-forming hybrids."

SUNDAY EVENING, August 25

L.W. Enquist and R.A. Weisberg, NICHD, NIH, Bethesda, Maryland: "A method for isolation, characterization and mapping of integration and excision defective mutants of lambda."

D. Freifelder, N. Baran, L. Chud, A. Folkmanis and E.E. Levine, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: "Requirements for insertion of λ DNA into the DNA of *E. coli.*"

D. Freifelder and E.E. Levine, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: "Formation of polylysogens during infection of *E. coli* by λ."

R.L. Roehrdanz, R. Purcell and D. Schultz, McArdle Laboratory, University of Wisconsin, Madison: "Lambda *sif*, a gene whose product inhibits site-specific recombination."

S. Gottesman and M.E. Gottesman, NIH, Bethesda, Maryland: "Site-specific recombination in vitro."

H.A. Nash, NIMH, NIH, Bethesda, Md.: "Integrative combination in vitro."

MONDAY MORNING, August 26

M.M. Gottesman and J.L. Rosner, NIAMDD, NIH, Bethesda, Maryland: "The acquisition and loss of a determinant for chloramphenicol resistance by coliphage λ."

L. Mac Hattie and J. Jackowski, Departments of Medical Genetics and Medical Biophysics, University of Toronto, Canada: "Insertion/deletion heterogeneity in the DNA of λ pcam and its progeny."

D.L. Brown and A. Campbell, Department of Biological Sciences, Stanford University, California: "Induction of deletions in λ-att2."

K. Kylberg and H. Drexler, Department of Microbiology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: "Time of production of T1 transducing particles."

J.L. Rosner, NIAMDD, NIH, Bethesda, Maryland: "P1-pro: An oversized P1 prophage."

G. Weinstock and D. Botstein, Massachusetts Institute of Technology, Cambridge: "The fate of lambda parental DNA."

D.G. Ross and D. Freifelder, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: "Maturation of a single λ phage from a dimeric λ DNA circle."

N. Sternberg and R. Weisberg, NICHD, NIH, Bethesda, Maryland: "Particles produced by an excision defective lysogen."

MORPHOGENESIS SECTION

83 Participants

MONDAY EVENING, August 26

J. Goldstein and S.P. Champe, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey: "Gene-dependence of T4 proteolytic cleavage in vitro."

A. Tsugita and M. Showe, Department of Microbiology, Biozentrum, der Universitat Basel, Switzerland: "Phage T4 head-gene proteins: Purification, characterization and in vitro cleavage."

U.K. Laemmli and S.F. Quittner, Department of Biochemical Sciences, Princeton University, New Jersey: "The proteins of the tubular polyhead of phage T4 and in vitro cleavage of the head proteins."

L. Onorato and M. Showe, Department of Biology, Haverford College, Pennsylvania: "Characterization of T4 23*ts* 'crummy heads' and the protease activity associated with them."

M. Woolf, G. Pruss and D. Bowden, Department of Molecular Biology, University of California, Berkeley: "In vitro cleavage of phage P2 head proteins."

R. Hendrix, Department of Biophysics and Microbiology, University of Pittsburgh, Pennsylvania: "Protein processing in petit λ assembly."

P. Ray, L. Jara and H. Murialdo, Department of Medical Genetics, University of Toronto, Canada; Faculty of Sciences, University of Chile, Santiago: "The role of gene Nu3 in λ morphogenesis."

D.A. Shub and D.H. Smith, Department of Biological Sciences, State University of New York, Albany: "Cleavage of SP01 head protein precursor."

TUESDAY MORNING, August 27

W. Earnshaw,* S. Casjens* and S. Harrison,† *Department of Biology, Massachusetts Institute of Technology, Cambridge; †Gibbs Laboratory, Harvard University, Cambridge, Massachusetts: "Structure of P22 head intermediates."

L.D. Simon,* D. Snover* and A.H. Doermann,† *Institute for Cancer Research, Philadelphia, Pennsylvania; †University of Washington, Seattle: "A bacterial mutation affecting T4 phage DNA synthesis and tail production."

J.R. Paulson and U.K. Laemmli, Department of Biochemical Sciences, Princeton University, New Jersey: "The structure of the core of T4 polyheads."

L.D. Simon, Institute for Cancer Research, Philadelphia, Pennsylvania: "T4 polyheads."

M.E. Bayer* and D.J. Cummings,† *Institute for Cancer Research, Philadelphia, Pennsylvania; †Department of Microbiology, University of Colorado Medical Center, Denver: "The surface morphology of T4 'lollipops.' "

R.W. Bolin and D.J. Cummings, Department of Microbiology, University of Colorado Medical Center, Denver: "The mechanism of T4 head length determination: The induction of lollipop monster phage."

R.K.L. Bijlenga and E. Kellenberger, Department of Microbiology, Biozentrum, der Universitat Basel, Switzerland: "Giant T4 phage with *ts* mutants in gene 24 grown at intermediate temperature."

H.-W. Ackermann, L. Berthiaume, S. Sonea and S. Kasatiya, Department of Microbiology, Faculty of Medicine, Laval University, Quebec; Institute of Microbiology and Hygiene of Montreal, Ville-de-Laval; University of Montreal Department of Microbiology, Montreal; Quebec Department of Social Affairs, Ville-de-Laval, Canada: "Malformations in group A staphylococcus phages."

TUESDAY EVENING, August 27

P.D. Sadowski and M.J. Holland, Department of Medical Genetics, University of Toronto, Canada: "The proheads of bacteriophage T7."

M. Syvanen, D. Kaiser and T. Masuda, Department of Biochemistry, Stanford University School of Medicine, California: "Packaging of phage λ DNA into head protein."

T. Hohn and B. Hohn, Department of Microbiology, Biozentrum, der Universitat Basel, Switzerland: "The prehead-head transition in bacteriophage λ ."

P. Dawson,* A. Skalka* and L. Simon,† *Roche Institute of Molecular Biology, Nutley, New Jersey; †Institute for Cancer Research, Philadelphia, Pennsylvania: "Lambda head morphogenesis: Studies on the role of DNA."

U.K. Laemmli, Department of Biochemical Sciences, Princeton University, New Jersey: "A possible DNA packaging mechanism for phage T4."

J. King and S. Casjens, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Coupling between protein synthesis and morphogenesis in P22."

WEDNESDAY MORNING, August 28

A. Becker and M. Gold, Department of Medical Genetics, University of Toronto, Canada: "Isolation of the phage λ A-gene product."

N. Sternberg and R. Weisberg, NICHD, NIH, Bethesda, Maryland: "Particles produced by an excision defective lysogen."

N. Sternberg, NICHD, NIH, Bethesda, Maryland: "The regulation of *W* protein activity."

D.K. Chattoraj and R.B. Inman, Biophysics Laboratory and Biochemistry Department, University of Wisconsin, Madison: "Location of DNA ends in P2, 186, P4 and λ bacteriophage heads."

E.C.-T. Wong and V.C. Bode, Biology Division, Kansas State University, Manhattan, Kansas: "Morphogenetic implications of abnormal DNA in FII mutant heads."

R.G. Shea, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Two proteins necessary for P22 DNA injection."

WEDNESDAY AFTERNOON, August 28

M. Salas, A. Camacho, J.L. Carrascosa and E. Viñuela, Department of Molecular Biology, Instituto G. Marañón, Madrid, Spain: "Morphogenesis of *Bacillus subtilis* bacteriophage GE φ29."

D. Anderson, E. Hagen, C. Peterson, B. Reilly, C. Schachtele and M. Tosi, University of Minnesota, Minneapolis: "Phage φ29 morphogenesis."

D.A. Marvin, E.J. Wachtel and R.L. Wiseman, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "Molecular structure of filamentous bacterial viruses."

Y. Kikuchi and J. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: "T4 baseplate assembly."

P.G. Arscott and E.B. Goldberg, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: "Tail fiber action on the baseplate of bacteriophage T4."

L.M. Kozloff and L.K. Crosby, Department of Microbiology, University of Colorado Medical School, Denver: "T4D-induced dihydrofolate reductase (dfr) and thymidylate synthetase (td) as capsid tail plate proteins."

W.B. Wood and M.P. Conley, Division of Biology, California Institute of Technology, Pasadena: "Role of the whiskers in T4 tail fiber attachment."

THURSDAY MORNING, August 29

J.W. Jarvik and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Genetic analysis of protein interactions in P22."

K. Kennedy, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "Temperature-sensitive mutants of bacteriophage PM2."

D. Henderson and J. Weil, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "Bacterial mutations affecting morphogenesis of lambda deletion mutants."

L.D. Simon, D. Snover, T. McLaughlin and C. Grisham, Institute for Cancer Research, Philadelphia, Pennsylvania: "A membrane alteration in an *E. coli* mutant that blocks T4 head assembly."

LYTIC SECTION

65 Participants

THURSDAY EVENING, August 29

J. Olah and W. Sauerbier, Department of Biophysics and Genetics, University of Colorado Medical Center, Denver: "Transcriptional organization of bacteriophage T4 transfer RNAs."

A.R. Brautigam and W. Sauerbier, Department of Biophysics and Genetics, University of Colorado Medical Center, Denver: "Physical mapping of the in vivo transcription units of bacteriophage T7."

C. Pachl and T. Young, Department of Biochemistry, University of Washington, Seattle: "Preparative polyacrylamide gel electrophoresis of T7 mRNA."

E.G. Niles and R. Condit, Departments of Therapeutic Radiology and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "T7 RNA polymerase transcribes the total late region of the T7 DNA in vitro.

K. Hercules and W. Sauerbier, University of Colorado Medical Center, Denver: "Decay of T7 early mRNA species."

FRIDAY MORNING, August 30

R.N.H. Konings, Laboratory of Molecular Biology, University of Nijmegen, The Netherlands: "Transcription and translation of purified genes and gene fragments of bacteriophage M13 DNA in vitro."

S. Clark, R. Losick and J. Pero, Biological Laboratories, Harvard University, Cambridge, Massachusetts: "A new RNA polymerase from phage PBS2-infected *Bacillus subtilis.*"

A.R. Price, Department of Biological Chemistry, University of Michigan, Ann Arbor: "Effects of inhibitors on macromolecular synthesis after PBS2 phage infection."

J. Segall, R. Tjian and R. Losick, Biological Laboratories, Harvard University, Cambridge, Massachusetts: "A novel effect of chloramphenicol on the rate of phage ϕe transcription in sporulating *Bacillus subtilis.*"

M.L. Walsh and P.S. Cohen, Department of Microbiology and Biophysics, University of Rhode Island, Kingston: "Deoxynucleotide kinase polysomes."

B. Singer, MCD Biology, University of Colorado, Boulder: "An interesting mutation which affects rIIB translation."

M. Van Montagu, W. Van Assche and J. Vanderkerckhove, Laboratory of Histology and Genetics, State University of Ghent, Belgium: "Spontaneous missense mutants of RNA bacteriophage MS2."

FRIDAY EVENING, August 30

H.R. Warner, R.F. Drong and S. Berget, Department of Biochemistry, University of Minnesota, St. Paul: "Metabolism of deoxyribonucleotides produced by T5-induced degradation of *E. coli* DNA."

M.S. Center, Division of Biology, Kansas State University, Manhattan, Kansas: "Studies on an endonuclease activity associated with the T7 DNA-membrane complex."

C. Mickelson and J.S. Wiberg, Department of Biology and Department of Radiation Biology and Biophysics, University of Rochester, New York: "Partial characterization of T4 gene 46 and 47 proteins."

J.B. Flanegan, P.K. Tomich, C-S. Chiu and G.R. Greenberg, Department of Biological Chemistry, University of Michigan, Ann Arbor: "Evidence that the T4 DNA replication complex regulates the biosynthesis of deoxyribonucleotides."

SATURDAY MORNING, August 31

R.F. Ramig and C.D. Yegian, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder; Department of Cell Biology, Albert Einstein College of Medicine, New York: "Determinants of localized high recombination in T4."

G. Mosig, A. Breschkin, R. Dannenberg and G. Garcia, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "Mutual suppression of gene 32 and rII mutations of phage T4."

J.S. Kotval, T.V. Potts and J.R. Christensen, Department of Microbiology, University of Rochester, New York: "Bacteriophage T1 recombination as it overcomes prophage-P1 restriction."

R. Kahmann, Max-Planck-Institut for Molekulare Genetik, Berlin, W. Germany: "Transfection in *Bacillus subtilis.*"

K. Horiuchi, G.F. Vovis, V. Enea and N.D. Zinder, The Rockefeller University, New York: "Physical mapping of the B-specific methylation sites of bacteriophage F1."

G.F. Vovis, K. Horiuchi and N.D. Zinder, The Rockefeller University, New York: "Restriction and modification of bacteriophage F1 heteroduplex DNA by endo R·*Eco*B."

SATURDAY EVENING, August 31

D. Montgomery, R.J. Frederick and L. Snyder, Department of Microbiology, Michigan State University, East Lansing: "RNA polymerase mutants and the role of T4 gene products in replication and transcriptional regulation."

A.W. Senear and J.A. Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "Specific RNA: Protein interactions in the replication of $Q\beta$ RNA."

A. Rodriguez and J. Weil, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "Mutants in a nonessential gene of T4 and a conditional lethal host mutant which restricts them."

G.S. Gill and L.A. Mac Hattie, Departments of Medical Genetics and Biophysics, University of Toronto, Canada: "Uniqueness of the extruded end of DNA in a DNA-ghost complex of coliphage T1."

SUNDAY MORNING, September 1

C.C. Pao and J.F. Speyer, University of Connecticut, Storrs: "Mutants affecting the development of phage T7."

R.C. Condit and J.A. Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: "Female-specific phage restriction: T7 RNA and protein synthesis in vivo and in a coupled in vitro system."

A.F. Morgan and D.J. Mc Corquodale, Division of Biology, University of Texas at Dallas: "Colicinogenic factor Ib (Col Ib) mediated arrest of bacteriophage BF23."

DNA REPLICATION

Arranged by
BRUCE ALBERTS, *Princeton University*
CHARLES RICHARDSON, *Harvard Medical School*

156 Participants

TUESDAY EVENING, September 3

Chairman: Friedrich Bonhoeffer, Max-Planck Institut für Virusforschung, Tubingen, Germany

D.A. Glaser, C.T. Wehr, C. Sevastopoulos, M. Konrad, J.L. Couch and J. Raymond Department of Molecular Biology, University of California, Berkeley: "Cold and heat sensitive DNA mutants of *E. coli.*"

E.B. Konrad, J.W. Thorner, D.C. Eichler and I.R. Lehman, Department of Biochemistry, Stanford University Medical Center, California: "Isolation and characterization of *E. coli* mutants with retarded joining of DNA replicative intermediate fragments."

P.F. Schendel, Department of Biochemistry, University of Wisconsin, Madison: "Genetic and biochemical analysis of the *dnaB* complementation group."

K.G. Lark* and J.A. Wechsler,† *Department of Biology, University of Utah, Salt Lake City; †Department of Biology, Columbia University, New York: "DNA replication in *dnaB* mutants of *E. coli:* Gene product interaction and synthesis of 4S pieces."

W.H. Kurtz and J.A. Wechsler, Department of Biological Sciences, Columbia University, New York: "Rifampicin-sensitive linear DNA synthesis in a *dnaE* mutant at the nonpermissive temperature."

H.E.D. Lane and D.T. Denhardt, Department of Biochemistry, McGill University, Montreal, Canada: "Altered replication of the chromosome of an *Escherichia coli rep* mutant."

J.-P. Merile* and Y. Hirota,† *Institut Pasteur, Paris, France: †National Institute of Genetics, Mishima, Japan: "*E. coli* factors essential for FDNA replication."

WEDNESDAY MORNING, September 4

Chairman: Lawrence Grossman, Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts

R. Bird, J. Louarn, M. Funderburgh and L. Caro, Department de Biologie Moleculaire, Universite de Geneve, Switzerland: "A more precise mapping of the replication origin of *Escherichia coli* K12."

L. Caro, R.E. Bird and M. Chandler, Department de Biologie Moleculaire, Universite de Geneve, Switzerland: "Chromosome replication in *E. coli dnaA* integratively suppressed by R or F factors."

A. O'Sullivan and K. Howard, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: "Location of a unique replication terminus and genetic evidence for partial bidirectional replication in the *Bacillus subtilis* chromosome."

N. Sueoka and J. Hammers, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: "Isolation of DNA-membrane complex in *Bacillus subtilis.*"

R.G. Wake, P. Upcroft, H. Callister and H.J. Dyson, Biochemistry Department, Sydney University, Australia: "Initiation and termination of chromosome replication in *B. subtilis.*"

S. Riva, Laboratorio di Genetica Biochimica ed Evoluzionistica del C.N.R., Pavia, Italy: "A new *Bacillus subtilis* mutant temperature sensitive in the initiation of DNA replication."

O. Pierucci and C.E. Helmstetter, Roswell Park Memorial Institute, Buffalo, New York: "Chromosome replication and the division cycle of *E. coli* and *B. megaterium.*"

WEDNESDAY EVENING, September 4

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

R. Sternglanz, H.F. Wang and J.J. Donegan, Department of Biochemistry, State University of New York, Stony Brook: "Hybridization studies with nascent DNA chains from *Bacillus subtilis* and from T7-infected *Escherichia coli.*"

B. Olivera, R. Lundquist, P. Fernandez, D. Hillyard and F. Bonhoeffer,* Department of Biology, University of Utah, Salt Lake City; *Max-Planck Institut für Virusforschung, Tubingen, Germany: "Discontinuous DNA synthesis in vitro."

R. Werner, Department of Biochemistry, University of Miami School of Medicine, Florida: "Replication by crystallization."

M. Anwar Waqar and J.A. Huberman, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Nucleotide sequences at the junction between RNA and nascent DNA in the slime mold, *Physarum polycephalum.*"

J.H. Taylor, L.C. Erickson and J.C. Hozier, Institute of Molecular Biophysics, Florida State University, Tallahassee: "Units of replication in chromosomal DNA."

B. Tseng, W. Oertel, J. Mendelsohn and M. Goulian, Department of Medicine, University of California, San Diego, La Jolla: "DNA synthesis in human lymphocytes."

W. Messer and R. Schindler-Tippe, Max-Planck Institut für Molekulare Genetik, Berlin, W. Germany: "Transcriptional control of DNA replication in *E. coli* isolation of initiation RNA (1-RNA)."

THURSDAY MORNING, September 5

Chairman: G. Robert Greenberg, Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan

D.M. Livingston and C.C. Richardson, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: "DNA polymerase III of *E. coli*."

W. Wickner and A. Kornberg, Department of Biochemistry, Stanford University Medical Center, California: "Novel holoenzyme forms of RNA polymerase and DNA polymerase in replication."

C.L. Smith and R.E. Moses, Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas: "DNA polymerase II activity in *mutTI E. coli*."

I.J. Molineux and M.L. Gefter, Massachusetts Institute of Technology, Cambridge: "The DNA binding (unwinding) protein of *E. coli:* Its properties and mode of action."

F.D. Gillin and N.G. Nossal, National Institutes of Health, Bethesda, Maryland: "Wild type of mutant T4 DNA polymerases and the T4 gene 32 DNA unwinding protein"

A.J. Levine, P.C. van der Vliet, J. Rabek and B. Rosenwirth, Department of Biochemical Sciences, Princeton University, New Jersey: "Adenovirus-specific single-stranded DNA binding proteins."

THURSDAY EVENING, September 5

Chairman: Gisela Mosig, Department of Molecular Biology, Vanderbilt University Nashville, Tennessee

J.E. Clements, J.D'Ambrosio and N.C. Brown, Department of Biochemistry, University of Massachusetts Medical School, Worcester: "Inhibition of *B. subtilis* DNA polymerase III by phenylhydrazinopyrimidines: Demonstration of a drug-induced DNA enzyme complex."

N.R. Cozzarelli, R.L. Low, S.A. Rashbaum and C.L. Coulter, Departments of Biochemistry and Anatomy, University of Chicago, Illinois: "Mechanism of inhibition of *Bacillus subtilis* DNA polymerase III by arylhydrazinopyrimidines."

J. Andersen and A.T. Ganesan, Department of Genetics, Stanford University School of Medicine, California: "Nascent DNA fragments in *Bacillus subtilis* and the role of polymerase III."

L.A. Loeb, Fox Chase Institute for Cancer Research, Philadelphia, Pennsylvania: "On the mechanism of catalysis by DNA polymerases."

L.M.S. Chang, Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut: "The distributive nature of enzymatic DNA synthesis."

W.D. Sedwick, T.S.F. Wang and D. Korn, Stanford University, California: "Structure and properties of the cytoplasmic DNA polymerase from human cells."

S. Spadari and A. Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey: "RNA- and DNA-primed DNA synthesis in vitro by HeLa cell DNA polymerases."

FRIDAY MORNING, September 6

Chairman: Karl Lark, Department of Biology, University of Utah, Salt Lake City, Utah

J. Tomizawa* and Y. Sakakibara,† *National Institutes of Health, Bethesda, Maryland; †National Institute of Health, Tokyo, Japan: "Replication of colicin E1 plasmid DNA in cell extracts."

M. Lovett, L. Katz, D. Guiney, D. Blair, J. Kupersztoch and D. Helinski, Department of Biology, University of California, San Diego, La Jolla: "Studies on the role of relaxation complex in plasmid DNA replication."

S.M. Jazwinski and A. Kornberg, Department of Biochemistry, Stanford University, California: "Gene H spike protein of ϕX174: Functions in adsorption, replication and transfection."

B. Francke, The Salk Institute, San Diego, California: "In vitro polyoma DNA synthesis: Requirement for cytoplasmic factors."

L.F. Liu and J.C. Wang, Chemistry Department, University of California, Berkeley: "DNA synthesis on covalently closed double-stranded template."

J.J. Champoux and B.L. McConaughy, Department of Microbiology, University of Washington, Seattle: "Priming of superhelical SV40 DNA by *E. coli* RNA polymerase for DNA synthesis in vitro."

W. Keller, Cold Spring Harbor Laboratory, New York: "Direct determination of the number of superhelical turns in SV40 DNA using a DNA relaxing protein from tissue culture cells."

FRIDAY EVENING, September 6

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

R. Schekman, A. Weiner and A. Kornberg, Department of Biochemistry, Stanford University Medical Center, California: "Enzymes of ϕX174 replicative form synthesis."

S. Wickner* and J. Hurwitz,† *National Institutes of Health, Bethesda, Maryland; †Albert Einstein College of Medicine, New York: "ϕX174 DNA replication catalyzed by purified *E. coli* proteins."

E. Milewski, P. Forterre and M. Kohiyama, Institut de Biologie Moleculaire, Universite Paris, France: "Isolation of factors involved in in vitro DNA synthesis."

T. Kornberg, A. Lockwood and A. Worcel, Department of Biochemistry, Princeton University, New Jersey: "Replication of the *Escherichia coli* chromosome with a soluble enzyme system."

P. Boerner, C. Kemper and D.W. Smith, Department of Biology, University of California, San Diego, La Jolla: "Stimulation of ATP-dependent in vitro DNA replication by factors from the periplasmic space of *E. coli.*"

S. Hayes and W. Szybalski, McArdle Laboratory, University of Wisconsin, Madison: "Role of the *oop* RNA in initiation of coliphage λ DNA replication: A *cis* effect of the *O* gene product."

SATURDAY MORNING, September 7

Chairman: Noboru Sueoka, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado

C.C. Richardson, D.C. Hinkle, W.E. Masker and P.L. Modrich, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: "Bacteriophage T7 DNA replication in vitro."

C.F. Morris, D. Mace, N. Sinha, M. Bittner, L. Moran and B. Alberts, Department of Biochemical Sciences, Princeton University, New Jersey: "Studies on in vitro DNA synthesis using purified T4 bacteriophage replication proteins."

C.-S. Chiu, J.B. Flanegan, G.R. Greenberg, P.K. Tomich and M.G. Wovcha, Department of Biological Chemistry, University of Michigan, Ann Arbor: "The nature of the complex of enzymes synthesizing deoxyribonucleotides and DNA after phage T4 infection."

R. Dannenberg, A. Breschkin and G. Mosig, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: "On the roles of phage and host genes in T4 DNA replication and recombination."

A.W. Kozinski, Department of Human Genetics, University of Pennsylvania, Philadelphia: "Complete and repetitive DNA replication of T4 bacteriophage with incomplete, short, genome."

R.C. Miller, Jr.* and L.A. McNicol,† *University of British Columbia, Vancouver, Canada; †University of Pennsylvania, Philadelphia: "Biological activity of T4 DNA synthesized in *E. coli* treated with toluene."

SATURDAY AFTERNOON, September 7

Chairman: Jerome Vinograd, The Chemical Laboratories, California Institute of Technology, Pasadena, California

J. Chen and D.S. Ray, Department of Biology and Molecular Biology Institute, University of California, Los Angeles: "M13 DNA replication in a mutant of *E. coli* defective in the $5' \rightarrow 3'$ exonuclease associated with polymerase I."

S. Eisenberg and D.T. Denhardt, Department of Biochemistry, McGill University, Montreal, Canada: "Location and distribution of gaps in ϕX174 RF II DNA isolated at the time of RF replication."

Y. Sugino and J. Tomizawa, National Institutes of Health, Bethesda, Maryland: "RNA sequences covalently joined to colicin E1 plasmid DNA."

R. Rownd, D. Perlman, D. Womble, N. Goto, T. Twose and E. Appelbaum, Laboratory of Molecular Biology and Department of Biochemistry, University of Wisconsin, Madison: "Structure and replication of R factor DNA."

D.B. Clewell, Y. Yagi and B. Bauer, University of Michigan, Ann Arbor: "Plasmid determined tetracycline resistance in *Streptococcus faecalis:* Evidence for a gene amplification process occurring during cell growth in the presence of tetracycline."

N.D.F. Grindley, L.A. Metlay, R.W. Kolter and W.S. Kelly, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: "Effects of DNA polymerase I on plasmid replication."

SUNDAY MORNING, September 8

Chairman: Lucien Caro, Department of Molecular Biology, University of Geneva, Geneva, Switzerland

V.L. Mackay and S.M. Linn, Department of Biochemistry, University of California, Berkeley: "In vitro studies of the *recBC* DNase of *E. coli.*"

L. Grossman, Biology Department, Brandeis University, Waltham, Massachusetts: "A model of repair involving *rec* and *pol* gene products."

J.D. McGhee, D.E. Jensen and P.H. Von Hippel, Institute of Molecular Biology, University of Oregon, Eugene: "Studies of the interactions of 'melting proteins' with DNA."

G. Herrick, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: "Hypotrichous ciliate macronuclear DNA structure."

P.H. Weigel and P.T. Englund, Department of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland: "Inhibition of *E. coli* DNA synthesis by KCN, CO and dibromophenol."

M.E. Furth* and S. Takahaski,† *McArdle Laboratory and †Biophysics Laboratory and Biochemistry Department, University of Wisconsin, Madison: "Transition from early to late mode of λ DNA replication in a recombination-deficient system."

J.-R. Wu and Y.-C. Yeh, Department of Biochemistry, University of Arkansas Medical Center, Little Rock: "Replicative intermediate of *dar,* a late gene of phage T4, involved in DNA synthesis."

This meeting was supported in part by a grant from the National Cancer Institute, National Institutes of Health.

PROTEASES AND BIOLOGICAL CONTROL

Arranged by
EDWARD REICH, *The Rockefeller University*
DANIEL RIFKIN, *The Rockefeller University*
ELLIOTT SHAW, *Brookhaven National Laboratory*

180 Participants

TUESDAY EVENING, September 10

Chairman: Robert M. Stroud, California Institute of Technology, Pasadena, California

K.A. Walsh, Department of Biochemistry, University of Washington, Seattle: "Unifying concepts among proteases."

J.S. Fruton, Kline Biology Tower, Yale University, New Haven, Connecticut: "the specificity of proteinases toward protein substrates."

H. Neurath, Department of Biochemistry, University of Washington, Seattle: "Limited proteolysis and zymogen activation."

WEDNESDAY MORNING, September 11

Chairman: Laszlo Lorand, Northwestern University, Evanston, Illinois

E.W. Davie, K. Fujikawa, M.E. Legaz and H. Kato, Department of Biochemistry, University of Washington, Seattle: "Role of proteases in blood coagulation."

C.M. Jackson, C.T. Esmon and W.G. Owen, Department of Biochemistry, Washington University, St. Louis, Missouri: "Activation of bovine prothrombin."

J. Stenflo,* P. Fernlund* and P. Roepstorff,† *Department of Clinical Chemistry, University of Lund, Malmo, Sweden; †Danish Institute of Protein Chemistry, Horsholm, Denmark: "Vitamin K-dependent modifications of glutamic acid residues in prothrombin."

S. Magnusson, L. Sottrup-Jensen, T.E. Petersen and H. Claeys, Department of Molecular Biology, University of Aarhus, Aarhus, Denmark: "Complete primary structure of prothrombin."

J.E. Folk and S.I. Chung, National Institutes of Health, Bethesda, Maryland: "Blood coagulation factor XIII: Relationship of some biological properties to subunit structure."

J. Jesty, J. Maynard, R. Radcliffe, S. Silverberg, F. Pitlick and Y. Nemerson, Yale University School of Medicine, New Haven, Connecticut: "Initiation and control of the extrinsic pathway of blood coagulation."

C.G. Cochrane, R.J. Ulevitch and S.D. Revak, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: "The activation of Hageman factor (clotting factor XII) by nonenzymatic and enzymatic mechanisms."

WEDNESDAY EVENING, September 11

Chairman: Abraham Osler, Public Health Research Institute, New York, New York

J. Pisano, National Heart and Lung Institute, Bethesda, Maryland: "Introduction to the chemistry and biology of the kallekrein-kinin system."

E. Muller-Eberhard, Scripps Clinic and Research Foundation, La Jolla, California.

T.E. Hugli, Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California: "Serum anaphylatoxins: Formation, characterization and control."

O. Götze, Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California: "Proteases of the properdin system."

S.C. Kinsky and H.R. Six, Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri: "A model for the lytic action of complement."

THURSDAY MORNING, September 12

Chairman: Kenneth C. Robbins, Michael Reese Research Foundation, Chicago, Illinois

K.C. Robbins, L. Summaria and G.H. Barlow, Michael Reese Research Foundation, Chicago, Illinois; Abbott Laboratories, North Chicago, Illinois: "Activation of plasminogen."

P. Wallen and B. Wiman, Department of Physiological Chemistry, University of Umea, Sweden: "On the generation of intermediate plasminogen and its significance for the activation."

E. Reich, The Rockefeller University, New York: "Tumor-associated fibrinolysis."

G.H. Barlow, A. Rueter and I. Tribby, Experimental Biology Division, Abbott Laboratories, North Chicago, Illinois: "Production of plasminogen activator by tissue culture techniques."

THURSDAY EVENING, September 12

Chairman: Michael Laskowski, Jr., Purdue University, Lafayette, Indiana

N. Heimburger, Behringwerke AG, Marburg/Lahn, W. Germany: "Proteinase inhibitors of human plasma: Their properties and control functions."

P.C. Harpel, M. Mosesson and N. Cooper, New York Hospital-Cornell Medical Center, New York; Downstate Medical Center, Brooklyn, New York; Scripps Clinic and Research

Foundation, La Jolla, California: "Studies of the structure and function of α_2-macroglobulin and C1 inactivator."

J.-O. Jeppsson and C.-B. Laurell, Department of Clinical Chemistry, University of Lund, Malmo, Sweden: "Function and chemical composition of α_1-antitrypsin."

T. Aoyagi and H. Umezawa, Institute of Microbial Chemistry, Tokyo, Japan: "Structure and activities of protease inhibitors of microbial origin."

E. Shaw, Biology Department, Brookhaven National Laboratory, Upton, New York: "Synthetic protease inhibitors by affinity labeling."

FRIDAY MORNING, September 13

Chairman: Christian De Duve, The Rockefeller University, New York, New York

A.J. Barrett, Tissue Physiology Department, Strangeways Research Laboratory, Cambridge, England: "Lysosomal proteinases."

Z.A. Cohn, The Rockefeller University, New York: "The role of proteases in macrophage physiology."

J.C. Unkeless* and S. Gordon,† *Massachusetts Institute of Technology, Cambridge; †Oxford University, England: "Secretion of plasminogen activator by mouse macrophages."

R.T. Schimke and M.O. Bradley, Department of Biological Sciences, Stanford University, California: "On the turnover of intracellular proteins."

D.F. Steiner, W. Kemmler and H.S. Tager, Department of Biochemistry, University of Chicago, Illinois: "Proteolytic mechanisms in the biosynthesis of polypeptide hormones."

W.R. Dayton, D.E. Goll, W.J. Reville, M.G. Zeece and M.H. Stromer, Muscle Biology Group, Iowa State University, Ames: "Possible significance of a Ca^{2+}-activated protease in myofibrillar protein turnover in skeletal muscle."

FRIDAY EVENING, September 13

Chairman: Zanvil Cohn, The Rockefeller University, New York, New York

P. Bornstein and J.M. Davidson, Department of Biochemistry, University of Washington, Seattle: "Biosynthesis of procollagen and its conversion to collagen by limited proteolysis."

J. Gross and E. Harper, Developmental Biology Laboratory, Massachusetts General Hospital, Boston; Harvard Medical School, Boston, Massachusetts: "The animal collagenases."

K. Ohlsson, Department of Clinical Chemistry and Surgery, University of Lund, Malmo, Sweden: "Granulocyte collagenase and elastase and their interactions with α_1-antitrypsin and α_2-macroglobulin."

J. Janoff, J. Blondin, R.A. Sandhaus, A. Mosser and C. Malemud, Department of Pathology, State University of New York, Stony Brook: "Human neutrophil elastase: Physiological and pathological actions."

B.D. Korant, Central Research Department, DuPont Co., Wilmington, Delaware: "Regulation of animal virus replication by protein cleavage."

U. Laemmli, Princeton University, New Jersey: "Proteolytic cleavage associated with the maturation of the head of bacteriophage T4."

(A) SATURDAY MORNING, September 14

Chairman: H.G. Williams-Ashman, University of Chicago, School of Medicine, Chicago, Illinois

L.J.D. Zaneveld,* K.L. Polakoski† and G.F.B. Schumacher,** *Population Research Center, IIT Research Institute, Chicago, Illinois; †Department of Obstetrics and Gynecology, University of Chicago, Illinois: "The proteolytic enzyme system of male and female genital tract secretions and spermatozoa."

L.L. Espey, Department of Environmental Studies, Trinity University, San Antonio, Texas: "Evaluation of proteolytic activity in mammalian ovulation."

H. Fritz, Institut fur Klinische Chemie and Klinische Biochemie der Universitat Munchen, W. Germany: "Boar, bull and human sperm acrosin—Isolation, properties and biological aspects."

(B) SATURDAY MORNING, September 14

Chairman: Harry Eagle, Albert Einstein College of Medicine, New York, New York

R.W. Holley, The Salk Institute, San Diego, California: "Factors that control the growth of 3T3 cells and transformed 3T3 cells."

H.-P. Schnebli, Friedrich Miescher Institute, Basel, Switzerland: "The effects of protease inhibitors on cells in vitro."

D.D. Cunningham, Department of Medical Microbiology, University of California, Irvine: "Effect of proteases on concanavalin A-specific agglutinability and proliferation of density-inhibited fibroblasts."

SATURDAY AFTERNOON, September 14

Chairman: Robert W. Holley, The Salk Institute, San Diego, California

J.K. Christman and G. Acs, Department of Pediatrics, Mt. Sinai School of Medicine, New York, New York: "Plasminogen activator: Purification, biological properties and correlation with tumorigenicity."

D.B. Rifkin and L. Beal, The Rockefeller University, New York: "Control of plasminogen activator synthesis."

A.R. Goldberg, B.A. Wolf, P.A. Lefebvre and S.G. Lazarowitz, The Rockefeller University, New York: "Plasminogen activators of normal and transformed cells."

R. Roblin, I.-N. Chou and P.H. Black, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts: "Role of fibrinolysin T activity in properties of 3T3 and SV3T3 cells."

R. Pollack,* R. Risser,* S. Conlon,* V. Freedman,† D. Rifkin** and S. Shin,† *Cold Spring Harbor Laboratory, New York; †Albert Einstein College of Medicine, New York; **The Rockefeller University, New York: "Production of plasminogen activator and colonial growth in semi-solid medium are in vitro correlates of tumorigenicity in the immune-deficient nude mouse."

L. Ossowski and J.P. Quigley, The Rockefeller University, New York: "The relation between cell migration and plasminogen activation."

M. Weber, Department of Microbiology, University of Illinois, Urbana: "Role of protease activity in malignant transformation by Rous sarcoma virus."

SUNDAY MORNING, September 15

Chairman: Daniel B. Rifkin, The Rockefeller University, New York, New York

W. Troll, T. Rossman, M. Levitz and T. Sugimura, New York University Medical Center, New York; Cancer Center, Tokyo, Japan: "The role of proteases in tumor promotion and hormone action."

L.M. Greenbaum, Department of Pharmacology, Columbia University, New York: "Cathepsin-D generated pharmacologically active peptides (leukokinins)."

P.M. Blumberg and P.W. Robbins, Department of Biology, Massachusetts Institute of Technology, Cambridge: "Relation between the effects of proteases on the chick fibroblast cell surface and the changes induced by transformation."

R.O. Hynes, J.A. Wyke and J.M. Bye, Department of Tumor Virology, Imperial Cancer Research Fund Laboratories, London, England: "The possible role of proteases in altering cell surface proteins in transformed cells."

A. Vaheri and E. Rouslahti, University of Helsinki, Finland: "Fibroblast surface antigen (SF) molecules: Relation to malignant transformation and growth control."

This meeting was supported in part by a grant from the National Institutes of Health.

IN-HOUSE SEMINARS

Cold Spring Harbor in-house seminars were initiated to provide a semi-formal avenue for communication between the various research groups at the laboratory. They are particularly useful for research personnel who have joined the laboratory during the summer. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in defending, organizing and presenting their research. In addition to those listed below, seminars were given by many others involved in research at this laboratory.

October

12th. Dr. Claudio Basilico, Department of Pathology, New York University Medical School, New York: "Temperature-sensitive mutants of mammalian cells."

16th. Dr. Jerrold Schwaber, University of Chicago, La Rabida; University of Chicago Institute, Illinois: "Human x mouse somatic cell hybrid clone secreting immunoglobulins containing heavy chains from both parental cells."

19th. Dr. Bonnie Collins, Department of Microbiology, Harvard Medical School, Boston, Massachusetts: "The separation of the messenger RNA's of Newcastle disease virus."

November

9th. Dr. Kenneth Takemoto, Department of Health, Education and Welfare, Bethesda, Maryland: "Current knowledge concerning SV40-related human papovaviruses."

16th. Dr. Hamilton O. Smith, Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland: "Mechanism of action of the *H. influenzae* ATP-dependent DNase and speculations on its function."

30th. Dr. Frank Lilly, Department of Genetics, Albert Einstein College of Medicine, New York: "Genetic control of murine viral leukemogenesis."

December

7th. Dr. Dana Caroll, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: "Studies on the organization of the genes for 5S RNA in *Xenopus*."

14th. Dr. Leroy Stevens, The Jackson Laboratory, Bar Harbor, Maine: "Teratocarcinogenesis and parthenogenesis in mice."

January

4th. Dr. Carol Prives, Biochemistry Department, The Weizmann Institute of Science, Rehovot, Israel: "Cell-free translation of SV40 messenger RNA."

Dr. Volker Vogt, Swiss Institute for Experimental Cancer Research, Lausanne: "Protein synthesis in cells infected with RNA tumor virus."

7th. Dr. Mark Achtman, Max-Planck Institute for Molecular Genetics, Berlin, Germany: "The *tra* cistron of F factors."

11th. Dr. Kenneth Keegstra, Department of Microbiology, State Univeristy of New York, Stony Brook: "Sindbis virus envelope glycoproteins as probes of changes in host carbohydrates during transformation."

18th. Dr. James C. Wang, Department of Chemistry, University of California, Berkeley: "Twisted DNAs as enzyme substrates."

22nd. Dr. Jim F. Williams, Institute of Virology, Glasgow, Scotland: "Genetic experiments with adenovirus type 5."

25th. Dr. Carlo Croce, The Wistar Institute, Philadelphia, Pennsylvania: "Assignment of the SV40 T antigen, U antigen, TSTA genes and SV40-transformed human cells."

February

13th. Dr. John Wilhelm, University of Geneva, Switzerland: "RNA polymerase activities of *Xenopus laevis* oocytes."

15th. Dr. Gerald R. Fink, Section of Genetics, Development & Physiology, Cornell University, Ithaca, New York: "Double-stranded RNA and killer strains of yeast."

19th. Dr. Steven Rosenfeld, Harvard Biological Laboratories, Cambridge, Massachusetts: "On the chemical nature of transfer factor."

21st. Dr. A.L. Goldberg, Harvard Medical School, Boston, Massachusetts. "Control of protein degradation in animal and bacterial cells."

22nd. Dr. Robert Nowinski, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: "Immunogenetic control of mouse leukemia virus."

26th. Dr. Mike Kuhel, Immunology Department, Albert Einstein College of Medicine, New York: "Somatic cell genetics of immunoglobulin producing cells."

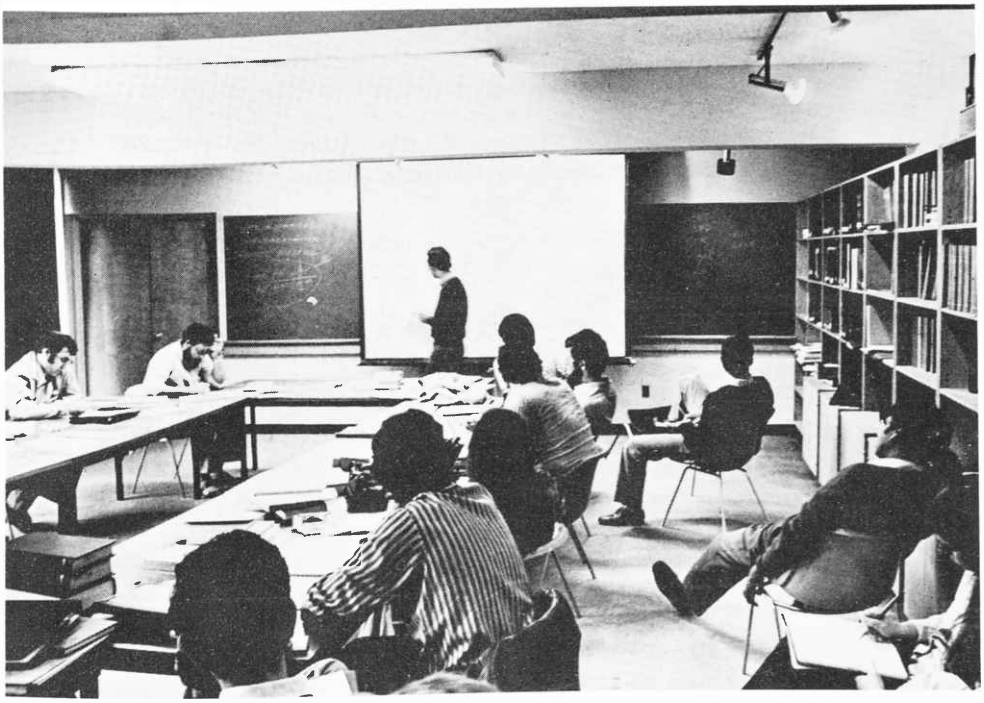
March

8th. Dr. Helene Smith, University of California, Berkeley; Naval Biomedical Research Laboratory, Naval Supply Center, Oakland, California: "Tissue culture systems as models for cancer research."

14th. Dr. John Cairns, Imperial Cancer Research Fund, London, England: "The statistics of *human* cancer."

15th. Dr. G. Albrecht-Buhler, Department of Biochemistry, J. Hillis Miller Health Center, University of Florida, Gainesville: "Properties of marker particle movements in the surface of 3T3 mouse fibroblasts."

Dr. Wally Mangel, Imperial Cancer Research Fund, London, England: "The initiation of RNA synthesis."



Seminar being given in James Laboratory Library (*Photo by Ross Meurer*)

21st. Dr. Tom Broker, Department of Chemistry, California Institute of Technolo Pasadena: "The mapping of transfer RNA by electron microscopy."

22nd. Dr. John J. Dunn, Department of Biology, Brookhaven National Laborato Upton, New York: "Processing of RNA in *E. coli* by RNase III."

29th. Dr. Jim Goldman, Public Health Research Institute, New York: "Biochemi studies of neurotransmitters in identified neurons of aplysia."

April

1st. Dr. Howard Steinman, Department of Biochemistry, Duke University Medic Center, Durham, North Carolina: "Superoxide dismutases: Structure functi relations studied through sequence analysis."

3rd. Dr. Peter Duesberg, Virus Laboratory, University of California, Berkeley: "Tum virus genes."

4th. Dr. David Secher, MRC Laboratory of Molecular Biology, Cambridge, Englan "Spontaneous mutations in immunoglobulin genes."

5th. Dr. Karen Artzt, Department of Anatomy, Cornell University Medical Colleg Ithaca, New York: "Early embryonic cell surface antigens."

8th. Dr. Ta-hsiu Liao, The Rockefeller University, New York: "Chemistry of pancreat deoxyribonuclease."

11th. Dr. B.L. Padgett, University of Wisconsin, Madison: "SV40-like viruses humans."

12th. Dr. Gary S. Hayward, University of Chicago, Illinois: "Agarose electrophoresis viral DNA."

18th. Dr. Vladimir Glisin, Department of Molecular Biology, University of Belgrad Yugoslavia: "RNA isolated by CsCl centrifugation: Some physical, chemical an biological properties."

19th. Dr. Giampiero di Mayorca, Department of Microbiology, University of Illinoi Medical Center, Chicago: "BK virus — A human virus of the polyoma virus family."

22nd. Dr. Walter Eckhart, Salk Institute for Biological Studies, San Diego, California "Polyoma gene functions."

26th. Dr. Bill Reznikoff, Department of Biochemistry, University of Wisconsin, Madison "The structure of the *lac* controlling elements."

May

10th. Dr. Albert Dahlberg, Division of Biological and Medical Sciences, Brown University Providence, Rhode Island: "Two forms of the *E. coli* 30S ribosomal subunit."

28th. Dr. Christina Kennedy, Unit of Nitrogen Fixation, University of Sussex, England: "The genetics of nitrogen fixation in *Klebsiella pneumoniae.*"

UNDERGRADUATE RESEARCH PARTICIPATION PROGRAM

Summer 1974

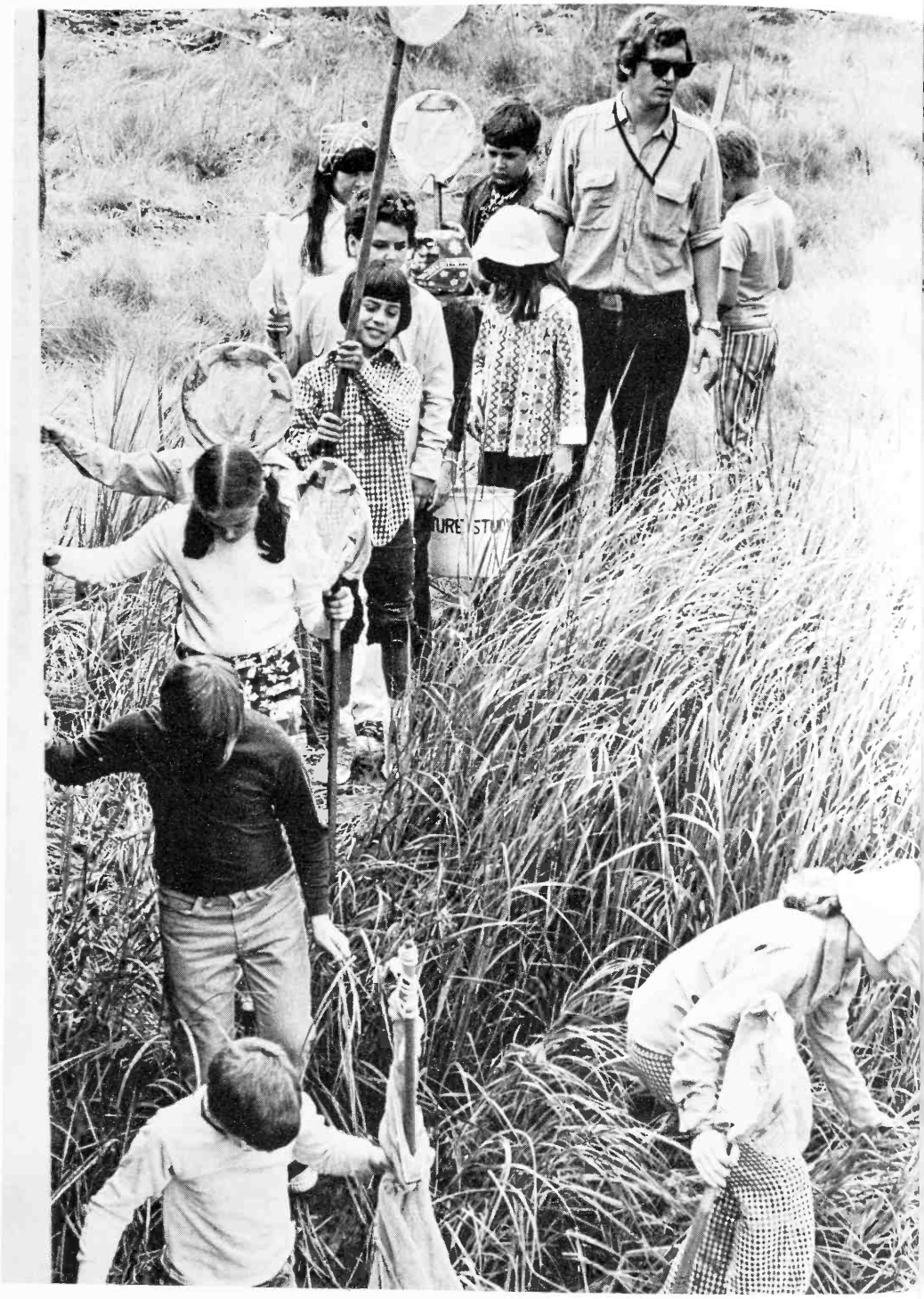
Another aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time Laboratory staff members. The program was initiated in 1959, and since that year 155 students have completed the course.

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.

The following students, listed with their laboratory sponsors and topics of research, were enrolled in the program, which was supported this year by Laboratory funds.

Margaret Jean Hightower, University of Alabama *Supervisor:* R.F. Gesteland	Purification of yeast killer particles
Keith E. Mostov, University of Chicago *Supervisor:* R. Roberts	A search for a mutant of the restriction endonuclease *Eco*RI
Harker Rhodes, Harvard University *Supervisor:* B.N. Apte	In vivo and in vitro degradation of reinitiation polypeptides
Hilary Jane Ronner, Barnard College *Supervisor:* A.I. Bukhari	Assay of DNA-unwinding proteins from plasmid-containing strains of *Escherichia coli*
Howard I. Rutman, Harvard University *Supervisor:* M. Botchan	Size determination of SV40 virion proteins from virus containing deleted DNA genomes
Vicky Valverde-Salas, Massachusetts Institute of Technology *Supervisor:* A.I. Bukhari	Temperature-sensitive β-galactosidase mutants of *Escherichia coli*
Gary Weiss, Columbia University *Supervisor:* R. Roberts	A search for new specific endonucleases
Mariana Wolfner, Cornell University *Supervisor:* R.F. Gesteland	Analysis of yeast killer RNA and cell-free protein synthesis in yeast extracts

URP Gary Weiss with his supervisor Richard Roberts (*Photo by Ross Meurer*)



Instructor Jim Romansky exploring with some of the children who participated in this year's Nature Study Course (*Photo by Ross Meurer*)

CHILDREN'S NATURE STUDY PROGRAM

This program offers young people the opportunity to explore the plant and animal life and the geological history of Long Island. A total of 401 students attended the 1974 program. Most of the courses were held outdoors, using the surrounding beaches, tidal pools and woods as sources of material. Jones Laboratory and the newly built classrooms at Uplands Farm provided instruments and equipment for the laboratory study of specimens collected by the students.

The Heritage Federal Savings and Loan Association, Huntington, provided scholarships for twenty children. The Three Harbors Garden Club donated two scholarships.

STAFF

Thomas Stock, M. Ed., Earth Science Teacher, Centereach, N.Y.; Program Director
Lois Alfano, M.S., Hofstra University; Naturalist leader, BOCES
Fred Maasch, M. Ed., Biology Teacher at Islip High School; Former ranger-naturalist at Fire Island National Seashore.
Alex McKay, B.A., Queens College; M.A., Long Island University; Past President, Huntington Audubon Society; Teacher, Finley Junior High School.
Jim Romansky, B.S., Cortland State; M.S. Biology, Albany State; M.S. Geology, Union College; Ranger, Fire Island National Seashore; Biology Teacher, Bay Shore High School; Education Chairman, Great South Bay Audubon Scoiety.
Dick Rosenman, M.A., Biology Teacher, Cold Spring Harbor High School

COURSES

General Nature Study
Advanced Nature Study
Elementary Geology
Bird Study
Seashore Life
Geology
Animals with Backbones
Icthyology – Herpetology
Marine Biology
Introduction to Ecology
Nature Photography
Fresh Water Life
Ecology of the Estuary

NATURE STUDY WORKSHOP FOR TEACHERS

This summer seventeen elementary and secondary teachers attended the 19th annual Workshop in Nature Study. Designed to help teachers in their classroom science activities, the workshop consisted of field trips, laboratory work and lectures.

Participants were offered a general review of the major groups of animals and plants, the geological features of Long Island, the ecological relationship of living things to their environment, and the natural history of living things and their economic importance to man. Instruction included methods of collecting and preserving specimens.

Otto Heck, instructor for the course, has been connected with this program for eighteen years. Mr. Heck is currently Assistant Professor of Biology at Trenton State College, where he teaches Biology, Ecology and Vertebrate Zoology.

LABORATORY STAFF

November 1974

Director

J.D. Watson

Administrative Director

William R. Udry

Research Scientists

1 Guenther Albrecht-Buehler
2 Carl Anderson
3 Bal Apte
4 Michael Botchan
5 Ahmad Bukhari
6 Raymond Gesteland
7 Terri Grodzicker
8 Walter Keller
9 James Lewis
10 Michael Mathews
11 Carel Mulder
12 Robert Pollack
13 Richard Roberts
14 Joseph Sambrook
15 Klaus Weber
16 Mary Weber
17 David Zipser 20

Postdoctoral Fellows

Janet Arrand
John Arrand
John Atkins
Ellen Daniell
Richard Gelinas
Martha Howe
Regine Kahmann
Dietmar Kamp
Rex Risser
William Topp
Sayeeda Zain

Graduate Students

Daniel Klessig
Elias Lazarides 15
James Manley
Brad Ozanne

Research Assistants

Gregory Ballantyne
Peter Baum

Cathleen Casserly Sup
Susanne Conlon
Barbara Doretsky Rob
Peter Edson Ellen
Gary Felsten Rob
Cynthia French Bru
Susan Froshauer Agn
Ronni Greene Will
Paula Grisafi Mar
Alan Johnston Bet
Barbara Kempler Gla
Robert Lancaster Mar
Sandra Lee Hel
Anita Lewis Mac
Pat Maloy Ro
Bernadine Miller Ge
Phyllis Myers
Linda Rawluk Ca
Tashmeem Razzak Wa
Donald Ruthig Alt
Judith Schwartz Bar
Jane Schweitzer
Ruth Solem Pu
John Stravato
Carole Thomason Na
Ingrid Wendel Ju

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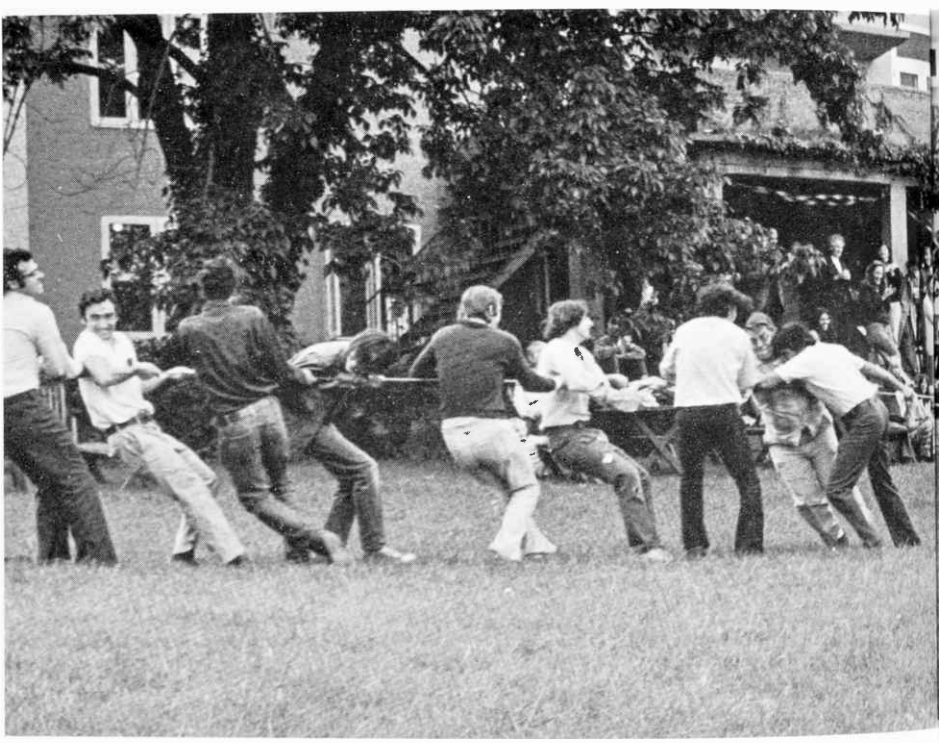

Staff vies in tug-of-war at end of the summer picnic (*Photo by Bob Yaffee*)

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Robert DeCarlo
Bruce DeTroy
Agnes Fisher
William Flanigan
Maria Hedges
Betty Jacques
Gladys Kist
Marie Moschitta
Helen Parker
Madeline Szadkowski
Robert Yaffe

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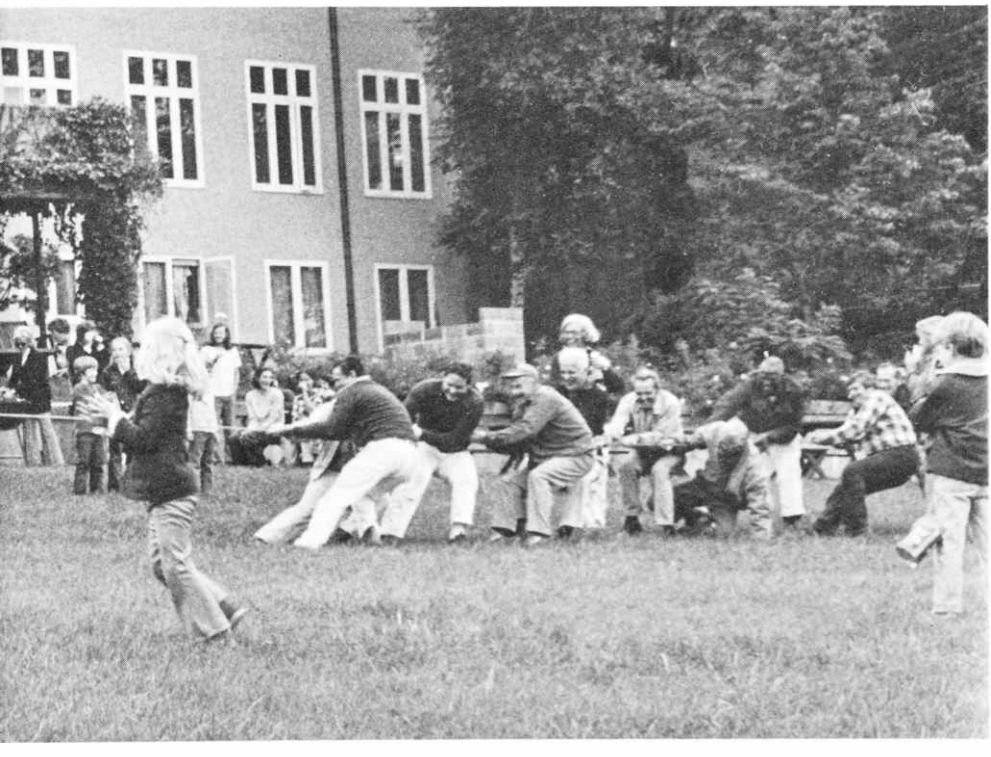

FINANCIAL STATEMENT

Balance Sheet
Year ended October 31, 1974
with comparative figures for 1973

ASSETS

	1974	1973
Current funds:		
Unrestricted:		
Cash	$ 204,029	$ 309,66
Accounts receivable	68,916	52,99
Inventory	84,781	68,8
Prepaid expenses	12,102	60,6
Total unrestricted	369,828	492,0
Restricted:		
Cash	291,615	68,51
Grants receivable	387,237	284,87
Total restricted	678,852	353,38
Total current funds	1,048,680	845,47
Plant funds:		
Unexpended cash	–	333,81
Investment	39,685	–
Due from current funds	233,697	–
Land and improvements	579,197	186,83
Buildings	1,723,521	1,416,11
Furniture, fixtures and equipment	423,766	280,44
Donated books and periodicals	365,630	–
Construction in progress	21,497	17,6
	3,386,993	2,234,84
Less allowance for depreciation and amortization	516,077	372,89
Total plant funds	2,870,916	1,861,94

LIABILITIES AND FUND BALANCES

Current funds:		
Unrestricted:		
Accounts payable	56,758	124,4
Accrued expenses	42,096	113,8
Due to plant funds	233,697	–
Fund balance	37,277	253,8
Total unrestricted	369,828	492,0
Restricted:		
Fund balance	678,852	353,38
Total current funds	1,048,680	845,47
Plant funds:		
Fund balance	2,870,916	1,861,94

Statement of Current Revenues, Expenditures and Transfers
Year ended October 31, 1974
with comparative figures for 1973

	1974	1973
Revenues:		
Grants	$1,505,426	$1,575,458
Indirect cost allowance on grants	663,050	569,832
Contributions	95,272	83,266
Robertson Research Fund contribution	320,012	155,827
Summer programs	129,821	117,303
Laboratory rental	18,765	18,244
Marina rental	30,000	—
Investment income	36,851	38,278
Book sales	297,247	213,856
Dining hall	105,971	84,241
Rooms and apartments	102,922	106,002
Other sources	2,967	2,810
Total revenues	3,308,304	2,965,117
Expenditures:		
Research*	1,515,896	1,427,716
Summer programs*	171,042	191,818
Library	80,637	69,240
Operation and maintenance of physical plant	421,311	372,366
General and administrative	344,173	294,280
Scholarships	7,920	3,120
Book sales*	227,452	128,384
Dining hall*	120,849	116,265
	2,889,280	2,603,189
Transfers:		
To unexpended plant funds	33,180	282,766
To invested in plant	602,382	165,447
	635,562	448,213
Total expenditures and transfers	3,524,842	3,051,402
Excess of expenditures and transfers over revenues	(216,538)	(86,285)

**Reported exclusive of an allocation for operation and maintenance of physical plant, general and administrative, and library expenses.*

NOTE: The complete financial statements, as certified by our independent auditors, Peat, Marwick, Mitchell, Co., are available on request from the Comptroller, Cold Spring Harbor Laboratory.

FINANCIAL SUPPORT OF THE LABORATORY

The Cold Spring Harbor Laboratory is a publicly supported educational institutio chartered by the University of the State of New York and may receive contributions whic are tax exempt under the provisions of the Internal Revenue Code, particularly Sectio 501C. In addition, the Laboratory has been formally designated a "public charity" by th Internal Revenue Service. Accordingly, it is an acceptable recipient of grants which wou result in the termination of "Private" foundations.

The Laboratory depends upon the generous contributions of its sponsors, participati institutions and friends for central institutional needs and capital improvements. addition, the development of any new programs, such as year-round research neurobiology and the marine sciences, can only be undertaken with substantial suppo from private sources.

Methods of Contributing to Cold Spring Harbor Laboratory

Gifts of Money

Can be made directly to Cold Spring Harbor Laboratory.

Securities

There are several ways to contribute securities:

(1) Your broker or bank may sell the securities and remit the proceed directly to the Laboratory.

(2) If you wish to send the stock directly to the Laboratory, either a) endors the certificate(s) in blank (no name transferee) by signing your name o the back of the certificate(s). Have your signature guaranteed on th certificate(s) by your bank or broker. Send the certificate(s) by *registere mail* to the Laboratory; or b) send *unsigned* certificate(s) along with covering letter. Under separate cover, send a stock power executed i blank (one for each certificate and again with a signature guarantee along with a copy of the covering letter. Both should be sent by firs class mail to the Laboratory. Depreciated securities should be sold fo your own account to establish a tax loss, then make your contribution t the Laboratory by check.

Bequests

Probably most wills need to be updated. Designating the Cold Spring Harbo Laboratory as a beneficiary insures that a bequest will be utilized as specifie for continuing good.

Appreciated Real Estate or Personal Property

Sizeable tax benefits can result from such donations, some of which th Laboratory can use in its program, others can be sold after donation.

Life Insurance & Charitable Remainder Trusts

Can be structured to suit the donor's specific desires as to extent, timing an tax needs and, at the same time, increase the resources available for the work of the Laboratory.

Conversion of Private Foundation to "Public" Status or Termination

This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation could be accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a "supporting organization of the Cold Spring Harbor Laboratory."

For additional information, please contact the Administrative Director, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, N.Y. 11724, or call area code 516-692-6660.

GRANTS

November 1, 1973 — October 31, 1974

Grantor	*Principal Investigator–Program*	*Total Award*	*Duration of Grant*
NEW GRANTS			
National Science Foundation	Dr. Bukhari - Research	$ 60,000	8/1/74-7/31/76
	Dr. Roberts - Research	50,000	6/15/74-5/30/76
	Dr. Gesteland - Research	41,400	9/1/74-8/31/75
	Dr. Zipser - Research	80,000	1/1/74-12/31/75
National Institutes of Health	Dr. Watson - General Research Support	148,850	1/1/74-12/31/74
	Dr. Gelinas - Fellowship	13,000	7/3/74-7/2/75
	Dr. Gesteland - Conference on Proteases	21,610	9/1/74-8/31/75
	Dr. Pollack - Neurobiology Training	168,480	5/1/74-4/30/77
	Dr. Pollack - Summer Workshops	187,200	4/1/74-3/31/77
	Dr. Zipser - Research	350,000	5/1/74-4/30/79
	Dr. Pollack - Career Development	125,000	6/30/74-6/29/79
American Cancer Society	Dr. Howe - Fellowship	8,500	1/1/74-12/31/74
Helen Hay Whitney Foundation	Dr. Daniell - Fellowship	28,500	3/1/74-2/28/77
International Agency Research on Cancer	Dr. Albrecht-Bühler - Fellowship	10,700	12/1/73-11/30/74
CONTINUING GRANTS			
National Science Foundation	W. Udry - Symposium Support	5,000	5/15/74-10/31/74
	Dr. Gesteland - Research	134,000	9/1/71-12/31/74
	Dr. Zipser - Research	85,200	1/1/72-12/31/73
	Dr. Pollack/Dr. Goldman - Research	115,600	5/1/73-4/30/75
National Institutes of Health	Dr. Pollack - Summer Training	354,459	1/1/70-5/30/75
	Dr. Gesteland - Career Development	92,000	1/1/69-12/31/73
	Dr. Zipser - Career Development	108,000	5/1/70-4/30/75
	Dr. Zipser - Research	200,195	1/1/70-4/30/74
	Dr. Watson - Symposium Support	96,000	4/1/74-3/31/79
	Dr. Watson - Cancer Research Center	7,500,000	1/1/72-12/31/76
	Dr. Watson - General Research Support	184,532	1/1/73-12/31/73
American Cancer Society	Dr. Sambrook - Fellowship	100,000	12/1/70-11/30/75
Jane Coffin Childs Memorial Fund for Cancer Research	Dr. Bukhari - Research	32,000	12/1/72-11/30/74
Alfred P. Sloan Foundation	Dr. Watson - Neurobiology Training	450,000	10/1/70-9/30/75
Volkswagen Foundation	Dr. Watson - Training Support	60,000	1/1/71-12/31/75
Atomic Energy Commission	Dr. Watson - Symposium Support	8,000	5/30/74-6/7/74
Grass Foundation	Dr. Watson - Neurobiology Training	5,000	6/1/74-9/30/74

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Mr. and Mrs. Henry S. Wingate

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Mr. Elton T. Young, II

LONG ISLAND BIOLOGICAL ASSOCIATION

Tent erected for 50th Anniversary Celebration held on the Laboratory grounds (*Photo by Ross Meurer*)

On October 5, 1974, 550 members and friends of the Long Island Biological Association joined in marking the 50th Anniversary of its founding with a celebration held in Jones Laboratory.

Opening the day's program, the Chairman of the Association, Edward Pulling, spoke briefly of its history. The Director of the Laboratory, Dr. James D. Watson, followed with some brief remarks on the Laboratory's research programs and introduced the featured speaker, Dr. Jacques Monod, Director of the Institut Pasteur, Paris. Dr. Monod discussed the importance of the central theory of biology, namely, the theory of evolution, in relation to ideas present in modern culture.

The remainder of the meeting was given over to dancing to the music of an orchestra led by Ben Ludlow, who generously contributed his services, and sampling the hors d'oeuvres and drinks prepared and served by lady member volunteers.

As pointed out by Mr. Pulling in his opening remarks, Jones Laboratory was particularly appropriate as the scene of the celebration. Constructed in 1893 with funds contributed by Mr. John D. Jones, for whom the building is named, Jones Laboratory dates back to the earliest days of the Laboratory when its research was initially sponsored and supported by friends and neighbors in the community. In use for almost 80 years for nature study, zoological and other research, this building is now scheduled to be renovated to provide adequate facilities for the new research program in the field of Neurobiology being undertaken by the Laboratory.

The Laboratory itself was founded in 1890 by several local philanthropists and the Brooklyn Institute of Arts and Sciences. The first Chairman of the Board of Managers was Eugene G. Blackford, who served from 1890 until his death in 1904. William J. Matheson succeeded him, serving until 1923. In that year, when the Brooklyn Institute of Arts and Sciences withdrew from Cold Spring Harbor, local supporters of the Laboratory formalized their efforts by incorporating as the Long Island Biological Association, with Colonel T. S. Williams its first Chairman. Jointly with the Carnegie Institution of Washington, LIBA continued to support and direct research at Cold Spring Harbor until 1962, at which time the Laboratory was reorganized as an independent institution with its own Board of Trustees. During the past 12 years, LIBA's chief function has been to widen the interest of the community in the Laboratory and to help support it financially.

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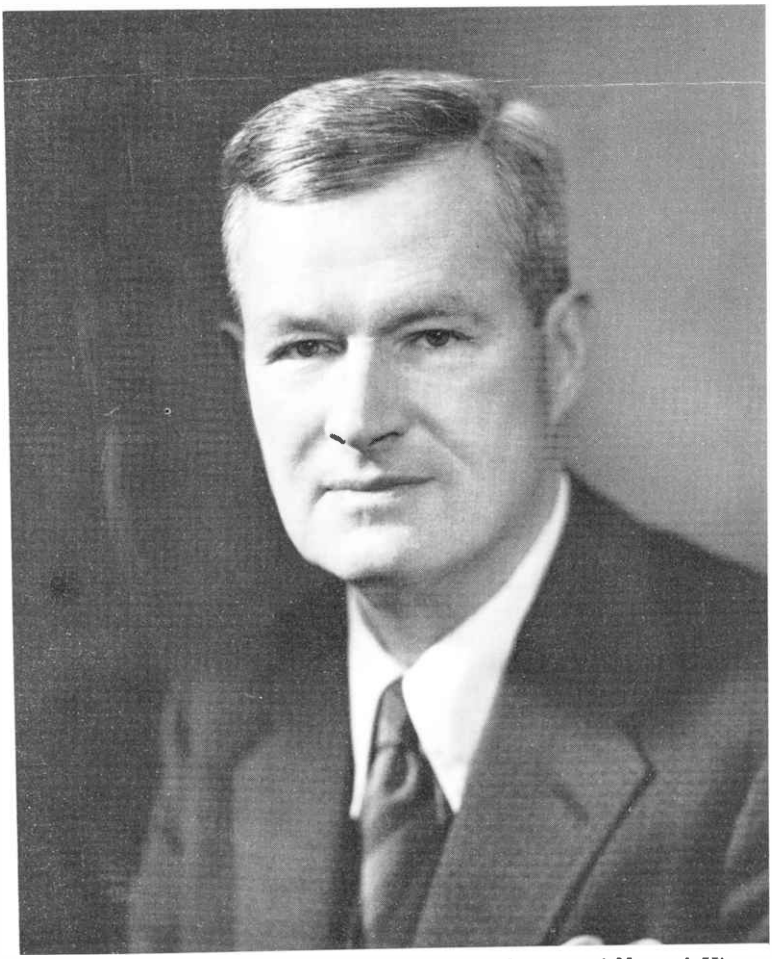

(Photo, taken in 1951, courtesy the American Museum of Natural History)

Robert Cushman Murphy
(1887-1973)

Robert Cushman Murphy was a native Long Islander, and very proud of it. From his youth he roamed the world on innumerable expeditions, yet he never lessened in his love for "fish-shaped Paumanok," as he wrote of it using Walt Whitman's colorful term. For most of his life, Bob Murphy was a museum man, first at the Brooklyn Museum and later at the American Museum of Natural History, where in time he became Lamont Curator of Birds. He was a foremost authority on oceanic birds, to which he was devoted from the time of his first voyage to the Antarctic, so vividly described in a classic travel story, *Logbook for Grace*, adapted from his letters to the young bride he had left at home. From those days, too, came his yarns about whaling and his rich store of sea chanties. Later travels the two shared together, even to a golden trip to Australia in the last year of Bob's life, when he and Grace were each in their vigorous ninth decade.

After retiring from the Museum in 1955, Bob and his wife devoted much of their effort to conservation activities on Long Island. It was a fitting postlude to the twelve years (1940-52) when Bob served with distinction as the courtly president of the Long Island Biological Association at a time when it was becoming more and more widely known throughout the world as the cradle of molecular genetics research. Dr. Murphy was no molecular biologist, but he fully recognized the importance of the novel revelations of the nature of life then being disclosed, and his leadership helped to bring the Laboratory to its present place of distinction.

— *Bentley Glass*