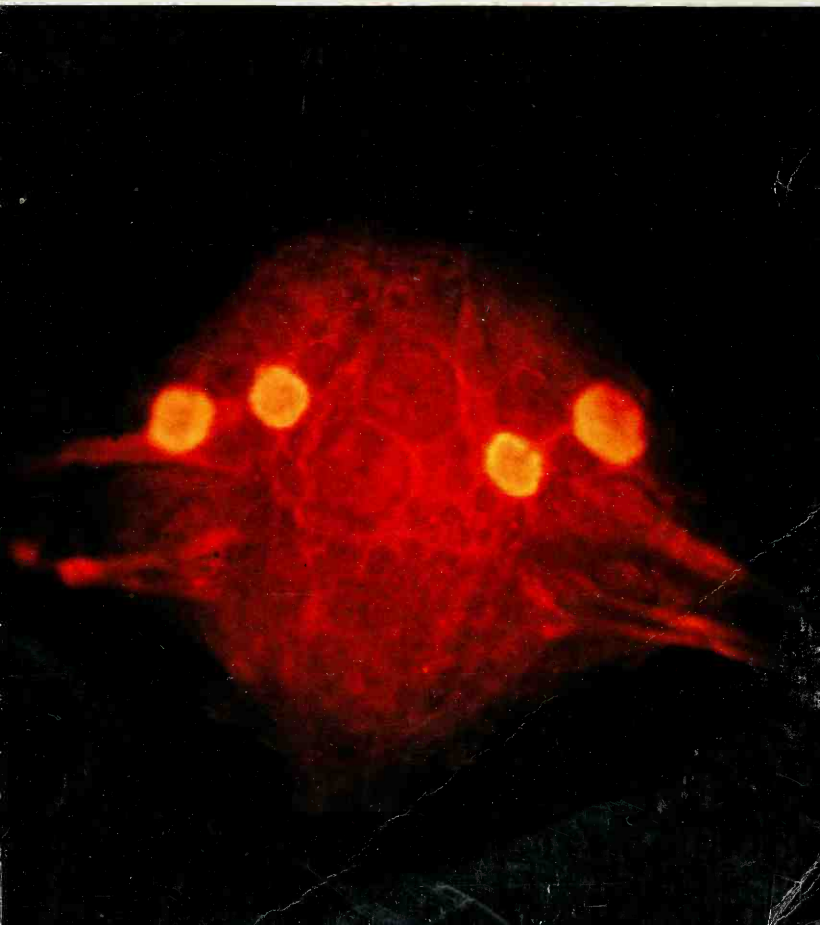
COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1980



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COLD SPRING HARBOR
LABORATORY

ANNUAL REPORT 1980

COLD SPRING HARBOR LABORATORY

COLD SPRING HARBOR, LONG ISLAND, NEW YORK

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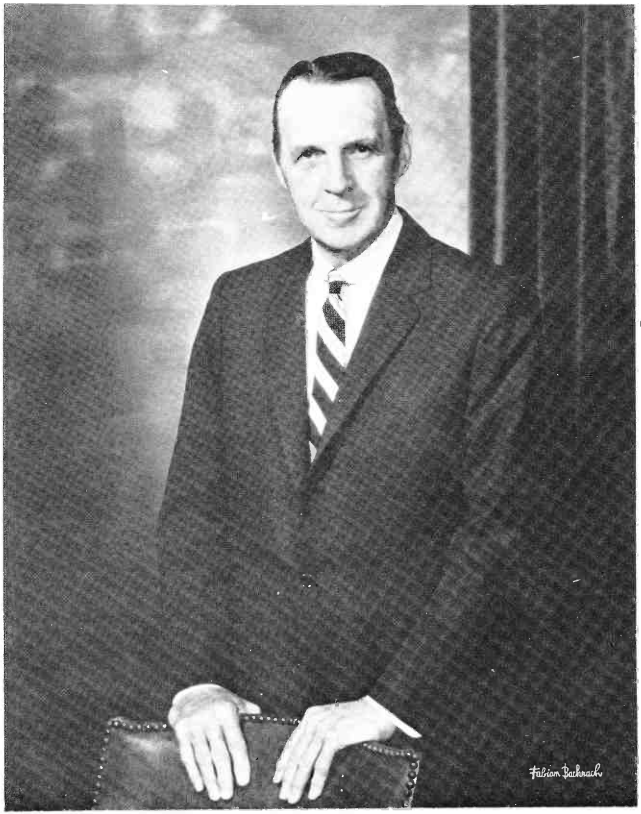


Charles S. Robertson

February 13, 1905–May 2, 1981

Charles Sammis Robertson, through his several gifts to this Laboratory, changed radically our nature, giving us the capacity again to do science at a level with the best and allowing us to escape out of a long cycle of physical decay that had been badly accentuated in the early 1960s upon the decision of the Carnegie Institution of Washington to discontinue support for its Genetics Program at Cold Spring Harbor.

We met first in the summer of 1972, soon after the death of his beloved wife, Marie. They had married in 1936, quickly assembling a large parcel of land in Lloyd Harbor that overlooked the waters of Cold Spring Harbor and that had for almost 200 years been farmed by members of his mother's family, the Sammises. On this land, they built a marvelous Georgian-style home that they had designed by the noted architect Mott Schmidt. In it and its surrounding fields and outbuildings they quietly raised their family of five children, Mimi, Anne, John, William and Kate. During the war years they also served as foster parents for two children evacuated from an England then at risk from the terror of German bombs and rockets.

Though always known as generous benefactors to local causes, with Mr. Robertson serving many years on the Board of Huntington Hospital and for several years as its president, the extraordinary nature of their private philanthropy remained a closely guarded secret for twelve years. To Princeton University, from which Charles Robertson graduated magna cum laude in 1926, the Robertsons, in 1961, donated 35 million dollars to be used to greatly expand and generously endow the Woodrow Wilson School of Public and International Affairs. Except for a privileged few, no one knew the identity of the benefactors until after Marie's death. Then the President of Princeton obtained Charles Robertson's permission to reveal their role. Princeton, like many other universities, was going through the travails occasioned by the Vietnamese War, and many students were under the illusion that only the CIA could be so generous and wish for anonymity.

Upon his wife's death, Charles Robertson became concerned over the long-term future of his house and property, worrying that upon his death, the Sammis lands that he had reassembled would again be dispersed. He came to us with the hope that, as an academic institution, we might utilize well his land and buildings, were he to properly endow them so they would not soon be regarded as a white elephant from which we should quietly disentangle ourselves. And wisely, perceiving that unless we were inherently stable, we could not give an intelligent, loving use to his estate, he also offered to provide several million dollars to create an endowment fund, the income of which was to be used to support research at the Laboratory itself.

It was an offer too wonderful to believe, or to consider even momentarily turning down, and we eagerly joined with the Robertson family to set up a separate corporate entity to be called the Robertson Research Fund. It came into legal existence in early June of 1973, accompanied by a gift of 8 million dollars from the Banbury Foundation. This was the Robertson family foundation created as a vehicle for the philanthropy made possible by the sale in the 1950s of their share of the stock in the Great Atlantic and Pacific Tea Company, which had been founded by Marie Robertson's family, the Hartfords.

Among the first uses of the Robertson Research Fund was the purchase of a Phillips 201 Electron Microscope to be placed in the newly renovated Davenport Lab. Soon after, this fund allowed us to totally renovate and equip Jones Lab as a center for Neurobiology and then to go on to complete the renovation of McClintock Lab, creating the new labs that allowed our Cell Biology Group to come into existence. Robertson monies also proved invaluable in allowing our summer research program for undergraduates to carry on when the NSF cut back on this program and in maintaining several key summer courses when the federal funds on which we had counted were lost due to last-minute recisions demanded by the OMB. Equally important, it has provided key monies to allow new staff members to start up their research at full blast, as well as funds for many of the Postdoctoral Fellows who have worked here over the past eight years.

In 1976, three years after the creation of the Robertson Research Fund, Mr. Robertson transferred to us his lovely estate of some 45 acres on Banbury Lane. The main house, which we now call Robertson House, came to us tastefully furnished with reproductions of the fine, antique, family furniture, which Mr. Robertson had moved to his winter home in Delray Beach, Florida, upon his retirement from the investment banking firm of Smith Barney. A second endowment fund, the Robertson Maintenance Fund, of $1.5 million dollars was then created to provide for the perpetual upkeep of the estate, along with a special gift of $100,000 to be used in preparing the estate for our special needs. We thus had the means to transform the elegant seven-car Georgian-style garage into a meeting complex and so create the Banbury Educational Center, a facility uniquely designed for the holding of small conferences and the teaching of summer courses. On June 10, 1977, the Banbury Meeting House was formally dedicated, with Francis Crick giving the first lecture. It was a very happy occasion, with Mr. Robertson and many of his family being in attendance. To complete the Banbury complex, a second residence, containing 16 bedrooms, was added in 1980 to supplement the housing for 20 visitors provided by Robertson House. Designed in the post-modern classical idiom by the celebrated American architect Charles Moore, it has been named Sammis Hall.

From the beginning of his formal association with us, Charles Robertson displayed an active interest in our Neurobiology Programs and early expressed the desire to help us further expand studies on the biological basis of behavior. Toward that objective, in 1977 he set aside further income from the Banbury Foundation to create the Marie H. Robertson Fund for Neurobiology. With these monies, we have been able not only to further increase the scope of our summer programs, but also to initiate a year-round research program. Though still very modest in size, our experimental effort in Jones Lab has already created new immunological tools that may radically alter the approaches taken by neurobiologists to understand how nerve cells become functionally interconnected into brain-like structures.

In all his dealings with me and other members of the Laboratory, Charles Robertson was the perfect benefactor, modest, yet highly intelligent and desirous of further learning, graciously serious, but always with a sense of humor, and deeply loving and loyal to those individuals and institutions that he admired. His coming into our lives was a marvelous, unexpected gift, his delight and interest in the programs he helped make possible was a joy, and his passing this spring an occasion of deep sadness and reverence.

May 20, 1981 *J. D. Watson*

DIRECTOR'S REPORT

The "War on Cancer" is now a decade old. As with most battles that go on too long, the hoopla and bravado are now muted, and the charismatic generals who were to lead us to certain victory have been rotated to less conspicuous commands. Today we are led by the better staff colonels of the immediate past, seasoned enough not to openly criticize the tactics of yesteryear, knowing well that if their former bosses are perceived not to have been equal to their charges, such lack of confidence may soon be transferred to them as well.

All too clearly the press is testy, and even Congress, old enough to be immediately at risk, cannot be counted on for still another generation of new recruits to march into battle against an enemy that still has not yet revealed its exact shape. Blind bombing for bombing's sake, although emotionally satisfying, was the wrong way to proceed against the Nazi beasts and might well be an equally inappropriate way to bring the cancer cells to their knees. Might it not now be better to tactically retreat to regroup our forces until we can strike with the precision of final victory?

If so, conceivably it could be no catastrophe, but, in fact, a blessing, for our official slasher, Mr. Stockman, to cut back the funding of a NCI that in growing so fast on the public purse, may have put on fat at the expense of muscle, and by possibly being so prematurely superfunded, has never acquired the intellectual confidence that comes from the making of real decisions. There was, in fact, an initial OMB thrust to cut away a billion dollars from the NIH (NCI), but it came to naught, and the money instead came from the poor and socially unfortunate. We thus must be aware that given the present national semi-consensus to spend more money on defense, we might only be able to retain our current ways of doing science by taking still more from the unblessed, hoping that the benefits of our research will trickle back to society either through the development of more-efficient medical practices or through the creation of the biotechnologic industries in which our nation has a competitive edge.

The questions thus must be faced of not only whether the medically oriented science we now do is really that good, but also whether we are doing it in a properly frugal manner. Are we still all too casually writing purchase orders to consume the vast sums that are popularly thought to have been flowing in our direction ever since Sidney Farber, Mary Laker, and Benno Schmidt coopted first Senator Yarborough, then Teddy Kennedy, and finally Richard Nixon to declare war on cancer. This was to be a campaign fought on many fronts, ranging from the curing of cancer to its prevention. Research as to its nature was to be somewhere in the middle, but not in any real way neglected. Soon we were to have at least a billion per year to spend, and so no one with even the chance of a sound idea need go wanting.

Here I will only comment on the ways we have gone about finding the origin and nature of cancer, since this is the area in which our laboratory has been directly involved. The popular perception now is that we put our money on the wrong horse by not worrying enough about the many chemicals that can cause cancer and instead mounting a large crash program to discover putative human tumor viruses. If viruses were the cause of much cancer and we could develop effective vaccines against the relevant ones, then within a decade or two the incidence of cancer should radically drop. But, after ten years of big funding, not one virus has yet to be implicated as the cause of a major human cancer. Moreover, many experts now increasingly believe that at least 90% of human cancer is due to lifestyle (e.g., smoking, the eating of fat, etc.) and environmental factors. Thus, with ever-increasing hostile scepticism about viruses being significant cancer-causing agents, shouldn't we now, better late than never, deemphasize the various tumor virus efforts. By so doing, the money that now supports them could go, instead, toward mounting a first-rate program on chemical carcinogenesis, a field still badly in need of intellectual feeding.

The truth, however, is not that simple, and, in fact, we are actually way ahead of the game by having directed so much early effort toward the understanding of tumor viruses. It is not that a decade ago chemical carcinogenesis was not inherently important. More relevant was the fact that there was no way this field could efficiently absorb vast sums of new money. It was intellectually in its infancy, and large numbers of new

scientists could have been brought into this field only to discover that they had no real working tools. The answers the public wanted could not have been obtained in a reasonable period of time. Now, however, thanks to the new procedures of recombinant DNA, it is at last possible to seek the exact genetic changes brought about by chemical carcinogenesis. Though this will not be an inexpensive endeavor, it is at last the time to back a big intellectual push for the nature of the genes that have become altered through the action of chemical agents.

This, however, should not be accomplished by wide-scale cutbacks in the tumor virus area. To be sure, we do not seem finally about to demonstrate that tumor viruses are, in fact, responsible for major killers like cancers of the lung or colon. Nonetheless, tumor viruses increasingly hold the center stage in cancer research. Through studies on the molecular level of both those tumor viruses that contain DNA and those that have RNA chromosomes, we are now on the verge of understanding the fundamental chemical features of cancer cells. This happy situation, which a decade ago we thought only marginally possible, is not the result of the work of a few talented scientists. Instead, it is the end product of the moving into tumor virology some ten years ago of a large number of our best younger molecular biologists. Then, recombinant DNA was not even a dream, and the smaller tumor viruses possessed the only eukaryotic DNA (RNA) that seemed simple enough to one day master. Given the bribe of massive funds from the War on Cancer, there really was no other career choice to make. So, within several years, a perceptible increase in the intellectual quality of cancer research was already apparent, and now the snickers that invariably once followed the description of a project as cancer research are gone. By transforming so much of cancer research into an intellectually respectable discipline, the War on Cancer has produced a most-needed victory.

Equally important have been the key research facts that have already come out of our virus efforts. Already the onocogenic segments of a number of tumor viruses, of both the DNA and the RNA classes, have been precisely defined, and we are in the process of clearly establishing the biological function of their protein products. SV40 DNA, for example, codes for two onocogenic proteins, one of which helps to initiate DNA synthesis, the other of still unknown cytoplasmic function. The Rous virus produces sarcomas because it has picked up accidentally a cellular gene (*sarc*) that codes for an enzyme (kinase) that phosphorylates key cellular proteins. When present on the viral chromosome, the gene is not subject to normal cellular control and overproduces its kinase products. The overphosphorylation leads, in ways that we have yet to discover, to the sarcoma phenotype.

Initially, the pace at which we could work on these viral cancer (onco) genes was of necessity both slow and expensive, with much luck being necessary just to obtain the mutant virus strains needed. Now, however, with recombinant DNA, the pace of tumor virus research has quickened almost frighteningly, and the normal cellular roles of the "cancer genes" carried by many more tumor viruses are likely soon to be known. With them in hand, we shall have an extraordinarily powerful set of tools to firmly establish the basic biochemistry of cancer.

The last stages of this victory, however, will not be a walkover. In moving our prime molecular attention from the tumor viruses to the cancer cells themselves, we face an extraordinary increase in complexity. The simplest human cells contain at a minimum some 2000 different proteins, the interactions of many of which must be dissected before we can be sure we have the right answers. The equipment we shall have to use will of necessity be more sophisticated than that employed in the past and, unless we are much luckier than we deserve, will not be cheap.

So, I don't foresee any way that we can maintain our momentum, much less increase it to the extent now technologically possible, if the NCI monies available for these fundamental studies are even mildly slashed. In a real sense, we are victims of our enormous success. Until recombinant DNA came along, we accepted the fact that all the prime parameters of cancer cells might not be in for many decades to come. Now with the smell of victory in the air, we see every reason to push ahead as fast as possible. This pursuit will take more, not less, money at a time when even the maintenance of our status quo is bound to strike many outsiders as basically indecent.

Moreover, soon we may be approaching the time when it actually makes sense to spend substantial monies looking for human tumor viruses. As we finally begin to master the basic molecular biology of the RNA tumor viruses (retroviruses), the more obvious it becomes that those scientists who, at the beginning of the War on Cancer, staked their careers on implicating retroviruses as human carcinogens never had a reasonable chance of success. They did not know enough or have the technological handles to proceed sensibly. Now that we appreciate the transposonlike qualities of retroviruses, we at last have a philosophy, admittedly incomplete, for analyzing the various retroviruslike entities that will be found over the next few years in a variety of human cells. By cloning them in bacteria, we can easily test whether or not they contain cellular (onc?) insertions into the basic retroviral genome. Even if we find "onc-like" segments, however, and then through transfection experiments show that they are real "oncs," we shall not yet have our "smoking guns."

To find them, we might best concentrate less effort on the isolation of new retroviruses and more on directly testing the DNA from primary

human tumors to see whether it can transform the appropriate normal cells into their cancerous equivalents. Preliminary attempts have already given positive results, and we have every reason to expect that a number of human "cancer genes" will soon be cloned in bacteria. Then, through DNA sequencing studies, we can find out whether any of the cancer genes are closely associated with retroviruslike transposons. If any such putative retrovirus sequences are identified, it should be relatively simple to look next for their expression in various human tissues as the first step to seeing whether in their RNA form they are horizontally transmitted from one cell to another as infectious retroviruses. Those experiments, at long last within our techological capability, will not be cheap. So unless there is the prospect of ample long-term funding for such efforts, we shall remain ignorant longer than necessary as to the role of retroviruses in human cancer.

The time may also soon be ripe for looking much more closely at the various papilloma (wart) viruses, about which there is no doubt as to their proliferative potential. Because they still have not yet been successfully grown in cultured cells, they were of necessity refractory to molecular analysis until recombinant DNA came along. Now we have the potential to routinely screen hundreds of human tumors for the presence of papilloma DNA. Definitive answers should also now be possible as to the oncogenic role, if any, of the various herpes viruses and in particular of EB, which has long been identified with the proliferative aspect of mononucleosis and so always a tantalizing candidate as the causative agent of one or more of the Hodgkin's-disease-like syndromes.

Even if it turns out that viruses cause only a minor fraction of human cancer, it still makes great sense to try to obtain the respective vaccines. The doing away with any minor cancer by such procedures would in a decade or so more than pay for all the monies so far spent on tumor virus research. So, given the availability of funds, we would be most remiss still to be held in check by our lingering embarrassment over the premature excesses of past human tumor virus searches. Instead, we should confidently take advantage of the fact that soon we shall have the appropriate base of DNA science to let us proceed in a logical, sensible manner.

Here we must not lose sight of the fact that a main reason why we are excited about the next decade in cancer research is that at last it has attracted many of the best younger scientists. If, however, the cancer money targeted toward the molecular genetics approach noticeably decreases, the better young Postdocs will see the writing on the walls and move elsewhere. It would thus be a tragic mistake now to rebalance money away from the virus area in order to help protect less-strong facets of the NCI program from the ravages of inflation and mounting entitlements. The effect on NCI could easily be that of a partial lobotomy.

We are now out of the pre-recombinant DNA days when tumor virus nucleic acids provided the best way to get at the eukaryotic genome. Tumor virology no longer seems the only place to go, independent of whether or not you are interested in cancer. It is only one of many fields in the process of intellectual explosion. Merely attending our annual RNA Tumor Virus meeting with 450 other highly competent retrovirologists has to bring up internal doubts as to whether today's intense competitive hassel is worth the obligatory psychic strain. For in today's fiscal stringency, your grant could have a priority score of 185 and not with certainity be funded.

If, however, you are on the other side of the fence with the consumer desire to at last cut back the ravages of cancer, then the more clever intellects that we can hold in cancer research the better. Only dismal would be the prospect of a drift back to the deserved second-rate reputation that all too long shackled cancer research.

Even, however, if federal cancer money continues to flow, we must face the increased competition for bright young brains that is already here from the creation of the many new recombinant DNA companies as well as from the major chemical giants that now privately proclaim that they may only have a long-term future if they not only embrace, but also succeed, in biotechnology. No longer do we worry that academia may not provide enough slots for our Postdocs. Instead, our concern is that the ever-descending level of academic pay will prove inadequate to attract and hold the superior faculties in molecular biology that will be required to turn out the students that industry says it will devour. It is thus by no means certain that cancer research can maintain its present upward intellectual spiral even if we maintain its funding at an inflationary corrected steady level.

I thus see no choice now but for the NCI and its NIH parent to look much more closely at its various divisions to see which programs really are effective and which are really there only because no one wants to blow the whistle on good intentions gone astray. Do we, for example, really need an ever-growing super National Library of Medicine, or would medical science be effectively the same were the striking new NIH edifice never built? And how much does the clinical research done by doctors playing part-time medical scientist, using NIH grants for the technicians who do their lab work, actually advance the pace of medicine? Were we overly ambitious in trying to turn virtually all medical schools into high-quality centers of medical research? To cut off the less-productive schools from most of their NIH lifelines, however, would cause havoc with countless careers. But do we have a choice if we are to provide our best scientists with the funds that will permit them to remain capable of deep, as opposed to play, science?

It is important here to emphasize that, except

for the NCI, we have been painfully shedding the fat from our individual NIH grants ever since the last days of Lyndon Johnson; and for the last several years, the same has been true for the NCI. Significant further retrenchments will most certainly be at the expense of future quality. The average salary paid academic scientists has not kept pace with inflation, and we have reached the state where either two bread winners per family or some moonlighting has become a prerequisite for a modestly adequate living. We are, furthermore, not only suffering from the use of equipment past its prime, but also from having to live with falling supply budgets, which soon must lead to a cutback in the number of good experiments. At least in this laboratory, I don't see much room for additional supply savings that will not compromise our productivity, and my impression is that a similar situation exists in most other leading institutions. Almost everywhere the general picture seems to be of overruns in supply budgets that soon must lead to actual lab shutdowns until the deficits are paid back.

Thus, with the nature of science getting more sophisticated and inherently more expensive per scientist, there is no way we can live with even an inflationary corrected level of supply funds without cutting back the number of scientists in our labs. Only with a steadily increasing real budget can we remain the same, and that we shall be lucky to get from Congress. It thus seems almost inevitable that the number of scientists doing federally sponsored cancer research will steadily fall, with many of the better younger scientists leaving pure research to do industrial research. In a real sense, we may soon be witnessing the replacement of competent scientists by microprocessor-enlightened machines that can do their jobs cheaper, if not better. Of course, if we had more money, we could have even more scientists as well as high technology. At some funding level, however, society has to say it can give its scientists no more, and that is the message that we now hear everywhere.

The fostering of high-quality research on cancer thus may now require the serious pruning away of the not quite best. How to distinguish the best from the almost best will never be easy, and in doing so we are bound to make wrong choices and cut individuals out of scientific careers that they have been given every indication to expect. Wielding this particular axe will be a most unpleasant task, but it is a job that the NIH (NCI) directors can no longer avoid. As long as we were living on growing budgets, the hard choices could be avoided. Now, either reaffirming the importance of tumor virus research and keeping up its magnificent upward crescendo or upgrading the equally important area of chemical carcinogenesis research cannot be passive deeds. Such acts can only be done by deciding that other aspects of cancer research have lower chances of making home runs, and such pronouncements would be bound to offend many long-time, powerful supporters of cancer research. But, as I already know too well, the function of a director is to lead, not follow, and we can only hope that the leaders of NCI (NIH) and their counselors will do their jobs well.

Highlights of the Year

An Extraordinary Set of Monoclonal Antibodies Directed against the Nerve Cells of the Leech

Often characterized as the ultimate biological challenge and of key interest to virtually all scientists is the way in which nerve cells are specifically linked together to form the neural networks that underlie the phenomena of perception, memory, and learning. That a problem is intrinsically interesting, however, does not mean that the time is at hand for its solution. Witness the difficulties embryologists have had in making any fundamental conceptual advances over the last fifty years. Good intentions by themselves get us nowhere, and the most savvy of the scientists who work on nerve cells have been careful not to ask now for the ultimate of answers, knowing that until the circuitries of the brains that intrigue them are known, speculation on their functioning will be a hopeless pursuit.

That being so, there are many reasons to seek out the simplest of brains that control demonstrable behavioral traits and to study them with every conceivable neuroanatomical trick. Here we have chosen to work on the interconnected nerve cells of the medicinal leech. We were first introduced to it seriously in 1974, when John Nicholls began teaching a summer course on its neurobiology and had among his first group of students Birgit Zipser. Excited by what she learned, Birgit soon after took up the leech as her main research tool and brought this system to Jones Lab when she joined our staff two years ago. Each leech nerve cord contains some 12,000 nerve cells, organized into some 30 segmentally compartmentalized groups that are called ganglia. Each ganglion, like the leech itself, has bilateral symmetry, and the some 400 nerve cells of each ganglion are organized into identical, equally sized, left and right groups. All the ganglia are not exactly the same: those at the head and tail are more complex and function in brainlike capacities, and two of the ganglia, called five and six, have special cells that control sexual behavior. We also now semi-know the roles played by several other morphologically distinct cell types, as well as the locations of a number of the synapses that connect together functionally related cells. Totally lacking yet, however, are any general ideas about how the nerve cells make their connections to each other during embryological development or detailed knowledge about the many intercon-

necting nerve circuits that must exist to generate the complex behavioral responses of the leech. Until recently, even dreaming about collecting such information was out of the question. Except for a few cells that have easily distinguishable morphologies, most of the nerve cells look roughly alike, and knowing with which specific cells we are actually dealing is often beyond our capability.

To generate better ways of tagging nerve cells, Birgit joined forces here with Ron McKay, who had just arrived from England, where he had been introduced to monoclonal antibodies in Walter Bodmer's Laboratory in Oxford. There he had already attempted to generate monoclonal antibodies to mammalian nerve cells, but no interesting specificities had come out. Upon coming here and learning about the leech, Ron first injected leech nerve cords into mice. Then, after the mice began to generate specific antibodies to the leech nerve cells, he fused the respective antibody-producing spleen cells with mouse myeloma cells to generate a large number of hybridoma cell lines. Most excitingly, Ron and Birgit already have identified some 40 different hybridomas, each of which produces a specific monoclonal antibody that binds to one or more specific nerve cells in each ganglia.

This finding of so many different specific antibodies surprised us, for until the experiment was done, we thought we would be very lucky to generate even one or two. The prospect of coming up with 40 different specificities at one blow was much too good to have as a serious objective. Still unknown are the nerve cell antigens to which our antibodies bind. As a first step to solve their identity, we have been fortunate in persuading Susan Hockfield to join us to use immunoelectron microscopic techniques to see to which cellular component(s) the various monoclonal antibodies bind. Before coming here, Susan was at UCSF, in Alan Bassbaum's Lab, studying pain receptors and came to learn of Birgit and Ron's monoclonal antibodies through her participation in our Pain Workshop held this past summer.

Now we are very excited by the possibility that many of our monoclonal antibodies are directed against those components of nerve cells that provide the biological specificities used in telling nerve cells how to link up with each other. Before we found them, we had no idea as to how to even begin looking for such linker molecules. Hopefully, monoclonal antibodies will provide the means to find the molecules that determine the high specificity of neural connections. Clearly, we are at the beginning of a very exciting chapter in Neurobiology, and we are most pleased that we may have almost immediately been thrust into a vanguard role.

Most crucial for our ability to push the monoclonal antibody approach in a big way has been the help provided by the Marie H. Robertson Fund for Neurobiology. Not only did it let us act swiftly in adding Susan Hockfield to our staff, but it also provided funds for a most useful small meeting in November on Monoclonal Antibodies to Neural Antigens. This was the second of two meetings on the nervous system held this year at the Banbury Conference Center. Like the first such meeting, on Molluscan Nerve Cells, the proceedings will be published by the laboratory as a Cold Spring Harbor Report in the Neurosciences. We plan relatively short volumes of some 200 pages each for this new series, which will arise exclusively out of the Neurobiology programs at the laboratory.

Our Summer Neurobiology Program Also Continues to Grow

Again this summer we put on a most impressive collection of courses and workshops in Neurobiology. Most had been successfully held in previous years. We did, however, add a new workshop on Perfusion Techniques. It brought together individuals from different home labs who had independently devised techniques to perfuse nerve cells. The merits of their respective methodologies could thus be directly compared. We also included as participants an equal number of experienced neurobiologists who wished to extend their repertoires to include perfusion. Everyone agreed that this workshop went very well, and we were most fortunate that the Marie H. Robertson Fund for Neurobiology could provide the funds that made it possible.

This summer marked the tenth consecutive year during this modern era that we have offered courses in Neurobiology. This program has consistently attracted very high level students and is an effort that we and its many instructors can be most proud of. From its inception, we have thought about rotating instructors and course topics to give us the renewed vigor that comes from new ideas and new faces. In fact, our program now bears little resemblance to our initial offerings. We should point out, however, that our course in Experimental Techniques has been most ably run by JacSue Kehoe since its beginning ten years ago, and this coming summer JacSue will again be in charge. Year after year it receives nothing but strong praise, and we see no point in replacing it for reasons of theory. Equally successful has been our Leech Course, led for the seventh consecutive time last year by John Nicholls and again to be offered this coming summer.

Most happily, Sir Bernard Katz, from University College London, was able to participate in our Synapse Course. Virtually no other neurobiologist has equalled his many contributions to the biology of synapses, and our program was indeed honored by his presence. While here, he and Lady Katz enjoyed the gracious hospitality that we can provide at Robertson House. The nature of our summer program was also most pleasantly en-

livened by having our Trustee from Yale University, Chuck Stevens, and his family in residence at Osterhout Cottage all summer.

Unfortunately, the ever-growing success of our Neurobiology summer program has not yet generated the prospect of long-term financial stability that we need for the successful planning of its future. Except for the first several years, when we had our initial Sloan Foundation Grant, we have had to use significant internal funds in order to keep its momentum growing, as well as spend weeks, if not months, of each year attempting to find private monies to supplement the never quite dependable federal funds, which in the last analysis must carry the main burden for a program now as large as ours. Here I would like to express our thanks to the Klingenstein Family, to Milton Cassel and the Rita Allen Foundation that he helps direct, and to the Volkswagon and Grass Foundations for giving us much essential last-minute assistance. Their inability to help would have meant the cancellation of one to several specific courses and workshops.

Now given the prospect of even less federal money for conferences, workshops, and advance training, we must undertake even more serious efforts to find support from the few major foundations that have the potential to help us in a big way during what clearly will be a rough time for so much of science. Our program has now gone on long enough to erase any doubts as to whether we do our job well. It thus becomes a question of whether the outside private world will give us the vote of confidence that will allow us to maintain a major national asset whose objectives are not likely to be taken up elsewhere if we were to pull back. Hopefully, such help will be forthcoming, since the financial burden we have had to carry internally for so many years may soon be more than we can bear.

Isolation of Viral Cancer Genes Remains a Prime Objective

When we initiated our research on the DNA tumor viruses we did so with the hope that our experiments, together with parallel work in other labs, would show that tumor virus DNAs contained regions that coded for proteins that could make cells cancerous. This expectation has been more than fulfilled, and cancer genes are no longer speculative dreams, but crisp molecular entities whose biochemical role we now seek to work out precisely. Having first determined their existence, our next task has been to obtain their respective protein products in amounts sufficient to do serious biochemistry. At first, we had to rely heavily on chance events like the finding here of an adeno-SV40 hybrid virus that overproduces the SV40 T (tumor) antigen. This allowed Bob Tjian, then a member of our staff, to show clearly that the T antigen specifically bound to several sites on SV40 DNA around its origin of DNA replication. Now our best guess is that T antigen plays a key role in the initiation of DNA synthesis, an hypothesis that some years earlier had already seemed likely from properties of temperature-sensitive mutants within the T-antigen gene. To go further and show exactly how the T antigen functions most likely will require the setting up of the appropriate in vitro systems for eukaryotic DNA synthesis. This is a field that still has not caught real fire, and if we are to succeed here, we as a laboratory must return to a major commitment in the field of DNA synthesis. Toward this end, we have recently appointed to our staff Fuyuhiko Tamanoi, originally a student of Reiji and Tuneko Okazaki in Nagoya and most recently a Postdoc of Charles Richardson at Harvard Medical School, where he did important work on the in vitro synthesis of T7 DNA.

At this moment, most of our work on DNA synthesis involves adenoviral DNA, whose terminal "Bellet" protein has recently been shown, through the collective work of many of our scientists, to be encoded by the adenoviral DNA. As far as we can tell, the Bellet protein is not involved in adenoviral oncogenesis, with that role being played by several of the early proteins encoded by genes at the extreme left end of adenoviral DNA. As yet, we still have not obtained these proteins in sufficient amounts to let us test directly whether they play a role in DNA synthesis. A prime objective at this moment is to genetically engineer the adenoviral early genes so that their respective protein products are overproduced in quantities that will let us do real biochemistry. Most helpful here will be the obtaining of monoclonal antibodies that will facilitate their purification. Already a monoclonal antibody specific for the early-1B-region 55K protein has been obtained, and we anticipate that soon there will be a stable of others, many of which should prove to be indispensable tools for our long-term efforts to establish the fundamental chemical features of cancer cells.

Our Symposium Focuses on Movable Genes

Experiments showing that some genetic elements do not have fixed locations, but can move from one chromosomal site to another, were first done here by Barbara McClintock in the early 1950s using the corn plant. Now "movable genes" are cropping up everywhere in biology, and it seemed most appropriate for us to use the format of our 45th Annual Symposium to hold a major meeting on this topic. Ahmad Bukhari and Jim Hicks worked hard and most effectively as our main organizers, almost miraculously keeping the formal program within bounds. It attracted some 280 leading genetics-oriented biologists, happily including many of our best friends of past years. A splendid opening lecture was given by Allan

Campbell, and Michael Yarmolinsky gave what was generally regarded as the most intelligently polished summary of at least the past decade.

Only slightly more than one-half the attendees were able to listen to the lecture in Bush Auditorium, with the rest watching in other rooms on our closed-circuit TV system, again most ably presided over by Herb Parsons. This is far from a perfect situation, and we have now realistically accepted the need to build a major new auditorium. This past year we committed funds to commission our architects, Moore, Grover, and Harper, to come up with a schematic design for a building to seat 360 and to be located across Bungtown Road from Bush Lecture Hall. At the current value of the dollar, we project a cost of around 1.5 million dollars, a sum that now seems too large to be raised by a conventional fund drive. So we are in active pursuit of a suitably enlightened single philanthropist, who by giving us the new auditorium that we must have if our symposium is to retain its premier rank also will be providing our community with a building we are already sure will be a visual delight.

The Pace of Recombinant DNA Research Quickens

By now so many scientists here are doing recombinant DNA experiments that it is hard to remember back to even three years ago when the then-effective NIH Guidelines still virtually blocked any such experimentation with tumor virus DNA as well as greatly slowed down most experiments with higher eukaryotic DNA. Now the guidelines are almost gone and the P3 facilities we once briefly almost uniquely possessed have little statutory use and soon may be totally unnecessary. Because we already possessed containment areas for tumor virus research, we luckily did not need to spend vast sums, unlike many other institutions, to bring ourselves into the recombinant DNA game. Now our need to prepare the occasional "memorandum of understanding" continues to waste valuable hours, but hopefully even that requirement will soon be gone. What a pleasant contrast with those bleak days, just five years ago, when many in the general public feared that DNA might be a major environmental hazard and so need the most stringent regulation.

Major Program Project Support for Our Studies on Vertebrate Gene Structure and Expression

Through the successive additions of Mike Wigler, Dave Kurtz, Steve Hughes, and John Fiddes to our staff, we now possess a very powerful group of younger scientists collectively interested in the structure and expression of vertebrate genes. All utilize as their main experimental tools the various recombinant DNA procedures, and each should benefit from maximum intellectual interaction with each other. To help facilitate such cohesive efforts, last May we applied to NCI for a moderately large program project grant to cover key salary support, major items of shared equipment, and necessary supplies such as the never inexpensive restriction enzymes. I assumed the task of program director and, like the others, anxiously awaited the site visit that came in October. Happily, we were judged well, with formal grant support starting April 1, 1981.

Among the most attractive features of the grant are the key-staff salary support that it guarantees for five years, thereby giving us a most-needed source of semi-long-term financial stability. Now every two weeks we hold Vertebrate Gene Group

Advanced Bacterial Genetics course participants and instructors

seminars in which current research is discussed, and a happy future for this new scientific division seems assured.

To help provide the new research facilities needed for this program, an extensive remodelling of James Lab has occurred. In effect, a new lab was provided for John Fiddes, Steve Hughes, and their collaborators, and much totally modernized working space became available both for our tumor virus group and for the courses that are taught each summer on the second floor. Now, perhaps for the first time in its history, James Lab lacks that stale smell that necessarily accompanies the improverished makeshift.

Unparalleled Interest in Our Summer DNA Courses on Genetics and Cancer

This past summer the number of students applying for our courses on genetics and cancer reached a new high. Our new Cell Culture Course, emphasizing the employment of hormones as growth factors and taught by Gordon Sato and David Barnes, was very well received, as was our Transformed Cell Course, to which was added a section on teratomas and teratocarcinomas taught by Gail Martin. Over the past several years, we had watched a perceptible falling off of interest in our Animal (Tumor) Virus Course that we had been presenting for over a decade, reflecting the fact that this material is now taught well in most major academic centers. To replace it, we started up a brand new course on Molecular Cloning, with Tom Maniatis, Ed Fritsch, and Nancy Hopkins as instructors. It quickly proved to be the most popular course we have ever offered, with 172 applicants for its 16 places. Also still very popular was our course on Advanced Bacterial Genetics, which has been taught for the past four years by the never-quiet trio of David Botstein, Ron Davis, and John Roth. Unfortunately, we have not been able to tempt them to return again this coming summer, but we remain much in their debt for the intellectual esprit they have so long conveyed. Teaching the Yeast Course this year were Brian Cox and Cal McLaughlin, substituting well for Jerry Fink and Fred Sherman, who, after ten years, had a summer sabbatical during which they taught a parallel course in Brazil. Happily, Jerry and Fred have consented to return as instructors this coming summer. Because of its popularity in 1979, we offered for a second time a course on the Molecular Biology and Developmental Genetics of *Drosophila*. It was again most ably taught by Mary Lou Pardue and Bill Gelbart. This coming summer we shall be offering in its time slot a new course on the Molecular Biology of Plants to introduce molecular biologists to the many opportunities to revolutionize plant breeding that recombinant DNA techniques may bring.

The Transformation of Davenport into Delbrück Laboratory

With the growing use of biochemical procedures in our genetics courses, the necessarily cramped quarters provided by Davenport Lab have proven increasingly inadequate from the point of view of both students and instructors alike. Equally troublesome has been the strain placed on our Yeast Group by having to give up their research space during the summer months. New laboratory space was clearly demanded, and toward this end we began construction in October of a major addition to Davenport that will effectively double the space available for research and summer teaching. Designed to fit in harmoniously with Davenport's Colonial Revival style, the new addition converts this building into a complex that can function on its own and not be dependent upon the availability of facilities in nearby buildings. Thus, it seemed appropriate to give the complex a new

Delbrück Laboratory under construction

name. So, beginning last fall, we began to call it Delbrück, to honor Max Delbrück and the Phage Course that he initiated there in the summer of 1945. The harshness of this past winter slowed down the early construction phase, but work on it is now almost back on schedule. The Yeast Group can thus occupy its new labs before the start of the summer season, with the formal dedication to occur at the end of August during the Phage Meeting.

The Carnegie Dorm Becomes Davenport House

By November we finished the renovation of the interior of the large Victorian building on the corner of 25A and Bungtown Road that was constructed between 1882 and 1884 by John D. Jones, the founder of this Laboratory. Long the residence of Dr. Charles B. Davenport, who directed the lab between 1898 and 1934, it later was turned into a dormitory for use by the Department of Genetics of the Carnegie Institution of Washington and gradually became known as the Carnegie Dormitory. With time, its physical shape gradually fell into greater and greater disrepair. Now, thanks to the super remodelling effort presided over jointly by our Superintendent Jack Richards and by my wife Liz, it has been brought back to its original Victorian flavor. Particularly pleasing are the stencilling and wood graining that we luckily were able to have done by the talented English artist and craftsman Tony Greengrow. Now at last it has become a very functional home for eight of our scientists. To celebrate its refinished state and the formal change of its name to Davenport House, in late November a special Sunday dinner was prepared in its new kitchen for the several descendents of Mr. Jones who still live in our midst and who so long provided support for us through their Wawapex Society.

A key feature of the renovation effort was the redoing of the main parlor room into a Music Room, to which we have just added a marvelous new Baldwin piano. Its acquisition was made possible by gifts specifically targeted for this purpose. We now possess a room appropriate for music at its finest, and we look forward to the cultural enrichment that it shall bring to our village, scientific style.

The Naming of Our New Cancer Research Facility after Reginald G. Harris

Construction finally started in November on the major new cancer-research-oriented building that we shall use to house small animals and to provide more suitable space for the generation of large numbers of monoclonal antibodies. It will be called the Reginald G. Harris Building, to honor the far-sighted director (1924–1936) of the Long Island Biological Research Laboratories, then directly operated on our lab grounds. Particularly important was the initiation by Harris in 1933 of our Annual Symposium as an occasion for the quantitative thinking that would help bring into Biology the then newly discovered concepts of modern Physics and Chemistry. Without his Symposia and the worldwide reputation that they brought to Cold Spring Harbor, serious science might have long vanished from this scene. It is only appropriate, therefore, that the Harris name be

Liz and Jim Watson in Music Room of renovated Davenport House

indelibly fixed onto one of our major research facilities. After many delays due to bad weather, the Harris Building is now rapidly assuming its final shape, and we hope to begin using it late this coming fall.

Indispensable Help from the Robertson Research Fund

From the moment of its inception, the Robertson Research Fund has given us the flexibility to start up new projects. Particularly important during 1980 was the help it provided in letting us totally renovate and equip major new labs for John Fiddes and for Fred Hefferon and Maggie So. It also proved invaluable in letting us attract two talented young scientists who already have had several postdoctoral years of research. Coming this year as our first Robertson Research Fellows have been Kenji Shimizu to work on cancer genes in Mike Wigler's lab and Kim Nasmyth to join the Yeast Group and its work on the mating-type gene complex. In addition, through Robertson funds we had at our disposal adequate funds for a number of projects for which federal funding could not cover true costs.

Major New Additions to Our Scientific Staff

This past summer we lost from our scientific staff two of its most invaluable members: Jim Lewis left to join the staff of the Fred Hutchinson Cancer Center in Seattle, and Dan Klessig moved on to Salt Lake City to become an Assistant Professor of Microbiology at the University of Utah Medical School. Both played key roles in the discovery of RNA splicing and we shall long miss them. Leaving here following their postdoctoral work have been Mike Dubow to move to McGill University, Bill Kilpatrick to go to Wake Forest University, Leah Lipsich to return to SUNY, at Stony Brook, and Nigel Stow to take up a staff position at the Institute of Virology in Glasgow.

Appointed to Staff Investigator slots at the completion of postdoctoral periods here have been Manuel Perucho, Ron McKay, Bruce Stillman, Jim Stringer, and Jim Lin. Coming here in November, also at the Staff Investigator level, was Fuyuhiko Tamanoi. And arriving in Jones Lab as a Staff Investigator just before the year ended was Susan Hockfield. Promoted from Staff Investigator positions to Senior Staff Investigators were Jim Feramisco, Amar Klar, Dave Kurtz, Jeff Strathern, and Yakov Gluzman. Newly appointed to our staff as a Senior Staff Investigator in James Lab to begin January 1, 1981, is Lee Silver, formally a postdoc with Dorothea Bennett at Sloan Kettering, who brings to us detailed knowledge of mouse genetics and embryology.

Promotion to the Senior Scientist Position

Our rank of Senior Scientist carries with it a continuous rolling commitment of five years of salary support. Given our inherent dependence on "soft" federal funds, this commitment represents our closest approximation to a tenure position and signifies that we believe the scientists concerned have the capability for sustained, self-motivated, high-level research. Acting at their late spring meeting, our Board of Trustees approved the well-deserved promotion to Senior Scientist of Mike Mathews, bringing to seven the number of such positions on our scientific staff.

We Continue to Have Very High Level Support and Administrative Staff

That the doing of science here seems such a natural activity owes much to the dedicated efforts of our highly talented support and administrative staff. Here I should point out our great indebtedness to Agnes Meyer, who retired this January after 15 years of cheerful, efficient service as our switchboard operator; Winifred Modzekeski, who, following her remarriage, left our Meetings Office where for 3 years she personified helpful consideration; Eileen Oates, who for 11 years worked so successfully in our Accounting Office; and Jim Stanley, who retired from our Buildings and Grounds Department where he had assisted our Superintendent Jack Richards so faithfully for the past 11 years. I must also mention the abrupt and tragically premature death of Robert Anderson in February, 1981. Coming to Long Island from Scotland some 20 years ago, Bob, in his capacity as head of our Housekeeping Division, was a loyal friend to all and we continue to mourn his loss.

Robert Anderson

Our List of Superior Books Continues to Grow

Again we report another year of high-level publishing. Three new books in our Monograph series were published this year. Two concerned *Transfer RNA*, one on its structure and physical properties, the other on its biological aspects. Both were edited well by Dieter Söll, Paul Schimmel, and John Abelson. *DNA Tumor Viruses*, edited by John Tooze, represents an almost total revision of material published in much less complete fashion in our earlier volume *The Molecular Biology of Tumor Viruses*, which also was edited by John. We anticipated that *DNA Tumor Viruses* would sell better than the average monograph and so priced it lower than if we had based its price upon the number of pages. Already the first printing is sold out, and we are in the process of bringing out an even lower priced revised paperback edition, which we hope will further increase its readership. This practice of going into paperback after we have covered our publishing costs was most effectively employed with *The Operon*, edited by Miller and Reznikoff. In less than a year, we have almost doubled its sales.

The publishing of our annual Symposium (Viral Oncogenes) was as expected again a major task. Because of delays in getting type set in Dublin, we did not have finished volumes until mid-July. So this year we have returned the typesetting process to this side of the Atlantic with the hope that it will be out before the start of the next year's Symposium. Also published this year was a two-volume set on *Viruses in Naturally Occurring Cancers*, which represents the proceedings of our intellectually most valuable Conferences on Cell Proliferation.

Appearing late this year to be sent to more than 1000 advance orders was the first of our lab Manuals for Genetic Engineering. Arising out of our Advanced Bacterial Genetics Course, it was prepared by the course instructors, Botstein, Davis, and Roth, and it already seems destined to become a minor best seller.

Our total sale of books, abstracts, and manuals for 1980 totalled 16,041 in comparison to the very similar figure of 16,300 for 1979.

Another Summer of Talented Undergraduate Research Students

Beginning in 1958 we started to bring here, on a formal basis, a group of ten college undergraduates to do research for periods of 10 to 12 weeks under the supervision of members of our scientific staff. For many years, this Undergraduate Research Program was supported by the NSF, but in early 1973 the NSF changed its rules to preclude awards to non-degree-granting institutions. To continue this program, we then had to dip into our always scarce internal funds designated for research. So we have greatly welcomed the support given to this program over the past two years by the Camille and Henry Dreyfus Foundation. Most fortunately, they will again support us this coming summer. So we again await the arrival of a student group that we find increasingly motivated toward future careers in pure molecular and cell biology as opposed to medicine.

I also wish to acknowledge the funds provided by our own Robert Olney Memorial Fund, which now supports one additional student per summer. Unlike those students supported by Dreyfus Funds, who afterward return to college, generally for their senior year, the Olney Fellowship is usually given to a student who has just completed his undergraduate years.

Increasing Use of Our Banbury Lane Facilities

A number of high-level small conferences were held at our Banbury Center during the spring and fall, in addition to the use of this site for our summer lecture courses in Neurobiology and for the housing of guests during our major meetings on the main laboratory grounds. Three key meetings related to biological risk assessment were organized by Victor McElheny. One was devoted to labeling dangers, another to the role of diet in gastrointestinal cancer, and the third to hormones and breast cancer. We also used the Banbury Meeting House for two specialized Neurobiology Meetings supported by the Marie H. Robertson Fund for Neurobiology. Both of these meetings were so organized as to give rise to short books consisting of reports prepared by advanced students in attendance and vetted afterwards by the meeting organizers. The first such book, *Molluscan Nerve Cells*, has appeared, already with most favorable reviews, giving us hope that we may have found the right formula for reporting these meetings to the much larger audience that books can reach. We also brought to the Banbury Center some 50 scientists in early December to discuss their recent work on Cloning Vectors for Mammalian Cells, now a very hot topic in the world of recombinant DNA.

Almost completely finished and about to be dedicated this coming July is our new residence hall, Sammis Hall. It provides us with 16 additional bedrooms, bringing to 36 the number of guests that can simultaneously be housed at Banbury. Until its completion, we happily had at our disposal Fort Hill, the magnificent home of the Mrs. Willis D. Woods that overlooks Center Island and the entrance to Oyster Bay Harbor. Late this year, at the age of 98, Mrs. Woods passed away after several years of impaired health that unfortunately followed more than 90 years of vigorous, inspired living.

LIBA Successfully Completes Its Fund Drive for the Carnegie Land Purchase

An institution that has consistent, strong, and loyal backing from its local neighbors is indeed lucky, and this has been our situation for all of our 90 years. This support is now most effectively channeled through the Long Island Biological Association (LIBA). Mr. Edward Pulling, its chairman, and its Board of Directors are always on the lookout for new projects that they can help bring to fruition through one of their customary low-keyed fund drives. Over the past two years they have mounted a $200,000 campaign to cover the costs of the land that we purchased from the Carnegie Institution of Washington. This was a most-necessary acquisition, for we needed part of this land to site our new Harris Building. Completion of this drive came ahead of schedule, allowing Ed Pulling to announce its success at our Annual Meeting held early this January.

We are most fortunate to have such wonderful friends.

Dr. Harry Eagle

Major Changes in Our Board of Trustees

The running of this lab has never been a simple matter, and as the Director I need many sources of advice and encouragement. I have been very fortunate in that I have always served under a Board of Trustees who have been unstinting in their ability to do real work on our behalf and whose long-term affection for us has never been in doubt. During these years no Trustee has been more invaluable than Harry Eagle, who for the last six years has served as Chairman of the Board. Harry became a Trustee, representing the Albert Einstein College of Medicine, in 1962, following the reorganization events made necessary by the withdrawal of support by the Carnegie Institution of Washington. Quickly he proved invaluable to the weak, fledgling organization and helped John Cairns to make the many improvements present when I took over as the Director early in 1968. Because our by-laws limit consecutive service on the Board of Trustees, Harry went off the board in 1967, happily to return in 1973. A year later, in 1974, he became our Chairman, functioning thereafter very closely with me and the other members of our Executive Committee. Always optimistic in an intelligent fashion and with a firm, no-nonsense command of his Board, he has helped lead us to heights unimaginable without his presence. Thus, his obligatory retirement this fall after six years as our Chairman is a deep loss, though we know we can still count on his help in potential moments of crisis in his capacity as an Honorary Member of our Board.

In his replacement as Chairman, Walter H. Page, we shall continue to be led by a person of the highest experience and integrity, and we have no reasons to fear our future. Bayard Clarkson, also of long past experience on the Board, became Vice Chairman; Robert Cummings is our new Treasurer, and Norton Zinder our Secretary.

Also retiring from our Board at our Annual Meeting were Clarence Galston, who has served with much faithful dedication as our Treasurer; Vittorio Defendi, on whom we could always count for invaluable council while on our Executive Committee; and Walter Guild. Newly elected to represent NYU was Claudio Basilico; Bob Webster returns after a three-year absence to again represent Duke University; and Matthew Scharff becomes the Trustee representing the Albert Einstein College of Medicine. Elected as a private Trustee was our Lloyd Harbor neighbor John Carr, a major figure at the Grumman Corporation, where he is now Vice Chairman of the Board.

Our Currently Sound Financial Picture Must Be Monitored Closely

This year, like those of the immediate past, we have operated under a conservative budget. In preparing for each coming year we try to project income on the low side and expenses on the high side, so balanced that our cash position at the end of the year will be equal to that at the start. We do this almost automatically by being very cautious about the sales of our books while projecting on the low side the interest income from the cash balances we hold in reserve to cover construction projects that we are already committed to start. Until now, this way of proceeding has generally led to positive cash flows that more than account for the increasingly large sums that we automatically set aside to cover the depreciation on our

buildings and our equipment going out of date. As soon as we know that we are again going in the black, we commit the newly added cash for still further improvements to our physical plant. It is through such procedures that we have been able to return so much of our formerly dilapidated physical plant to its now pleasingly effective state.

I worry now, however, that the next several years will not be so fiscally rosy. In the last analysis, the sales of our books depend upon the receipt of government grants by their purchasers, and if individual grants are squeezed much further, the choice to buy our books won't even exist. We may be particularly vulnerable because the massive lengths of so many of our volumes demand correspondingly high prices just to cover our expenses, much less to produce a minor profit with which we can further upgrade ourselves. We should thus be prepared to produce more short books, aimed at the rapid, clear appearance of the newest facts, as opposed to the highly comprehensive monographs that have so characterized our lists from the past decade. Already we think we see an increasing preference for our less comprehensive books, but it will take several years before the nature of our list can appreciably change.

Further clouding our fiscal health are the rapidly escalating oil, gas, and electric bills from which we try to escape but cannot. And for too many years we have skimped more than makes long-term sense on the maintenance of our newly restored buildings. Our best guess thus is that our current way of proceeding will soon no longer produce the substantial infusion of free money that has been at the heart of our physical revival and that must continue to be available if we are to remain truly innovative.

We thus have given serious consideration to the upgrading of our efforts to raise funds from the national, as opposed to our local, community. Over the past decade we have probably devoted less resources to "developmental activities" than any equivalent institution of our size. Now my occasional letter or visit to a major foundation office is our only such thrust. Over the next few years, however, we probably have no choice but to abandon our anomalous ways and seek more professional help. I must confess, however, that the biting of this bullet will not be easy.

We May Need to Immerse Ourselves Increasingly in the Outside World

Because of the high quality of our science and the prominent role that our courses and meetings now play in American science, we shall probably only be moderately hurt, not badly mangled, by the initial thrust of the current administration effort to take the government off the back of its people. Obviously, we have fared very well recently with federal support, much better than we ever did when private benefactors made or broke us. So we do not in any way mourn for the supposedly good old days of unrestrained private initiatives like those existing in 1932. Then our director Reginald Harris in his Annual Report listed suicide as one of the alternatives open to his lab as our nation sank deeper and deeper into the great depression that filled our streets with able men out of work. Now, wounded in our discovery that we cannot remain the best while resting, we nevertheless remain a nation with the capacity to promote science for all its worth, and it is my belief that we have no other choice but to so act. The withdrawal of our government from the support of science, as now suggested by the high priest of monetarism, Mr. Milton Friedman, would be an act of national hari-kari, and his recent *Newsweek* articles only prove that intellectuals of the left do not have a monopoly on silliness.

Even assuming, however, that sizable sums of federal monies continue to flow our way, our next decade is unlikely to follow closely the pattern set in the 1960s and 1970s. Then we still operated effectively as pure academics, indifferent to the possibility that the marvelous edifice of modern Biology that we were creating was putting us in the same big league as the Chemistry and Physics that first produced the industrial, and more recently the electronic, revolutions.

Now, through recombinant DNA and monoclonal antibodies, we may have the capacity to help change the course of much of our industry and agriculture as well as of medicine. Thus, even if we should want to remain aloof, the outside world needs badly to buy into our lives, and the real question is not whether we make our forays out of our ivory towers, but how. These adventures will not come naturally. Yet, if we hesitate too long, proclaiming the sanctity of the pure mind, we may easily find that we are not among those institutions with the financial clout necessary for the promotion of truly innovative science.

If, however, we can continue to be intelligently nimble, employing our wits to seek out the best of the industrial as well as of the academic worlds, we may be able to leave this decade as happily as we now somewhat apprehensively enter it.

May 29, 1981 *J. D. Watson*

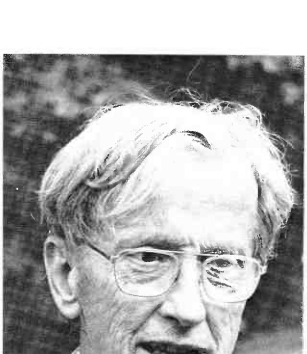
M. Delbrück and W. M. Stanley at 1941 Symposium

Vernon Bryson, Max Delbrück, and Rollin Hotchkiss at "Graduation Exercises" of the 1950 Phage Course

Max Delbrück

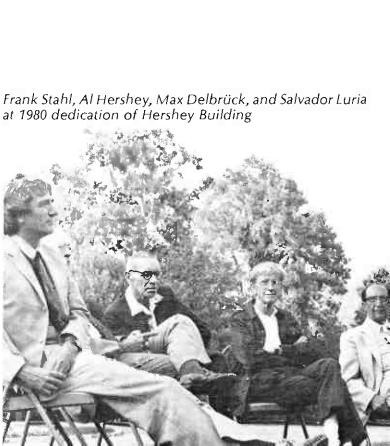
1906–1981

With great sorrow, I must report the passing of Max Delbrück on March 9, 1981 in Pasadena in his 75th year. Through his great intelligence and clarity of expression, his extraordinary integrity, and his affection for the many friends whose lives he helped to form, he cast an indelible mark on the development of Molecular Biology. His love for Cold Spring Harbor was manifested in many ways, and our future summers will never be the same without him.



Delbrück with wife, Manny, and daughters, Nicola and Ludina, at Cold Spring Harbor, 1963

Frank Stahl, Al Hershey, Max Delbrück, and Salvador Luria at 1980 dedication of Hershey Building




WARDOUR
ZANSKAR

YEAR-ROUND RESEARCH

TUMOR VIRUSES

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

MOVABLE GENETIC ELEMENTS

Molecular Genetics
Insertion Elements and Plasmids
Yeast Genetics

VERTEBRATE GENE STRUCTURE AND EXPRESSION

Mammalian Cell Genetics
Hormonal Control of Gene Expression
Genes for the Major Structural Proteins
Polypeptide Hormone Genes

CELL BIOLOGY

Cell Motility
Cell Biochemistry
Quest 2-D Gel Laboratory

NEUROBIOLOGY

Neurobiology Laboratory
Neurobiology Workshops:
- Invertebrate Neurobiology Workshop
- Intracellular Staining with Lucifer Yellow
- Exploratory Synapse Workshop
- Pain Workshop
- Perfusion Workshop

First row: M. Perucho, M. Wigler, D. Kurtz, D. Levy, C. Lama, D. Bishop, K. Shimizu; J. Stringer, A. Barros, C. Stephens, W. Boorstein, J. Fiddes
Second row: M. So; F. Heffron, R. Bialt; B. Parsons, R. Roberts
Third row: G. McGuiness, G. Chaconas, M. George; C. Adler, B.D. Jiang; L. Rodgers, L. Smith, D. Zipser
Fourth row: K. Nasmyth; J.B. Hicks, A. Klar, J. Strathern; S. Blose, J. Lin
Fifth row: A.-M. Francoeur, E. Woodruff, F. Tamanoi, P. Reichel, G.P. Thomas, M. Mathews, F. Asselbergs, B. Stillman; K. Burridge; B. Anderson, P. Pope, M. Anderson, C. Grzywacz

TUMOR VIRUSES

As can be seen from the research reports that follow, work on the molecular biology of adenoviruses and SV40 continues to comprise a major portion of the research effort at the laboratory. The several autonomous groups, whose work is described below, although located in different buildings, continue to collaborate in various ways. A major advantage of this form of organization is that different groups are able to attack the same problem from different directions.

MOLECULAR BIOLOGY OF TUMOR VIRUSES

J. Sambrook, *C. Adler, B. Ahrens, B. Anderson, M. Anderson, J. Bier, R. Frisque, L. Garbarini, Y. Gluzman, R. Greene, T. Grodzicker, C. Grzywacz, D. Hanahan, D. Holtzman, S.-L. Hu, R. Kelch, D. Klessig, R. McGuirk, R. McKay, C. Paul, P. Pope, M. Quinlan, M. Ramundo, P. Rosman, D. Smith, D. Solnick, C. Stephens, N. Stow, J. Stringer, W. Topp, J. Wiggins, K. Willison*

During the past year the Tumor Virus section has continued its efforts to understand how the genomes of viruses that malignantly transform cells are organized and expressed. The topics investigated fall into six major categories: (1) the organization of viral DNA sequences in malignantly transformed cells, (2) the mechanism of RNA transcription and splicing, (3) the properties of proteins associated with transformation, (4) the genetics of the transforming viruses simian virus 40 (SV40) and adenovirus 2 (Ad2), (5) the properties of malignantly transformed cells grown in culture, and (6) the interaction of SV40 with early mouse embryos.

The Organization of Viral DNA Sequences Integrated in the Genomes of Cells Transformed by Adenoviruses or SV40

Adenovirus-transformed Cells

J. Sambrook

Semipermissive cells transformed by adenoviruses (e.g., rat cells transformed by Ad2) often carry multiple sets of partial copies of viral DNA that are not colinear with the viral genome. The best-studied example is the cell line F4, where the integrated viral DNA, isolated by molecular cloning, has been analyzed by a variety of hybridization techniques and direct DNA sequencing. The general structure of the integrated sequences is shown in Figure 1. Starting from a *Hind*III site on the left, there occur, in order, a tract of highly repetitive cellular DNA about 1 kb in length and a tract of viral sequences 1543 nucleotides long, which begins 2 nucleotides from the right-hand end of the viral genome and extends through part of early region 4 (E4) to nucleotide 1545. These right-end sequences are then joined directly to sequences from the left end of the viral DNA by a blunt-end joint. There then follows a tract of sequences approximately 22 kb in length that are colinear with the viral genome as far as map position 63. They therefore include not only the transforming region (early regions 1A [E1A] and 1B [E1B]), but also a sizable chunk of late genes. Finally, there is another blunt-ended joint between the viral and host DNAs (which consists of unique or nearly unique sequences).

This arrangement, where sequences from the right end of the viral DNA are joined to those from the left, is not uncommon in adenovirus-transformed cells; similar configurations have been detected, by Southern hybridization, in several other lines of rat cells transformed by adenovirus type 5 (Ad5) (Visser et al., *Cold Spring Harbor Symp. Quant. Biol. 44:* 541 [1980]; J. Sambrook and R. Greene, unpubl.).

Several lines of transformed cells contain sets of viral DNA sequences that are amplified together with their flanking cellular sequences into tandemly repetitious structures. For example, F4 cells contain 16 copies of the arrangement shown in Figure 1. All 16 copies are identical. In theory, this situation could arise in three ways. First, integration may have originally occurred at only one chromosomal site, but F4 cells, which are known to be aneuploid, may have since accumulated 16 copies of that chromosome or a fragment of it. Although not conclusive, the available cytogenetic data argue strongly against this hypothesis. Second, integration may have occurred independently in 16 sites that lie at separate places in the rat genome but are identical to one another with regard to sequence. Although there is no formal evidence to exclude this possibility, such a series of events would run counter to virtually everything that has been established about the mechanism of integration of DNA viruses. Comparative analyses of a large number of cell lines transformed by SV40 or adenoviruses continue to indicate that integration is a random or quasi-random event. Yet, if the hypothesis set out above were correct, we would need to postulate that in the original cells that gave rise to the F4 line, a bizarre, inverted form of adenoviral DNA was created that managed to find and to recombine with a particular cellular sequence no less than 16 times. It seems much more likely that the set of viral sequences in F4 cells is the product of a single integration event. At some subsequent time the viral DNA, together with its flanking host sequences, was amplified 16-fold to generate a tandem array. The pressure to amplify could conceivably be generated by a need for large quantities of those viral products that are needed to achieve full expression of the transformed phenotype. How such amplifications might occur is obscure, but the two most likely mechanisms are either successive rounds of unequal crossover or saltatory replications followed by recombination. In either case, the final tandem array is probably genetically unstable and is likely to be expanding and contracting by unequal crossover. Presumably, the average of 16 copies per F4 cell is then a compromise between the constant selection for fast-growing cells that contain more copies of viral DNA and the

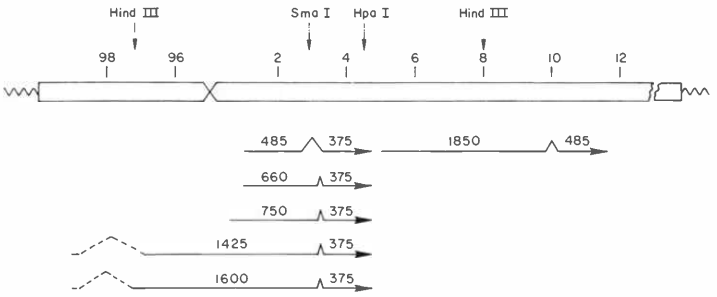


Figure 1
The organization of the integrated adenoviral DNA in the cell line F4. The viral insertion consists of two tracts of adenoviral DNA from the right- and left-hand ends of the viral genome. Also shown are transcription products found in F4 cells.

increased genetic instability that such expanded sets would bring.

Although all this is plausible, little direct evidence so far exists to support the idea of an interspersed tandem array of host and viral sequences. Cast-iron proof of this arrangement will come only when two adjacent viral inserts have been cloned in prokaryotic vectors together with the host sequences that connect them.

Not all lines of adenovirus-transformed cells contain such complex arrays of viral DNA sequences as those of F4 cells. For example, the cell lines F17 and F18 contain only two to three copies of a segment stretching from the left-hand end of the viral genome to map position 17, just downstream from the late promoter. These sequences have been cloned in prokaryotic vectors, and preliminary analysis indicates that they are colinear with the viral genome. The junctions between cellular and viral DNA sequences have not yet been analyzed in detail.

SV40-transformed Cells

J. Stringer

Transformation of cells by SV40 is stabilized by integration of all or part of the viral genome into that of the cell. Previous studies using restriction endonuclease mapping have shown that SV40 DNA can be linked to cellular DNA at many points on the viral genome and that there are multiple sites for SV40 integration in the cellular genome. During the past year we have used molecular cloning to examine directly the nucleotide sequences at cell-SV40 recombinant junctions and have shown that SV40 integrative recombination is nonspecific at the level of nucleotide sequence. DNA fragments containing the integrated viral DNA present in two SV40-transformed rat cell lines, SVRE9 and SVRE17, were cloned in prokaryotic vectors, and the DNA sequences linking SV40 and cellular DNAs were determined. Comparison of the DNA sequences at the SV40-cell junctions in SVRE9 and SVRE17 cells with those of a previously characterized viral insertion from SV14B cells shows that no specific viral or cellular sequences occur at SV40-cell junctions. In addition, the sequences at SV40-cell junctions differ from those present in other DNA insertions (such as bacterial transposons, Ty1 of yeast, *copia* elements in *Drosophila*, and retrovirus proviruses) in that the cellular sequences that flank SV40 insertions are not arranged as direct repeats. It can be concluded from the structure of SV40-cell recombinant junctions that the recombination system responsible for SV40 integration is not site-directed and that it operates via a mechanism different from that which mediates transposition and retrovirus integration.

Transcription and Processing of Viral RNA

The Late Promoter of Adenovirus 2

S.-L. Hu, M. Anderson

In collaboration with J. L. Manley (MIT), we have identified a region of the viral genome required for the initiation of transcription in vitro from the major late promoter of Ad2. A fragment of the adenoviral genome containing the cap site of the major late transcripts was cloned in pBR322. Deletions were then generated in vitro in and around the TATAAAA sequence located at position –25 to –31 nucleotides upstream from the cap site (Fig. 2). DNAs with these deletions were tested by the method of Manley et al. (*Proc. Natl. Acad. Sci. 77:* 3855 [1980]) for their ability to initiate transcription in vitro. Removal of sequences

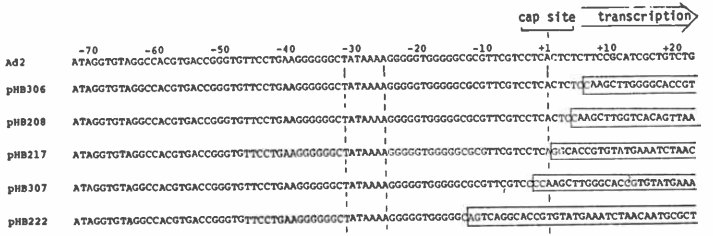


Figure 2
DNA sequence of deletion mutants around the cap site of the Ad2 major late promoter. Ad2 sequences deleted and substituted by pBR322 sequences are indicated by the open boxes. The upstream boundary of the Ad2 sequences in these clones is located at position –430, or coordinate 15.4, on the Ad2 genomic map.

upstream from position –47 or downstream from position –12 did not abolish transcription, but deletions extending into or beyond the TATAAAA sequence reduced transcription to less than one-tenth. Removal of the normal cap site slightly reduced, but did not abolish, transcription. These results indicate that the region of the genome upstream of the cap site, with boundaries within 15–17 nucleotides to either side of the TATAAAA sequence, is required for the initiation of transcription in vitro from the major late promoter of Ad2. Recently, we have constructed plasmids containing the T-antigen-coding region of SV40 inserted downstream of both wild-type and deleted derivatives of the Ad2 major late promoter (Fig. 3). We have assayed the expression of T antigen under the control of the Ad2 promoter by indirect immunofluorescence after microinjecting these plasmids into simian cells. Results obtained substantiate the conclusion drawn from in vitro studies.

RNA Synthesis and Splicing in Constructed Variants of Adenovirus

D. Solnick

A region-specific mutagenesis technique has been used to generate a series of mutants mapping in a portion of the adenoviral transforming region, E1A. One of the mutants was characterized in considerable detail. Using RNA mapping techniques, the mutant was shown to be defective in one of the splicing events normally responsible for producing the multiple RNA species from E1A. The defect acts in *cis*, and DNA sequence analysis indicates that it is the consequence of two nucleotide changes within a splice-site consensus

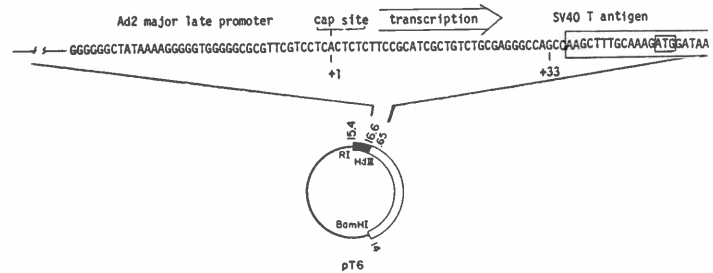


Figure 3
Structure of pT6 plasmid. The thin line on the circle represents the pBR322 sequence, the filled bar represents Ad2 DNA, and the open bar represents SV40 DNA. Numbers next to the filled bar and the open bar indicate the coordinates of Ad2 and SV40 DNAs in respective map units. The sequence at the junction of Ad2 and SV40 DNAs is presented at the top, with the cap site and the direction of transcription as indicated. The Ad2 major late promoter lies upstream of the cap site and the SV40 sequence lies downstream of the cap site, with the initiation codon of T antigen indicated by the box.

sequence. This is the first direct demonstration that the splice-site consensus sequence is required for normal processing of RNA.

Other insights into the control of adenoviral RNA syntheses have come from analysis of the specialized recombinants I have constructed. Viral recombinants were constructed in which a copy of the adenoviral major late promoter was inserted at an ectopic site in the viral genome and used to express downstream genes. The fragment harboring the late promoter extends from –400 to +33 nucleotides relative to the RNA start site, thereby excluding the downstream splice site (at +41) normally used in the formation of the tripartite leader. In the first such recombinant (Ad SVR101), the late promoter was inserted 270 nucleotides upstream from the early promoter for E1A. Both promoters retained transcriptional activity. Transcripts initiated at the inserted late promoter contained additional 5′ sequences but were spliced and polyadenylated at the sites normally used for the processing of E1A RNA. Consistent with recent results from several groups, transcription from the ectopic late promoter did not require viral DNA replication.

During a wild-type infection, the two largest mRNAs (0.9 and 1.0 kb) from E1A are synthesized prior to the onset of DNA replication, whereas the smallest mRNA (0.5 kb) requires DNA replication for its synthesis. The 0.5-kb mRNA generally is present after replication in an amount roughly equal to that of the 1.0-kb mRNA and somewhat greater than that of the 0.9-kb mRNA. During infection with R101, these ratios are essentially unperturbed. However, the 5′ extended derivatives of these RNAs are present in quite different amounts. Production of the analog of the 0.5-kb mRNA is enhanced roughly tenfold relative to that of the other extended mRNAs, which themselves are overproduced tenfold in comparison with their unextended forms. Thus, insertion of a late promoter in front of a region that primarily (but not exclusively) synthesizes early products shifts the emphasis in the expression of that region such that synthesis of a late product predominates.

A second recombinant (R102) was constructed in which the late promoter was inserted closer (80 nucleotides) to E1A. Again, both early and late promoters are used, and splicing and polyadenylation occur at the usual positions. For unknown reasons, however, the 5′ extended RNAs from both R101 and R102 are translated quite inefficiently both in vivo and in vitro.

Another type of recombinant (R105) was therefore constructed in which the late promoter was inserted upstream of a second insert, the SV40 early region (see Fig. 4). The SV40 sequence contains the coding, intervening, and 3′ untranslated regions for large and small T antigens but does not contain the early promoter. R105 produces RNAs analogous to the SV40 16S and 19S mRNAs, but which contain 33 nucleotides of adenoviral sequence at their 5′ ends. These RNAs are translationally active, producing both large and small T antigens, the former in particularly large amounts. R105 is therefore being used (by Ron McKay) in studies of the protein's DNA-binding functions. Moreover, the construction of R105 uncovered a number of unexpected phenomena related to the biogenesis of adenoviral late mRNA, among which is the fact that most of the tripartite leader sequences normally found on such RNA are expendable.

Using this adeno-SV40 hybrid as a prototype, recombinants are being constructed that will allow the insertion and expression of either cloned cDNAs or genomic DNAs up to 10 kb in length. A long-term goal is to use adenovirus as a vector for preparing DNA libraries, and then, using an immunoassay developed with the help of Ron McKay, to screen such libraries for recombinants expressing the gene of interest. The ability to obtain a gene given only an antibody to its product would be an immensely powerful tool.

Proteins of Transformed Cells

Transforming Proteins of SV40

R. McKay

One of the early gene products of SV40, large T antigen, remains the only example in higher organisms of a protein that controls gene expression by binding to specific regulatory DNA sequences. Large T antigen interacts with a specific DNA sequence and through this interaction both controls the expression of viral genes and initiates viral DNA replication. During the past year we have continued our studies of the interaction of T antigen with the regulatory DNA sequences in the viral genome. Using an immunoassay, we have shown that one of the three previously identified binding sites in the viral genome is a strong, independent binding site and that in the absence of this site, the other binding

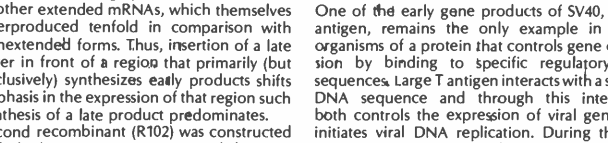


Figure 4
Structure of a new adeno-SV40 hybrid virus.

sites do not exhibit marked preferential binding. In collaboration with D. DiMaio (Johns Hopkins University), we have shown that SV40 variants carrying altered sequences in this region that binds strongly in wild-type SV40 DNA have a much lower affinity for SV40 T antigen. These observations provide direct evidence for the hypothesis that the altered phenotype of the mutant viruses is a consequence of diminished T-antigen binding.

We have continued to develop the technology of probing DNA-protein interactions with specific antibodies by devising an immuno-footprinting procedure. Using such a procedure, we hope to be able to screen mutant forms of T antigen rapidly for alterations in their ability to contact specific sites in the regulatory region of the viral DNA.

SV40 T antigen is a multifunctional protein, and in addition to binding DNA, it binds to a cellular protein of 53,000 daltons, has an ATPase activity, and is a tumor-specific transplantation antigen. A number of different experiments show that the early region of SV40 DNA coding for large T antigen induces the specific immune response when animals are challenged with SV40-transformed cells. Recently, a number of groups have used antisera against large T antigen to show that there is a large-T-antigen-like molecule on the surfaces of SV40-transformed cells. With L. Gooding (Emory University), we have used a number of monoclonal antibodies that bind to different sites on T antigen to show that surface T antigen shares all the antigenic determinants we have tested. We conclude that surface T antigen must be very similar to the much more abundant nuclear form of the protein. The very low background obtained when staining cells with monoclonal antibodies has allowed us to quantitate the expression of surface T antigen. We hope to be able to use the knowledge we gain about the structure of surface T antigen to ask directly how this tumor-specific protein functions as a target for the immune system.

Cellular Proteins Induced in Transformed Cells

B. Anderson

Previous studies initiated by David Lane (Lane and Crawford, *Nature 278:* 261 [1979]) indicated that the large T antigen of SV40 was bound to a host protein in a variety of SV40-transformed cell lines. The host proteins, denoted middle T antigens, coprecipitate when the large T antigen is immunoprecipitated from cell extracts reacted with various sera from animals bearing SV40-induced tumors. Using this approach, several additional SV40-transformed cell lines have now been screened for the presence of middle T antigens. Furthermore, methods now exist by which it is possible to determine whether anti-SV40 tumor sera contain antibodies directed against the middle T antigens, and these methods have been used to identify tumor sera containing anti-middle-T-antigen antibodies.

mRNA has been isolated from SV40-transformed rat cells producing middle T antigen and has been translated into polypeptides in a reticulocyte lysate cell-free translation system. Sera containing anti-middle-T-antigen-specific antibodies have been used to immunoprecipitate middle-T-antigen-related polypeptides from the mixture of total cell-free translation products. The cellular mRNA has been fractionated by sedimentation through a sucrose gradient, and a fraction relatively enriched in mRNA that can direct the cell-free translation of middle-T-antigen-related polypeptides has been identified.

The mRNAs contained in the sucrose gradient fraction rich in middle-T-antigen mRNA are to be used as templates for the synthesis of double-stranded cDNA molecules, which will be cloned in plasmids and propagated in bacteria. After identification and suitable characterization, the resultant middle-T-antigen cDNA clones will be used as probes in the isolation of the genomic copies of the middle-T-antigen genes.

Coincidentally, a human melanoma cell line under study because of its ability to secrete large amounts of plasminogen activator (see below) has been found to contain readily detectable levels of middle T antigen. This represents the first clear demonstration of the presence of stable middle T antigen in a human cell in which SV40 was not involved in the induction of the transformed state.

Induction of plasminogen activators is a classical characteristic of transformed cells. We are attempting to clone a plasminogen activator gene from human cells. The ultimate goal is to achieve expression of the cloned gene in a bacterial host, which would enable the preparation and purification of large amounts of plasminogen activator. In addition to their potential significance in transformation, highly purified plasminogen activators hold promise in the clinical management of thrombosis conditions.

The project is being undertaken in collaboration with D. Rifkin (New York University) who has provided both a human melanoma cell line that secretes the plasminogen activator of interest and an antiserum raised in rabbits by immunization with plasminogen activator purified from the tissue-culture fluid of growing melanoma cells. Polyadenylated mRNA has been isolated from the melanoma cells and translated into polypeptides in the cell-free reticulocyte lysate system. The anti–plasminogen-activator serum has been used to identify a plasminogen-activator-related polypeptide among the cell-free translation products. The mRNA has been fractionated by sedimentation through a sucrose gradient, and of the fractions collected, two adjacent fractions contained mRNA that has directed the synthesis of plasminogen-activator-related polypeptides in the cell-free translation system.

The next stage in this project will be to clone

cDNA copies of the plasminogen activator mRNA and to screen the clones obtained for those containing a copy of the entire coding sequence for plasminogen activator. Conventional genetic engineering techniques will then be used to achieve the production of the plasminogen activator polypeptide by linking the coding sequence, in the appropriate codon reading frame, to a bacterial gene promoter.

The Genetics of the Transforming Viruses SV40 and Adenovirus

Origin-defective Mutants of SV40

Y. Gluzman, R. Frisque

Replication of SV40 DNA begins at a unique site on the viral chromosome, coinciding with the *BglI* restriction site, and proceeds bidirectionally around the circular molecule. The viral promoter responsible for initiation of early transcription is also located near this site. We have isolated origin-defective mutants of SV40 (Gluzman et al., *Cold Spring Harbor Symp. Quant. Biol. 44:* 293 [1980]) and have determined the nucleotide sequences of five of them. All of the mutants are *BglI*-resistant and have suffered deletions that range in size from 4 to 241 nucleotides. Mutants with deletions of 4, 6, 9, and 58 nucleotides induce the synthesis of wild-type T antigen, even though the major portion of the untranslated 5' end of early mRNA is removed in the case of the mutant with a 58-nucleotide deletion. These mutants transform rat cells with an efficiency comparable to that of wild-type SV40 DNA. The mutant with a deletion of 241 nucleotides does not produce any detectable T antigen and does not transform cells. This deletion extends to nucleotide 220 (see pp. 799–833, Tooze, *The Molecular Biology of Tumor Viruses*, 2nd Edition, Part 2, Cold Spring Harbor Laboratory [1980]), and from comparisons with mutants that produce T antigen, the early viral promoter can be localized between nucleotides 5 and 220.

Deletions in the mutants producing T antigen are located between the AT-rich region at nucleotides 15 to 31 and the AUG codon for large-T-antigen and small-T-antigen proteins. The positions of the 5' termini of the principal early mRNAs produced in rat cells transformed by wild-type or mutant DNAs were determined by a method involving synthesis, separation, and sequencing of DNAs complementary to the 5' ends of early viral messages (in collaboration with P. Ghosh and P. Lebowitz, Yale Medical School). As previously reported (Reddy et al., *J. Virol. 30:* 279 [1979]), the 5' termini of wild-type mRNAs were found to lie 21–28 nucleotides downstream from the AT-rich sequence on the DNA template. The origin-defective mutants produced mRNAs with principal 5' termini that had been shifted downstream from the positions of the wild-type 5' termini. The size of this shift corresponded to the length of the deleted DNA segment (see Fig. 5).

We have also analyzed the mRNAs isolated from cells transformed by the viable mutant *dl*892 (Subramanian and Shenk, *Nucleic Acids Res. 5:* 3635 [1978]). This mutant has lost 19 nucleotides beginning 15 nucleotides upstream from the AT-rich sequence and produces the same principal 5' termini as does wild-type SV40 (see Fig. 5). These data suggest that a DNA sequence of 29 nucleotides, which includes the AT-rich sequence, contains a component(s) of a promoter for early transcription. This component functions in positioning the 5' ends of the principal early mRNAs 21–25 nucleotides downstream from the AT-rich sequence and acts independently of these downstream sequences.

From nuclease-S1 analysis of the early viral messages, we have observed that the quantity of viral RNA in cells transformed by the origin-defective mutants is greater than that found in wild-type transformants. The mutant DNAs have altered T-antigen-binding sites, and decreased binding of large-T-antigen protein to these sites may be responsible for the increased amount of viral mRNA. However, we have not detected a corresponding increase in the early viral proteins in the mutant transformants.

Transformation of Permissive Cells by SV40

Y. Gluzman

Three new lines of transformed monkey cells (COS-1, COS-3 and COS-7) have been isolated through transformation of CV-1 cells with an origin-defective mutant of SV40. The transformed cell lines obtained are permissive for infection with SV40 and express a functional viral T antigen. Thus, these cells support the growth of SV40 *tsA* mutants at the nonpermissive temperature and allow the propagation of pure populations of other early mutants of SV40. This is in contrast to the previously isolated SV40-transformed CV-1 cells (C2, C6, and C11), which were made by infection of CV-1 cells with UV-irradiated SV40. C2, C6, and C11 cells do not support the replication of early mutants of SV40. It is probable that these cells express a mutated T antigen that is able to transform cells but is defective in a viral replicative function (Gluzman et al., *J. Virol. 22:* 256 [1977]).

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

Y. Gluzman, J. Stringer

The mutant SV40 DNA insertion in C6 cells has been rescued through fusion of C6 and COS-1 cells. Fusion to COS-1 cells provides a functional T antigen in *trans* that allows the C6 SV40 insertion to excise and replicate in C6–COS-1 heterokaryons. The rescued C6 insertion was cloned in pBR322. The cloned DNA produces a T antigen that is

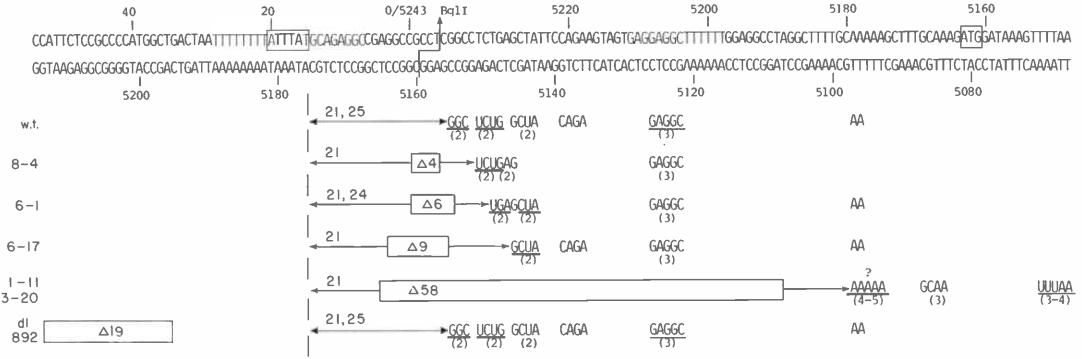


Figure 5

Schematic diagram of the region of SV40 DNA coding for the 5′ termini of the early viral mRNAs. Transcription occurs on the lower strand in a left-to-right direction. On the upper strand, a Goldberg-Hogness sequence and the AUG sequence for initiation of translation of large T antigen are indicated. In the lower portion of the figure are shown the deletions in the five origin-defective and dl892 mutants used in the present study and the RNA sequences within which the 5′ termini of the early viral mRNAs fall. Heavy and light lines under certain sequences indicate major termini and principal minor termini, respectively; nonunderlined sequences indicate sites of relatively scarce 5′ termini. Numbers in parentheses indicate number of cDNA 3′ termini and probably number of mRNA 5′ termini identified within the given stretches of nucleotides; where no numbers are given under sequences, only one mRNA 5′ terminus has been identified to date. Lines with terminal arrowheads indicate number of nucleotides from Goldberg-Hogness sequence to templates for principal 5′ termini of each virus. An A residue has been found at position 5177 in the DNA of the origin-defective mutants; however, a G residue has been found in this position in mutant 8-4 and 1-11 early mRNAs. The boundaries of deletions are 5190-4 for mutants 1-11 and 3-20, 5239-5242 for 8-4, 5237-5242 for 6-1, 5238-3 for 6-17, and 35-53 for dl892 (inclusive).

defective for lytic growth but still capable of transforming rat and monkey cells. The mutation responsible for the lytic defect has been localized by marker rescue to the *Hin*dIIIB fragment (0.42–0.65 map units).

A second mutant of SV40 that is competent for transformation but defective for lytic growth was discovered during the analysis of the integrated SV40 DNA present in the rat line SVRE9. The SV40 insertion in these cells contains a mutation in the viral large-T-antigen gene that renders the protein incompetent for the synthesis of SV40 DNA. SVRE9 cells, however, are transformed by the criteria of morphology, growth rate in low serum, and anchorage-independent growth. In addition, the cloned viral insertion retains the ability to transform rat cells in culture to density-independent growth. The mutation responsible for this new phenotype has been mapped both genetically and physically. Marker-rescue experiments localized the mutation between nucleotides 3187 and 3459. DNA sequence analysis of this region shows a single-base change at position 3342. This change generates a new *Pst*I site in the viral insertion and predicts an amino acid change of a serine to an alanine in the large-T-antigen protein. Apparently, this change abolishes the DNA synthesis function of the protein without affecting its transforming activity.

Cloning of DNA Fragments from the Left-hand Terminus of the Adenovirus-2 Genome and Their Use in Site-directed Mutagenesis

P. Rosman, R. Frisque, N. Stow

The left-hand 11% of the genomes of Ad2 and Ad5 contain genes that play important roles both in cellular transformation and in the early stages of lytic viral infection. This early region (E1) contains two separate transcription units, E1A (map coordinates 1.4–4.4) and E1B (map coordinates 4.5–11.2). Several overlapping spliced mRNAs are specified by both E1A and E1B, and a number of polypeptides encoded by these regions have been identified (see Fig. 6). The presence of overlapping genes considerably complicates the investigation of the roles of individual E1 polypeptide products during the lytic cycle and in transformation. To overcome this problem, we have used molecular cloning to introduce genetic lesions specifically within E1A and E1B.

Viral DNA fragments cloned in plasmid vectors provided convenient sources of substrate for mutagenesis. A variety of procedures were used to introduce mutations at either general sites or specific sites, and the altered viral sequences were then cloned and amplified. Using a dG-dC tailing procedure, we have cloned the Ad2 *Hpa*I E fragment (0–4.5 map units) and the *Bgl*II E fragment (0–9.0 map units) into the *Pst*I site of the plasmid vector pBR322. *Pst* sites regenerated at the ends of the insert enabled part or all of the insert to be excised. Cleavage of the plasmid with *Pst*I plus *Xba*I generated a 0–3.8-map-unit fragment, which was then ligated to the Ad5 mutant *dl*309A (3.8–100 map units) to generate unit-length genomes. Transfection with the ligation products resulted in the production of progeny virus that was able to replicate on both HeLa cells and line 293 cells, demonstrating the biological activity of the sequences rescued from the plasmid. Small deletions of about 10 bp were introduced around the *Sma*I site (map position 2.9) within the cloned viral insert, and the altered DNA sequences were reintroduced into progeny virus as described

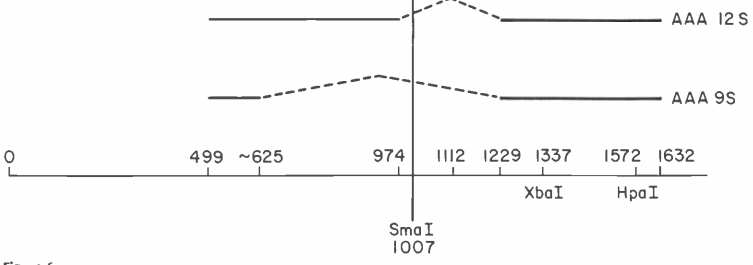


Figure 6
Location of the SmaI site in relation to the E1A mRNAs.

above. The mutant viruses grew well on line 293 cells (a line of human embryo kidney cells transformed by sheared Ad5 DNA) but plaqued with greatly reduced efficiency on HeLa cells, exhibiting a host-range phenotype similar to previously described mutants with lesions located within this region of the genome. Mutant-virus growth appears to be complemented (in at least some instances) by E1 products expressed by the line 293 cells.

Small deletions were also introduced at a variety of other sites in a plasmid (BE5) containing *Bgl*II E fragment; specifically, at the sites for *Xba*I at map position 3.8, *Hpa*I at map position 4.5, *Sst*I at map position 5.0, and *Kpn*I at map position 6.1. Several mutations at these sites, which lie at or to the right of map position 3.8, have been transferred to virus using an alternative technique. The plasmids were linearized within the pBR322 sequences with *Bam*HI and cotransfected with *dl*309A into line 293 cells. Infectious virus was produced as a result of homologous recombination within the DNA sequences shared by the viral insert and *dl*309A. Depending on the site at which the crossover occurred, wild-type or mutant virus carrying the deletion was generated. The mutant viruses are currently being studied in detail with regard to the nature of the defects during lytic growth and in cellular transformation.

Adenoviral DNA Replication

N. Stow

The terminal fragment clones of adenoviral DNA have also proved useful in the study of viral DNA replication. Surprisingly, when long sequences of pBR322 DNA are left attached to the left end of an adenoviral genome, the viral DNA retains its infectivity and the progeny virus has normal left-end DNA. Whether the additional pBR322 sequences are excised before replication of the ligated molecules or are lost as a consequence of the replication of these molecules is not yet known. If the latter situation is correct, it suggests that new viral DNA strands can be initiated at a specific sequence (corresponding to the terminal sequence) that can be recognized even when located within a viral DNA molecule. Alternatively, for DNA strands initiated at the right end of the viral DNA, a sequence-specific mechanism might determine the position at which the synthesis of the strand is terminated (corresponding to the normal left-hand terminus). Genomes constructed with deletions of about 50 bp at their left-hand termini were also infectious, and in this case the missing DNA sequences were restored in progeny virus DNAs. This experiment provides evidence that the inverted repeats at the two ends of the viral genome interact with each other during DNA replication. Preliminary evidence indicates that adenoviral DNA replication can occur even when both ends of the molecule are linked to foreign DNA. A plasmid containing two copies of the left end of Ad2 arranged head to head was constructed. When cells were coinfected with this plasmid and Ad2 helper DNA, the viral insert replicated and accumulated in the infected cells. This type of plasmid has considerable potential as a cloning vector for eukaryotic cells because it should be possible to introduce foreign DNA sequences within the viral insert and to study their replication and expression in cells coinfected with helper-virus DNA.

Construction and Analysis of Cell Lines That Express Unselected Adenoviral Genes by Cotransformation of the HSV-1 *tk* Gene and Adenovirus-2 DNA

T. Grodzicker, D. Klessig

We have started to obtain sets of human cell lines that express adenoviral genes and can be used as permissive hosts for the isolation and propagation of conditional mutants. The 293 line of human cells, which carries and expresses the transforming region of Ad5, was isolated by morphologically transforming human embryonic kidney cells with sheared viral DNA. This line has been extremely useful as a host for propagating transformation-defective mutants in E1. However, all attempts to make more cell lines using the same procedure have failed. We adapted the procedure of cotransformation that had been developed for mouse L Tk^- cells to introduce unselected Ad2 genes into permissive, thymidine-kinase (TK)-deficient human 143 cells.

Line 143 cells were cotransfected with fragments of Ad2 DNA (*Eco*RI A: 0–59; *Bam*I B: 0–29) and a cloned *Bam* fragment that carries the HSV-1 *tk* gene. Tk^+ cells were isolated after selection and growth in HAT medium. Several cotransformed lines were obtained that complement the growth of Ad5 *dl*312 (Δ1.2–3.7) and Ad5 *dl*434 (Δ2.6–8.7) deletion mutants that lack sequences from the left end of the viral genome. Mutant-virus growth was always due to complementation and not to recombination with viral DNA present in the cell lines. The amount and arrangement of viral sequences in the cotransformed cell lines have been analyzed by restriction endonuclease digestion and Southern hybridization. Most of the cell lines contain a single insertion of the HSV-1 *tk* fragment and a single insertion of adenoviral DNA. However, one line (B1) contains at least four different insertions, two of which are present in multiple copies. The adenoviral DNA in all cell lines is composed of sequences from the left end of the genome that extend for varying lengths in different lines. For example, two lines, A2 and A5, contain adenoviral sequences that extend from at least position 0.7 on the left to somewhere between positions 11 and 14.7 (A5) and 18.5 and 22

(A2) on the right. These lines, which complement E1A and E1B mutants, efficiently synthesize both E1A and E1B mRNAs. The A2 line does not synthesize viral RNA that lies beyond E1A and E1B. The B1 line contains multiple and amplified adenoviral insertions and synthesizes very low levels of E1A mRNA, higher levels of E1B mRNA, and a unique mRNA that maps to the right of the E1B family and extends to at least position 16.7. Although the B line contains DNA corresponding to E1, none of the insertions contain upstream left-end sequences because B1 cellular DNA does not release a *Bal* fragment (map position 0.7–5.8) upon restriction enzyme digestion. It seems likely, therefore, that the E1As integrated in B1 lack sequences needed for efficient transcription and are, in effect, promoter mutants. Some Tk^+ subclones support the growth of viral mutants as efficiently as the original parental line; others give reduced levels of complementation. For all Tk^+ subclones examined, the alteration or reduction in viral gene expression is independent of changes in the pattern of integration of viral DNA. To examine in detail amplification of viral sequences and the defective E1A promoter, we are attempting to clone the viral insertions in the B1 cell line.

In attempting to construct cell lines that carry and express adenoviral genes that lie beyond E1A and E1B, one must take into account the observation that in lytically infected human cells, expression of E1A is necessary for expression of many other adenoviral early genes. Thus, in cotransformation experiments using the HSV-1 *tk* gene and adenoviral DNA, it may be necessary to include left-end DNA sequences. As a plentiful source of E1A, we have used a plasmid, F4/41, that carries Ad2 DNA isolated from the rat transformed cell line F4. This clone contains left-end sequences that extend from a position 62 nucleotides from the left end of the genome to the *Hin*dIII site at position 7.9. It thus contains all of the coding sequences of E1A and part of E1B as well.

We transfected line 143 cells with a plasmid containing both the F4/41 insertion and the HSV-1 *tk* gene and have obtained five Tk^+ cell lines that carry approximately one copy of the F4/41 adenoviral sequences (D. Klessig and T. Grodzicker, unpubl.). They not only fully support the growth of E1A deletion mutants, but also partially complement the growth of an E1B mutant Ad5 *dl*313 (Δ3.6–10.1) and an E1A-E1B mutant Ad5 *dl*434. Thus, the truncated E1B in these cell lines (D10, D16, D21, D27, and D28) must be expressed to yield proteins that are biologically active and can carry out some of the E1B lytic functions.

Analysis of adenoviral mRNA from the D cell lines by the nuclease-S1 technique shows that all of the E1B sequences in these lines are present in poly(A)$^+$ cytoplasmic RNA. It has also been shown that rat cells transformed by F4/41 also make "E1B" transcripts (R. Frisque, S.-L. Hu, and J. Sambrook, unpubl.). It is striking that these cell lines make E1B mRNA because (1) the E1B transcription termination and poly(A)$^+$ addition sites are missing from the F4/41 clones, and (2) the splice acceptor site for one of the major E1B mRNAs and both the splice donor and acceptor sites for the other major E1B transcript are also missing from the F4/41 clone. How the E1B mRNAs in the D lines are transcribed and processed remains to be determined: whether they annex control signals from the plasmid, the *tk* insertion, or flanking cellular DNA. It should be noted that the adenoviral genes were unselected markers in construction of the D lines, which were selected to be Tk^+ only; yet all of them express, and are partially able to complement, E1B mutants. We are presently constructing cell lines that contain the left end of the viral genome as well as other internal adenoviral genes. We have obtained one cell line that contains the gene for the adenoviral early, 72K, single-stranded-DNA-binding protein. This line does not contain left-end sequences and expresses 72K sequences in poly(A)$^+$ RNA at very low levels.

Adeno-SV40 Hybrids That Express SV40 Large and Small T Antigens Under the Control of Conventional Adenoviral Promoters

T. Grodzicker, D. Solnick

Adeno-SV40 hybrid viruses have proved to be invaluable tools in the study of animal virus gene expression. We are now constructing new hybrid viruses to define further genetic regulatory mechanisms and to obtain viral genomes that are suitable for use as animal cell cloning vectors.

One of us (Terri Grodzicker), in collaboration with R. Tjian and C. Thummel (University of California, Berkeley), has obtained several novel adeno-SV40 recombinant viruses that express wild-type SV40 large and small T antigens under control of different adenoviral promoters.

To place the SV40 early region under adenoviral transcriptional control, the structural sequences that code for SV40 large and small T antigens were separated from the viral early promoter, purified and amplified in *E. coli* (Fig. 7A). As recipients for the SV40 early region, we used two Ad2/Ad5 hybrid viruses that each contain only two of the three *Bam* sites present in the Ad2 genome. These interserotypic viruses, designated 1x51i and 4x22Sb, were formed in vivo as wild-type recombinants from crosses between temperature-sensitive mutants of Ad5 and the nondefective Ad2-SV40 hybrid virus Ad2$^+$ND1. Neither of these viruses contains SV40 DNA. The SV40 early region was inserted at the *Bam* sites of adenoviruses to generate a population of defective recombinant viruses that contain SV40 insertion(s) replacing essential internal adenoviral genes. These were propagated in monkey cells in the presence of helper adenovirus. Expression of SV40 large T antigen by the recombinants provides a helper function for the growth of adenovirus in monkey

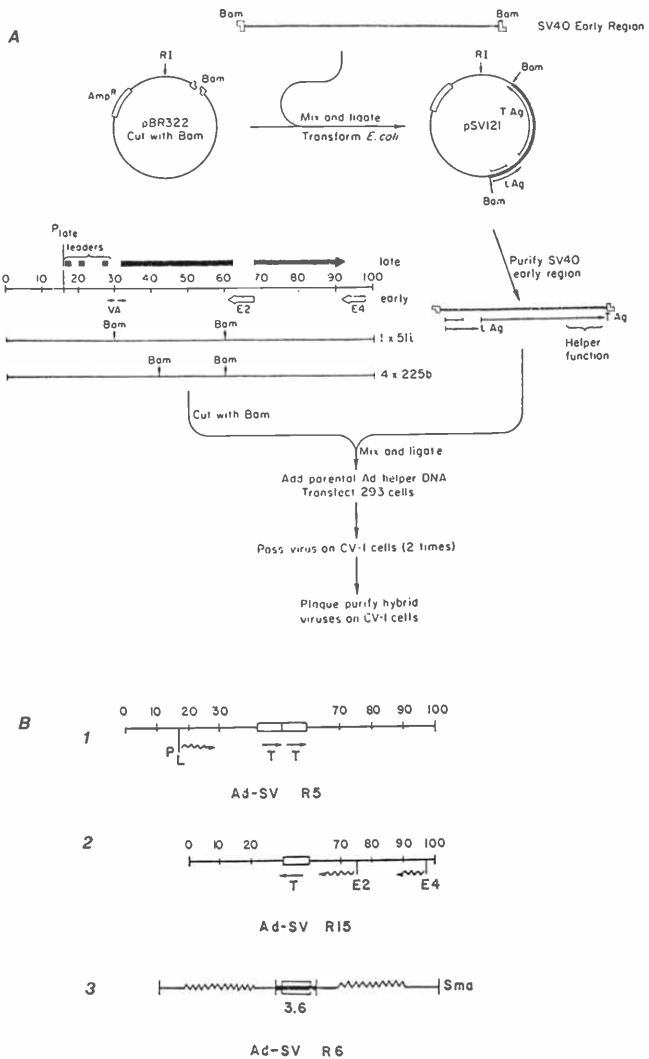


Figure 7
Construction and structure of adeno-SV40 hybrid viruses Ad-SVR5, Ad-SVR15, and Ad-SVR6.

cells, and this provides a selection for recombinants that express SV40 T antigen. Analysis of four hybrids indicates that they contain the SV40 early region inserted in different positions and orientations within the adenoviral genome (Fig. 7B). (1) Ad-SVR5 has a tandem insertion of SV40 inserted between the *Bam* sites at positions 42 and 60. The SV40 DNA is oriented with its 5′ end at position 42 and is transcribed from the same strand as the adenoviral major late transcripts. (2) Ad-SVR15 has one copy of the SV40 early region inserted between positions 29 and 60 and oriented so that the *A* gene is transcribed in the same direction as the E2 and E4 that lie upstream from it. (3) Ad-SVR1 and R6, which were made by insertion of SV40 into an Ad2/5 recombinant virus known as 1x51i, have grossly rearranged genomes, although the SV40 coding region is intact. No viruses with the 5′ end of the SV40 insert positioned at 29 have been found. The *Bam* site at position 29 bisects the VA RNA_I gene, leaving its promoter intact. It is possible that hybrid transcripts originating from this promoter cannot be properly processed or that this arrangement of genes interferes with transcription and/or processing from the major late promoter. Recent experiments using analyses of heteroduplexes of Ad-SVR1 (or R6) and adenovirus helper DNA by electron microscopy (in collaboration with Louise Chow, Electron Microscopy section) suggest that the SV40 early region is inserted in the adenovirus left-end 1b transcription unit.

Regardless of the arrangement and orientation of the SV40 insertions in the different hybrids, all produce wild-type SV40 early proteins. However, the hybrid viruses produce different amounts of large and small T antigens with different kinetics of synthesis. Ad-SVR15-infected cells yield low amounts of T antigen. Ad-SVR5 produces high amounts of T antigen as a late viral protein, as do Ad-SVR1 and R6, which make 3 to 5 times more T antigen than is found in SV40-infected cells. Large and small T antigens produced by all viruses have the same mobility as the wild-type SV40 proteins, as judged by immunoprecipitation with various T antisera and analysis of antigens on SDS-polyacrylamide gels. The Ad-SVR6 T antigen has been shown to be identical to SV40 T antigen by partial proteolysis with *Staphylococcus aureus* V8 protease and analysis of tryptic peptides. Using Ad-SVR6-infected cells, it has been possible to purify about 200 μg of T antigen from 8 liters of infected cells.

The SV40-containing RNAs made by the different recombinants are transcribed from different positions on the genome, although in all cases they contain intact SV40 early sequences and display normal splice patterns. However, the ratio of small-T-antigen mRNA to large-T-antigen mRNA in cells infected with hybrid viruses is reduced relative to the ratio found in mRNA extracted from lytically infected cells. Some hybrid mRNAs appear to be transcribed from the adenoviral late promoter and to contain the tripartite leader sequence (Ad-SVR5). Others (Ad-SVR15) are probably transcribed from *l*-strand early promoters. Thus, there seem to be several sites in the adenoviral genome into which the SV40 early region can be stably integrated to allow the synthesis of active mRNA. We are presently attempting to insert a variety of viral and human cellular genes into the adenoviral recombinants to see whether they can be expressed.

Using a different approach, David Solnick has also constructed a new adeno-SV40 hybrid virus that holds promise as a vehicle for the expression of cloned DNAs. This recombinant is shown in Figure 4 and is described above in Transcription and Processing of Viral RNA.

Transformation and Tumor Induction by SV40 and Adenovirus

This year has been one of transition for our group. We have finished the studies on SV40 early mutants, particularly mutants defective in the viral small-T-antigen protein, and SV40-transformed cells, which have occupied our group for the past several years. In addition, we have begun a number of new projects involving transformation and tumor induction by the human adenoviruses.

Characterization of Small-T-Antigen Mutants of SV40

W. Topp, M. Anderson, C. Paul

Christine Paul has sequenced a number of so-called viable deletion mutants of the small-T-antigen gene of SV40, which were isolated in our lab three years ago by Merilyn Sleigh. She has also examined, by immunoprecipitation, the small-T-antigen proteins that are encoded by these viruses. Although it has been reported that the levels of small-T-antigen mRNA and protein that are produced by a similar set of mutants correlates well with the location of the deletion, we find no such correlation. In fact, one mutant, *dl*2004, which in reality carries only a base-change mutation at the *Taq*I cleavage site, produces only very low levels of a normal-size small-T-antigen protein, suggesting that the picture might be considerably more complex than originally thought. Another surprising result is that the mutants *dl*883, *dl*884, and *dl*2005, all of which have deleted sequences around the splice point for small-T-antigen mRNA production, synthesize low levels of a small-T-antigen protein of the proper size for the corresponding deletion. Either these proteins are being translated off an unspliced message or there is a second, perhaps aberrant, splice that can be made elsewhere in the primary transcript.

Marilyn Anderson has studied the uptake of glucose in rat cells transformed by SV40 and by

SV40 variants defective in either the small-T-antigen or large-T-antigen proteins.

SV40-transformed rat embryo fibroblasts characteristically exhibit an enhanced 2-deoxyglucose transport rate relative to the untransformed parent cell. To investigate the role of small and large T antigens in this process, 2-deoxyglucose transport was studied using a number of cell lines derived from rat embryo fibroblasts transformed by wild-type SV40, *ts*A209, and the small-T-antigen deletion mutant *dl*884. The presence or absence of small-T-antigen protein was found to have no effect on 2-deoxyglucose transport in subconfluent cultures of the transformants. The transport rate of all transformants was 3 to 5 times higher than that of the normal rat embryo fibroblasts when measured over a 10-minute period at 37°C. Large T antigen was shown to be required for maintenance of the elevated glucose transport. Unlike the wild-type transformants, when *ts*A transformants were maintained for at least two generations at 40°C, glucose transport declined dramatically.

Isolation of a Thymidine-kinase-deficient Rat Cell Line

W. Topp

Transformation by the human adenoviruses is, in general, several hundredfold less efficient than that by SV40. Although many explanations for this effect are plausible, there is as yet no experimental evidence that bears on the problem. It is our belief that this may be so because the integration and expression of the transforming region of these viruses is by itself insufficient to bring about cellular behavior sufficiently altered that it could be detected as commonly assayed. Some undefined second event, perhaps amplification of the viral genes or a second site mutation, must then occur that results in the development of a fully transformed phenotype. To study this hypothetical stepwise progression, it is necessary to have a means of selecting infected cells solely for the expression of adenoviral transforming proteins. The means by which we intend to do this is through cotransfection of the adenoviral transforming region, either as viral DNA or as cloned fragments of viral DNA, with a second selectable gene. It is known that cotransfected genes frequently are both integrated and expressed even though only one has been selected for. To this end, we have isolated three cell lines, derived by serial passage in sequentially increasing concentrations of BrdU of the 3T3-like rat fibroblastoid cell line Rat-1, which are lacking in nuclear thymidine kinase (TK). These lines are easily transfectable by cloned HSV TK and other DNAs and are "normal" by all criteria tested, save that they are both established as cell lines and clonable. Marilyn Anderson, Dick Frisque, and Mary Ramundo are now using these cells to establish cell lines carrying and expressing the genes from E1A and E1B of human Ad2. Once isolated, these lines will be characterized and contrasted to lines similarly isolated expressing the SV40 transforming proteins.

Oncogenesis by Group-D Adenoviruses

W. Topp, D. Smith

The human adenoviruses can be divided into five groups on the basis of a variety of criteria. Of the 36 identifiable human adenovirus serotypes, only three, Ad12, 18, and 31 (group A), are highly tumorigenic in newborn hamsters. The substantial majority (groups B and D) are only weakly oncogenic, tumors appearing after 12 months in only 5–10% of the animals infected. All these tumors are almost invariably undifferentiated fibrosarcomas arising at the site of injection. The C-group and E-group adenoviruses have no detectable oncogenic potential. Similar results are obtained when groups A, B, C, and E are injected into newborn rats. However, it has been reported that the group-D adenoviruses behave differently in rats. One member of this group, human Ad9, induces, with nearly 100% efficiency, benign breast fibroadenomas within 3 to 4 months of injection into newborn female rats. The same result is obtained with adult rats. Diane Smith, in collaboration with Tom Broker and Louise Chow (Electromicroscopy Section) and C. Shellabarger and B. Lane (State University of New York, Stony Brook), has begun a series of studies first to repeat this unusual result and then to identify what feature of the little-studied group-D adenoviruses is responsible for this unique behavior.

Transformation of Culture Cells by Mutants of Adenovirus 2

R. Frisque, M. Anderson, N. Stow, P. Rosman, M. Ramundo

We have now cloned most of E1 of Ad2 in pBR322. In addition, two clones representing E1A and E1B have been made. A large number of deletion mutants have been constructed at specific sites in these clones, and we are now testing their transforming capabilities in an effort to determine which Ad2 proteins are involved in the initiation and maintenance of the transformed state. Because the transformation efficiencies are low when using DNA transfection techniques, we have attempted to increase the efficiency in several ways: by microinjecting the DNA into cells, by cotransfecting Tk^- cells with the *tk* gene and mutant DNAs, and by using reconstructed viruses containing the various deletions.

Molecular Biology of a Human Papovavirus

R. Frisque

JCV is a human papovavirus associated with the disease progressive multifocal leukoencephalopathy (PML). It is highly tumorigenic, causing a wide variety of tumors in hamsters and primates. Little is known about the molecular biology of JCV, in part due to the inabllity to obtain satisfactory yields of the virus. By cloning the viral DNA, we should be able to overcome this problem and begin a more thorough investigation of JCV. I have constructed several clones that contain either full-length or deleted species of the genome. In addition, DNA isolated directly from PML brains has been cloned. This will allow the study of the viral DNA without having to pass the virus in tissue culture, a step that appears to generate a large number of rearrangements in the DNA. The biological activity of these recombinant molecules is now being tested, and an extensive physical map is being produced. Initial experiments indicate that these clones transform primary hamster brain cells.

Interaction of SV40 with Preimplantation Mouse Embryos

K. Willison

Last year we described experiments that involved the introduction of SV40 DNA into adult mice by microinjection of 3.5-day blastocysts with a few tenths of a picogram of DNA. When total cellular DNA extracted from animals derived from injected embryos was analyzed by Southern analysis for the presence of SV40 sequences, we found very small amounts (approximately 1 copy per genome equivalent) of SV40 circular DNA in some of the samples. We were worried about problems of contamination, and therefore a collaboration was set up with F. Kelly and C. Babinet at the Institut Pasteur, Paris, France. In Paris, they produced 50 129Sv inbred mice that had been infected with SV40 on day 2.5 of development. We received a travel grant from the International Union against Cancer, Geneva, and Dr. Kelly spent 2 months here during the winter. We analyzed DNA extracted from the head portion of these newborn mice and found that 15 animals again had very small quantities of SV40 supercoiled DNA; the control experiments showed that accidental contamination could not be the explanation. By Southern analysis, these free genomes appeared to be identical to wild-type SV40. However, we could not isolate virus from the tail parts of the same SV40-DNA-positive newborn mice, nor could we rescue virus by transfection of their total cellular DNA onto permissive CV-1 monkey cells.

Consequently, a bacteriophage λ library was prepared from a pool of DNA from various of the SV40-positive mice. The DNA was banded on a CsCl gradient to isolate supercoiled molecules that would include the SV40 DNA molecules. This fraction was cut with *Eco*RI and inserted into λ*WES*·B. The resultant library was screened by probing with ^{32}P-labeled SV40 DNA, and a single recombinant phage was isolated. This phage, λSV1, had a single insertion of SV40. Detailed restriction mapping shows that it is altered, as compared with wild-type SV40. There is rearrangement on the late side of the origin of replication as well as elsewhere.

Virus stocks have been made by transfecting CV-1 cells with this DNA. This virus grows normally on CV-1 cells. At present, we are examining its growth and properties on various primary and established mouse cell lines. This is because we believe that there may be particular differentiated mouse cell type(s) that can support the replication of this virus. This is in contrast to wild-type SV40 infections of mouse cells, which only result in the establishment of the transformed state of the virus.

We hope that we may learn more about the differentiated state of cells during embryogenesis with this sort of approach. The examination of alterations in the controlling regions of SV40 genes required for growth on certain cell types may help us understand the control of the normal, endogenous genes in these cells.

Analysis of Surface Antigens Shared by Normal Embryos and Tumor Cells

It has long been known that embryonic and tumor cells often express some of the same antigens on their surfaces. These antigens may be involved in morphogenetic processes during normal development but, when they are expressed on tumor cells, are responsible for the uncontrolled growth and invasiveness of tumors. These antigens are generally detected by immunological techniques, and much effort has been expended in raising conventional antisera against one or the other type of cell and searching for cross-reactive antibody activities. The powerful monoclonal antibody technique has disclosed many new cross reactions as well. Indeed, one of the first monoclonal antibodies made by C. Milstein and coworkers (MRC Laboratory of Molecular Biology, Cambridge, England) was found to bind to early mouse embryos and embryonal carcinoma cells (ecc) the stem cells of teratoma and teratocarcinoma tumors. The antibody, M1·22·25·8, was shown to recognize a Forssman antigen determinant on sheep red blood cells (SRBC). Forssman antigen is a neutral glycolipid, and indirect data suggested that the M1·22·25·8-binding molecule on embryonal ecc was also a glycolipid. Several years ago, Hakamori (*Proc. Natl. Acad. Sci. 59:* 245 [1968]) had shown that Forssman antigen expression on the surface of hamster cells changed dramatically upon transformation with polyoma virus.

Interestingly, the monoclonal technology has generated many anti-glycolipid antibodies in the mouse embryo/ecc system as well as in other systems, such as the rat retina. We wondered whether they acted as immunodominant determinants and constituted a background problem in the dissection of complex biological systems by means of the monoclonal procedure. In collaboration with D. Marcus and his group (Albert Einstein College of Medicine, Bronx, New York), we decided to screen a series of conventional antisera (which were already known to react with different glycolipids) against ecc in order to determine which glycolipids were present in these cells. Immediately, we found that anti-globoside antisera bound very strongly to ecc in a double-layer indirect immunofluorescence assay using a fluorescence-activated cell sorter for analysis of the samples. We then screened preimplantation embryos for reaction with this anti-globoside antisera. It first appears on the surface of two cell embryos and reaches a maximum level on early morulae and then declines. This is different from the monoclonal anti-Forssman antibody, M1·22·25·8, which is only first expressed on early morulae and increases over the next 24 hours of development. Figure 8 is a fluorescence micrograph of two 10–14-cell embryos showing the strong reaction with anti-globoside antiserum. This antiserum can now be used as another differentiation marker for early embryogenesis. A. Suzuki (University of Tokyo) is examining the chemical nature of the neutral glycolipids on ecc and has found that the Forssman-carrying determinant of ecc is a different glycolipid from that found on SRBC. He is presently engaged in working out the detailed structure of the sugars. This observation of differences in the molecules carrying these glycolipid determinants on different cell types is important because it raises questions about the whole approach of looking for cross-reactive antibody specificities. If one wishes to make a connection between two cell types on this basis, it will be necessary to show biochemical and functional equivalences of the molecules carrying the antigenic determinants or the determinants them-selves if they are carried on different molecules. For the time being, the antibodies are useful for isolating live cells and for probing the structure of the cell surface in developing embryos.

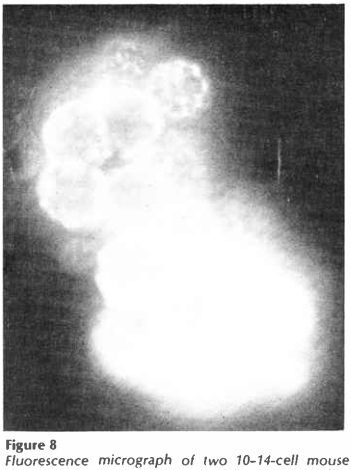


Figure 8
Fluorescence micrograph of two 10–14-cell mouse embryos showing the strong reaction with anti-globoside antiserum.

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NUCLEIC ACID CHEMISTRY

R. J. Roberts, *I. Battle, R. M. Blumenthal, J. E. Brooks, T. R. Gingeras, B. Jiang, M. Kelly, M. Moschitta, P. A. Myers, D. O'Loane, K. O'Neill, B. Parsons, P. Rice, M. Wallace, C. Yen*

During the past year the Nucleic Acid Chemistry group has continued to accumulate data toward the elucidation of the adenovirus-2 (Ad2) DNA sequence and the mapping of expressed regions of that sequence. Many computer programs have been written to aid both in the assembly and in the analysis of the growing body of sequence data now available. Our long-standing interest in restriction endonucleases has been maintained and expanded to include their cognate site-specific methylases. Efforts to clone the genes for restriction endonucleases have concentrated upon the prior cloning of methylases, and substantial progress has been made in cloning and characterizing the *dam* (DNA adenine methylase) gene of *E. coli*.

Adenovirus-2 Genome Sequence

T. R. Gingeras, C. Yen, R.J. Roberts, M. Kelly

The determination of the nucleotide sequence of the Ad2 genome has been one of the principal efforts of this group. On the basis of the nucleotide sequences thus far examined, the length of the entire genome is estimated to be approximately 36,500 nucleotides. At the present time, two main blocks of sequence have been collected. The first segment consists of 13,500 nucleotides located between 0% and 41% on the genome. The second segment is located at the right end of the genome (89–100%) and consists of 4000 nucleotides of contiguous sequence. The remainder of the sequence from the internal portion of the genome consists of several unconnected blocks of sequence that are frequently clustered about previously mapped restriction sites. These unconnected segments total an additional 8000 nucleotides. In addition, two other sources exist for nucleotide sequence information concerning this internal portion of the Ad2 genome. F. Galibert and his colleagues have deduced the sequences from coordinates 70% to 89% on the Ad2 genome (*Gene 6:* 1 [1979]; *Nucleic Acids Res. 8:* 2173 [1980] and unpubl.), and B. S. Zain (University of Rochester) and her colleagues have in excess of 4000 nucleotides from this same internal region (unpubl.).

Over the course of the past year, the strategy employed for sequencing the Ad2 genome has been expanded to include the collection of data using the M13 cloning and sequencing system. This approach not only provides a simplified means for obtaining second-strand sequence information within segments already sequenced and allows us to resolve persistent ambiguities, but it will also establish an M13 clone bank, consisting of single-stranded segments from both *l* and *r* strands of the genome. These clones will be of great use in the future for the purification and characterization of specific Ad2 mRNAs.

Analysis of the sequence presently available and its correlation with data gathered elsewhere (see, e.g., Baker and Ziff, *Cold Spring Harbor Symp. Quant. Biol. 44:* 415 [1980]; Perricaudet et al., *Proc. Natl. Acad. Sci. 77:* 3778 [1980]; Aleström et al., *Cell 19:* 671 [1980]) gives a reasonably complete picture of the organization of information at the left end (coordinates 0–17) of the genome, including early regions 1A (E1A) and 1B (E1B) and polypeptides IX and IVa2. Between coordinates 17 and 41 we have located the coding regions for the segments of the tripartite leader and the genes for VA_I and VA_{II}. We have also identified a number of significant stretches of open reading frames, although detailed assignments in this area must await the resolution of ambiguities in our sequence and more detailed sequence information about the splicing of mRNAs encoded there. In addition to the expected open reading frames present in the *l* strand, which are presumably associated with the newly discovered early region 2B (E2B), there are also open reading frames within the intervening sequences that separate the late leaders.

The most intriguing example of an unassigned open reading frame within an intervening sequence is found in early region 4 (E4) at the right end of the genome. This region displays a complex splicing pattern, as judged by electron microscopy (Chow et al., *J. Mol. Biol. 134:* 265 [1979]). This complexity can now be understood in terms of the sequence, as there are no less than four distinct stretches of open reading frames present. It appears that the observed spliced mRNAs have been designed to gain access to three of the four open reading frames. The fourth open reading frame contains the largest coding capacity (a polypeptide of about 28K is predicted), and yet this reading frame is inaccessible because it lies within an intron. Experiments are under way to ascertain whether this or any of the other intervening sequences are utilized.

Computer Program Development

R. M. Blumenthal, T. R. Gingeras, P. Rice, R. J. Roberts

From the start of the Ad2 sequencing project, we have been developing software designed to aid in both the DNA sequencing itself and the

subsequent analysis of that data. Such programs have elicited considerable interest because of the huge volume of sequence data being generated worldwide and because they are written in a language (FORTRAN) that is relatively "transportable" to other computers.

Several new programs have been written in the last year to facilitate the generation and storage of nucleotide sequence data. One such program, PRIMER, provides the user with strategies for sequencing the gaps in a partially complete sequence. PRIMER finds appropriate-sized restriction fragments close to the gap for use as primers in the Sanger-Coulson dideoxy sequencing procedure. A major improvement in the data-entry stage of sequencing resulted from completion of the program DIGITPAD (see Fig. 1). This program makes use of a digitizing tablet on which autoradiographs can be placed and allows the user to enter data directly from the autoradiograph using a pressure-sensitive position pen. The program also contains editing functions: A gel may be "read in" rapidly several times, and the entries are compared automatically. Recently, a "voicebox" has been added to this system so that the user can be sure of proper data entry without looking up from the autoradiograph. The program DRIVER was written 2 years ago and has been used to find overlaps in different blocks of sequence data and to place such data both in working files and into a master archive. DRIVER has recently been revised by the addition of several subroutines designed to handle larger amounts of data and to edit the archive files by removing duplicate entries and cross-checking these data with the final sequence.

A second area of program development has been in generating restriction maps for sequenced DNA. These programs have been used in developing cloning strategies, in sequencing strategies, and simply to check the accuracy of new sequence data. The program CUTTER, written in collaboration with J. Milazzo (Computing Center, State University of New York at Stony Brook) finds all the substrate sites in a sequence

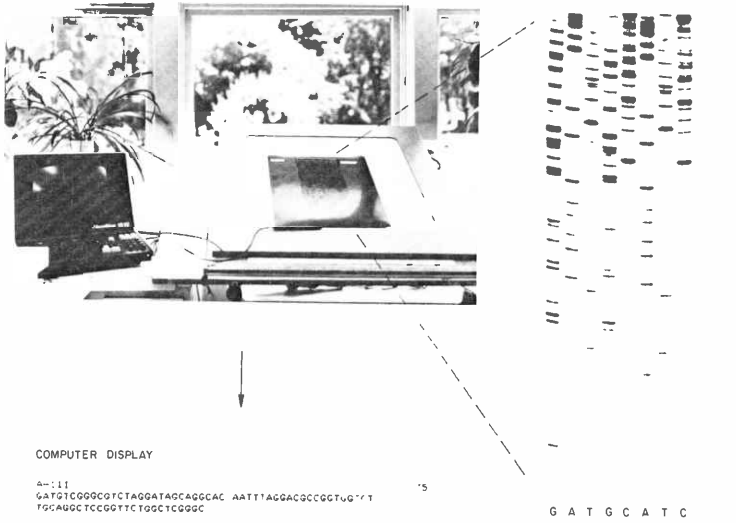

Figure 1
A semiautomated gel-reading system. Such a system consists of an autoradiograph mounted on a translucent digitizing tablet that is connected to a computer and cathode-ray-tube terminal. The sequence is read from the autoradiograph (using the signal pen) directly into memory. The nucleotide sequence is stored and displayed on the terminal screen. The limit of resolution of the tablet is 0.1 mm. A menu of other functions (editing, homology searches) is encoded on the surface of the tablet in an area not covered by the autoradiograph. These functions can be activated by touching the designated areas with the signal pen.

for every known restriction enzyme activity. This master list is then used by a variety of other programs. FRAGOS groups all sites for a given enzyme together and displays them both in order of occurrence and in order of size, lettering the fragments in the process. This program also allows multiple digests, accepting an indication of end-labeling between the digests, and indicates which final fragments are unlabeled or symmetrically or asymmetrically labeled. This is of considerable use in the Maxam-Gilbert chemical sequencing procedure. Currently, we are exploring the use of computer graphics in displaying these data more rapidly and clearly.

A third area of program development has been in the definition of open translational reading frames, both to localize gene boundaries and, again, to check the quality of new sequence data. Two such programs have been written. One, RFRAME, displays all occurrences of initiation and termination codons in all six reading frames (three in each direction). The second, DISPLAY, provides similar information graphically, showing all open reading frames longer than a user-defined minimum and allowing regions of interest to be shown in greater detail.

Finally, a number of programs to assist in designing gene cloning strategies are under development. One, REVCUT, is complete. This program is used when one wishes to clone a gene coding for a given protein of known sequence. The amino acid sequence of the protein is entered, and the program determines which restriction enzyme sites should *not* occur within that gene (barring their occurrence in intervening sequences). This information is used in selecting the enzyme with which to digest the chromosomal DNA and allows an in vitro selection against undesired genes by judicious redigestion of the clone bank.

At present, we are incorporating graphics capability into many of these programs and consolidating the programs into a limited number of multi-use sequence processing packages.

Restriction Endonucleases and Methylases

J. E. Brooks, B. Jiang, P. A. Myers

The list of restriction enzymes continues to grow, and of the 258 such enzymes now known, there are at least 69 different specificities. Of the nine new enzymes characterized by this group, eight have proved to be isoschizomers of previously described enzymes. The ninth, from *Moraxella bovis* (ATCC 17947), is still under investigation. In addition, we have shown that *Bst*EIII is an isoschizomer of *Mbo*I. One interesting observation concerns *Pae*R7, from *Pseudomonas aeruginosa*, which, under conditions of mild digestion, selectively cleaves certain *Xho*I sites. However, when gross overdigestion is employed, all *Xho*I sites can be cleaved.

Studies of the methylases that serve to protect the host DNA from the effects of the cognate restriction enzymes have also continued. The methylase from *Arthrobacter luteus*, which protects against *Alu*I (AGCT), also protects against *Pvu*II (CAGCTG), *Sst*I (GAGCTC), and *Hind*III (AAGCTT). The latter case is particularly interesting because the residue normally methylated in *Haemophilus influenzae* Rd is the first A residue in the sequence, whereas the *Alu* methylase must modify one of the four internal residues. This means that *Hind*III is sensitive to methylation at more than one position, a phenomenon that has not previously been shown to occur. Another interesting observation concerns an isoschizomer of *Hha*I (GCGIC) that has been found in *Spiroplasma citri*. This new enzyme, *Sci*I, cleaves at a different site within the recognition sequence (GICGC). However, both enzymes are unable to cleave when the first C residue (GĊGC) is methylated. This is unusual, as most restriction enzyme pairs that recognize the same sequence but cut at different bases are blocked by methylation at different bases.

dam Methylase

J. E. Brooks, T. R. Gingeras, R. M. Blumenthal

We are continuing efforts to clone the structural genes for a number of type-II restriction and modification enzymes. Our efforts are now directed to two-step clonings, first of the protecting methylase and then of the restriction endonuclease, as attempts to clone both simultaneously have met with very limited success both here and elsewhere. Accordingly, we have developed methods for the transfer and selection of methylase genes, starting with the *dam* gene of *E. coli*. The *dam* methylase modifies the sequence GATC, and *E. coli* strains overproducing the *dam* methylase should be good hosts for the cloning of restriction enzymes such as *Mbo*I (GATC) and several of its isoschizomers, as well as *Bcl*I (TGATCA) and *Cpe*I (TGATCA).

The *dam* gene was originally cloned as a 23-kb piece by selection for the adjacent *trpS* gene. This clone, made by G. Herman and P. Modrich (Duke University), has been subcloned here to a 1.14-kb piece by using screening and selection procedures that monitor the methylase itself. The screening procedure makes use of the increased sensitivity of *dam*$^-$ strains to the mutagen 2-aminopurine; *dam*$^-$ strains transformed with a *dam*$^+$ plasmid grow much better. The selection procedure makes use of the fact that *dam*$^+$ plasmid DNA will itself be methylated and resistant to the enzyme *Mbo*I; the subcloned plasmids are passaged through *dam*$^-$ cells, reiso-

lated, and cleaved in vitro with *Mbo*I to select for *dam*$^+$ plasmids.

In addition to using the *dam* clones as hosts for cloning certain restriction endonucleases, they are being used to study the *dam* methylase itself. The nucleotide sequence of the *dam* gene (coding for a 31,000-m.w. protein) has been determined. This, together with in vitro transcription and Northern blot analysis, has allowed the tentative identification of transcription and translation initiation and termination sites for the *dam* gene. These are currently being confirmed. In addition, in vitro mutagenesis studies of the *dam* gene have been started that may lead to identification of those regions of the protein and DNA important to the site-specific action of the *dam* methylase. The *dam* clone has also been used as a hybridization probe to Southern blots of digested DNA from the chromosomes of other bacteria. Thus far, two species, *Proteus vulgaris* and *Enterobacter cloacae*, both known to contain *dam*-type methylase activity, have been found to contain *dam*-hybridizing sequences. By hybridization selection of the DNA, it should be possible to clone these sequences to see whether they are actually *dam* genes and, if so, to compare them with the *E. coli dam* gene.

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ELECTRON MICROSCOPY

T.R. Broker, L.T. Chow, *J.A. Engler, M. Hallaran, B.A. Kilpatrick, M. Rossini, D.M. Smith, B. Urso, M.P. van Bree*

The Electron Microscopy section has continued its study of the DNA organization, RNA transcription and splicing patterns, and genetic regulation of human adenoviruses. Electron microscopic heteroduplex analyses, DNA cloning and sequencing, and microinjection of specific segments of viral DNA into the nuclei of cells in culture have been used. Representatives of rather distantly related adenovirus serotype classes A (Ad12), B (Ad3 and Ad7), C (Ad2 and Ad5), and D (Ad9) are being studied.

Other systems have also been examined. The DNA sequence of bacterial insertion element IS5 was determined. In collaborative projects, the structures of eukaryotic genes, including chicken β-actin and rat α_{2u} globulin, have been analyzed.

Identification of Adenovirus Early Region 2B RNAs and Proteins

L.T. Chow

To identify minor mRNAs specified by human adenovirus serotype 2 (Ad2), infected HeLa cells were treated with the antibiotic anisomycin. This drug inhibits protein synthesis and stabilizes mRNAs, resulting in their accumulation (Lewis and Mathews, *Cell 21:* 303 [1980]). In collaboration with Jim Lewis (Protein Synthesis section), Ad2 RNAs were extracted 3 hours and 6 hours after infection in the presence of 10 μM anisomycin and were characterized by cell-free translation and by electron microscopic heteroduplex analysis. Several rare early RNAs complementary to the viral *l* (leftward-transcribed) strand between coordinates 30 and 11 were detected (Fig. 1). The RNAs are derived from the early region 2 (E2) promoter at coordinate 75.1 and utilize the IVa2 mRNA terminator and polyadenylation site at coordinate 11.1. Their map coordinates are 75/± 68.5/39/26–11 (major species) and 75/39/30–11 (minor species). Another infrequently observed species has a main body between coordinates 23 and 11. Thus, the transcripts are an alternative product of the E2 promoter previously ascribed only to the message for DNA-binding protein (DBP). The leader segments at coordinates 75 and 68.5 are the same as those found on the DBP message. We propose that the DBP gene be renamed "early region 2A (E2A)" and that the new transcripts be described as being

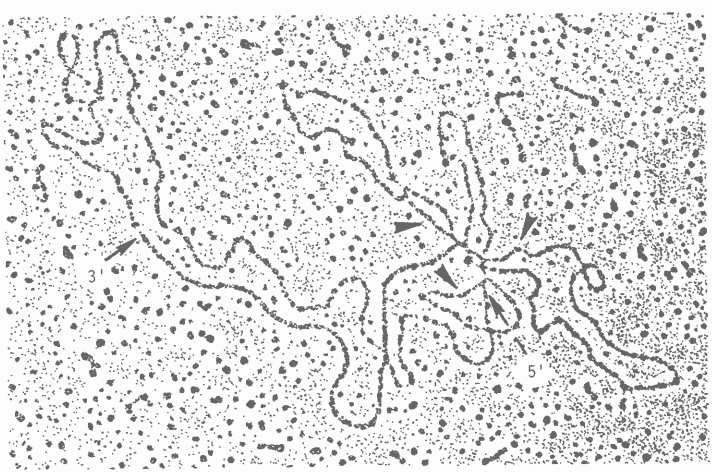


Figure 1
Ad2 DNA:early RNA heteroduplex revealing the structure of the predominant E2B RNA: (5') 75.1–75.0/68.5–68.3/39.3–39.0/26.0–11.1 (3'). The adenoviral DNA strand has circularized by virtue of its terminal inverted duplications. Arrows indicate the 5' and 3' ends of the RNA. Large arrowheads point to the three DNA deletion loops corresponding to the intervening sequences of the spliced RNA.

from "early region 2B (E2B)." The short internal leader from coordinate 39 is in close proximity to the 3' end of the major *r*-strand family-1 transcript synthesized at immediate-early times through intermediate and late times (coordinates 16.6/19.6/ ± 22.0-23.2/26.6/30.5–39.0). It is also close to the downstream splice point for joining the tripartite leader to the main body of the late *r*-strand family-2 RNAs (16.6/19.6/26.6/39.0-49.5) (Fig. 2). The 5' ends of the three different main bodies on the *l* strand are near the 5' ends of a minor member of late *r*-strand family-1 RNAs (30.5–39.0), the third *r*-strand RNA leader segment (26.5–26.8), and the 3' end of the *r*-strand "i" leader (22.0–23.2). Thus, as we have reported for many other regions of the adenoviral genome (*ICN-UCLA Symp. 14:* 611 [1979]), there is a clustering of transcriptional control sequences and RNA splice sites of transcripts both from the same strand and from complementary strands. No mRNAs were found with 5' ends (and, by inference, promoters) between coordinates 20 and 30 or with 3' ends near coordinate 20, such as those described on the basis of saturation hybridization analyses by Pettersson

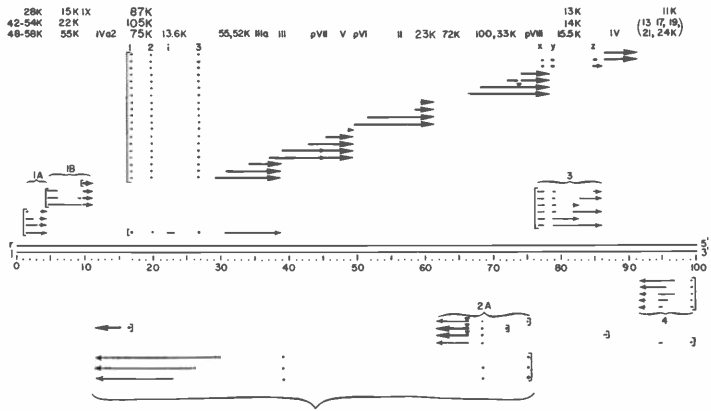


Figure 2

The human Ad2 cytoplasmic RNA transcripts characterized by electron microscopy of RNA:DNA heteroduplexes. The 36,500-bp chromosome is divided into 100 map units. Arrows indicate the direction of transcription along the r strand or the l strand of DNA. The 5' ends of the cytoplasmic RNAs fall within the boundaries of those of the primary nuclear transcripts (Evans et al., Cell 12: 733 [1977]; Berk and Sharp, Cell 12: 45 [1977]) and therefore refine the locations of transcription promoters (indicated by vertical brackets). The conserved segments constituting early RNAs (Chow et al., J. Mol. Biol. 134: 265 [1979]; Stillman et al., Cell 23: 497 [1981]) are depicted by thin arrows and those in late RNAs (Chow et al., Cell 12: 1; Chow and Broker, Cell 15: 497 [1978]; L. T. Chow and J. B. Lewis, in prep.) by thick arrows. The promoter for the late RNAs at coordinate 16.5 is also active at early times, but transcription terminates primarily at coordinate 39 (Chow et al., 1979 see above; Chow et al., Cold Spring Harbor Symp. Quant. Biol. 44: 401 [1980]). Gaps in arrows represent intervening sequences removed from the cytoplasmic RNAs by splicing. The ▼ marks optional splices. E1A, E1B, E2A, E2B, E3, and E4 are labeled. At intermediate and late times, E2 is expressed from several additional promoters and E3 RNA can be made under the direction of the major r-strand late promoter (Chow et al., 1979 see above). All derivatives of the late r-strand transcript have the same tripartite leader, the segments of which are labeled 1, 2, and 3. They form a number of 3'-coterminal families (Chow et al., Cell 11: 819 [1977]; Chow and Broker, 1978 see above). All late r-strand messages from the major promoter can have the i leader segment, but it is most commonly associated with the 55K,52K message, as shown, especially at early and intermediate times after infection or in cells infected in the presence of cytosine arabinoside (Chow et al., 1979 see above). Some of the RNAs for protein IV (fiber) can also contain some combination of ancillary leader segments x, y, z (Chow and Broker, Cell 15: 497 [1978]). The correlations of mRNAS with encoded proteins were based on cell-free translations of RNA selected by hybridization to DNA restriction fragments (Green et al., Virology 97: 275 [1979]; Harter and Lewis, J. Virol. 26: 736 [1978]; Lewis et al., Proc. Natl. Acad. Sci. 72: 1344 [1975], Cell 12: 37 [1977]; Cold Spring Harbor Symp. Quant. Biol. 44: 493 [1980]; Lewis and Mathews, Cell 21: 303 [1980]; Pettersson and Mathews, Cell 12: 741 [1977]; Spector et al., Cold Spring Harbor Symp. Quant. Biol. 44: 437 [1980]; van der Eb et al., Cold Spring Harbor Symp. Quant. Biol. 44: 383 [1980]; Stillman et al., 1981). Proteins are designated by K (1000 daltons) or by Roman numerals (virion components). Alternatively spliced RNAs complementary to E1A, E1B, E2B, E3, and E4 give rise to multiple proteins, some of which share common peptides (J. E. Smart, J. B. Lewis, and M. B. Mathews, pers. comm.).

et al. (*J. Mol. Biol. 101:* 497 [1976]) and by Galos et al. (*Cell 17:* 945 [1979]) in late and early RNAs, respectively.

These new RNAs have been correlated with proteins, with molecular weights of 105,000, 87,000, and 75,000, translated in cell-free systems programmed with fractions of the same RNA preparations that have been selected by hybridization to *Hin*dIII fragment B (coordinates 17.0–31.5). The sum of the molecular weights of these three proteins exceeds the coding capacity of the region between coordinates 11 and 30. Therefore, at least two of the proteins must have overlapping coding sequences. It has been demonstrated that the 87K protein is the precursor to the 55K protein linked to the 5′ terminus of each DNA strand and is necessary for DNA replication (see Protein Synthesis section; Stillman et al., [1981]). It is noteworthy that the relative amounts of the mRNAs from E2A and E2B (100:1) seem to reflect the amounts of DBP and terminal protein synthesized, which is possibly another reason they are expressed (and presumably coordinated) in the same transcription unit. The functions of the other two proteins of 105K and 75K are yet to be determined. We suspect that they also are involved in DNA replication.

Identification of Additional Early and Late RNA Transcripts

L.T. Chow

In collaboration with Jim Lewis (Protein Synthesis section), we previously characterized early adenoviral RNAs made in the absence of drugs or in the presence of cytosine arabinoside and cycloheximide (*J. Mol. Biol. 134:* 265 [1979]). In the course of the analyses of adenoviral transcripts from anisomycin-treated cells, an additional spliced product of E1B (coordinates 5′ 4.6–6.1/9.0–9.6/9.8–11.1 3′) and "fused" products of E1A and E1B and of E4 and E2A were characterized. The fused messages arise when termination and polyadenylation at the normal sites fail to occur and transcription extends into the downstream transcription unit and utilizes its termination signals. Because splicing of primary transcripts always conserves the 5′ end and 3′ poly(A), the products are new, hybrid messages composed of spliced segments derived from both the upstream and downstream transcription units.

Electron microscopic searches for new messages have also revealed two previously undescribed products of the major late *r*-strand transcription unit in late RNA preparations (Fig. 2). Both have the consanguinous tripartite leader or the tripartite leader plus i leader segment (coordinates 22.0–23.2) found on all products of the primary transcript. The main bodies of the RNAs are short and extend from coordinate 48.6 to coordinate 49.5 (at the 3′ end of late family 2) or from coordinate 59.7 to coordinate 61.8 (at the 3′ end of late family 3). The latter RNA is coincidental with an open translation frame found by DNA sequence analysis downstream from the hexon gene (Kruijer et al., *Nucleic Acids Res. 8:* 6033 [1980]; Akusjarvi and Pettersson, *Nucleic Acids Res. 9:* 1 [1981]) and therefore is inferred to be message for a virus-encoded endopeptidase identified by the phenotype of mutant *ts*1 mapped in that region. The *ts*1 mutant virus fails to process several virion precursor proteins to their mature forms (Bhatti and Weber, *J. Biol. Chem. 254:* 12265 [1979]) and in particular does not efficiently cleave the 87K precursor to the 55K DNA terminal protein (Challberg and Kelly, *J. Virol.* [1981], in press; Stillman et al., 1981.

Studies of Ad9 and Ad12

L.T. Chow

Ad9, a representative of class D, has been reported to cause tumors specifically in female rat breast tissue, unlike all other human adenoviruses tested (Ankerst et al., *Int. J. Cancer 13:* 286 [1974]). Male rats are unaffected. Bill Topp and Diane Smith (Tumor Virus section) are in the process of retesting these results and are constructing restriction enzyme cleavage maps and cloning Ad9 and related viruses. We are using electron microscopic heteroduplex techniques to (1) determine the regions of homology and divergence of the Ad9 chromosome with respect to the chromosomes of other adenoviruses; (2) map the RNA transcripts, which we have shown to have tripartite leaders and main-body coding regions quite analogous to those of Ad2, 3, and 7; and (3) align the transcription and restriction maps. In the future, we will examine the RNAs produced by Ad9 in various male and female rat cells or in cell cultures to attempt to define this unusual sex and tissue specificity of transformation.

Ashley Dunn (formerly of CSH Laboratory and now at the EMBO Laboratory in Heidelberg) has provided us with an Ad12 *Eco*RI-D clone (16.5–28.5) of Ad12 DNA. By electron microscopy, we have found that it encodes all the components of the tripartite leader. Jeff Engler is currently sequencing this segment for comparison with the leader regions and E2B regions of Ad2, Ad3, and Ad7.

Both Ad9 and Ad12 also specify i leader segments present in *r*-strand transcripts analogous to those of Ad2, Ad3, and Ad7, which we previously discovered. This reinforces our conviction that the i leader is essential for the virus and is consistent with our results in a collaborative study with Bill Kilpatrick and Jim Lewis (Protein Synthesis section) that the Ad2 i leader segment is actually an mRNA for a 13,600-dalton protein.

Sequencing of Ad3, Ad7, and Ad12 DNAs

J. A. Engler, M. P. van Bree, B. Urso, T. R. Broker

Previous electron microscopic studies by Kilpatrick et al. (*J. Virol.* **30:** 899 [1979]) suggested that

the leader segments encoded by DNAs of class-B Ad3 and Ad7 were not homologous to those of the class-C Ad2. Although sufficient divergence in sequence was found to explain the lack of cross-annealing, the DNA sequence analysis of cloned segments of Ad7 and Ad3 DNAs between genome coordinates 15.9 and 17.0 showed an unexpectedly high degree of homology with the comparable region of Ad2, as reported last year. This homology with Ad2 has now been found to extend from genome coordinate 11 to at least coordinate 19 in Ad7 (and, where sequenced, in Ad3 and in the class-A Ad12). The majority of the divergences are nucleotide transitions and transversions. The few deletions and insertions in this region are multiples of three nucleotides. Also, most transitions and transversions are found to be separated by multiples of three nucleotides. On the reasonable assumption that these divergences occur primarily in third codon positions, the protein-coding frame can be assigned. In the case of the IVa2 gene, 90% of the inferred amino acid sequence is conserved in all the serotypes studied. Furthermore, many of the changes in the amino acid sequence involve substitution of functionally similar amino acids. Another long open translation frame, with serotype divergences in the third coding position, starts to the right of genome coordinate 19 and extends leftward to a stop codon near coordinate 15 in the intervening sequence of the IVa2 gene. One or more of the *l*-strand transcripts from E2B, described above, may encode this anticipated polypeptide.

The DNA sequence of the protein-coding region for the Ad3 peptide IX gene (genome coordinates 9.5–11) has also been determined (Fig. 3). Although the aminoterminal halves of the nucleotide and amino acid sequences are highly conserved when compared with those of Ad2 and Ad5, the carboxyterminal halves are much more diverged. These two domains are separated by an alanine-rich chain about 10 residues long in all serotypes studied, sufficient to make an alpha-helical linker. Many deletions and insertions are apparent, but except for one near the carboxyl terminus, which changes the utilized protein termination codon, all occur as multiples of three nucleotides within the protein-coding region.

There are a large number of divergences between class-B viruses Ad3 and Ad7 and class-C viruses Ad2 and Ad5 near the 5′ and the 3′ termini of peptide IX RNA (Fig. 3). These divergences are located near or at the splice sites of E1B RNAs, the "TATA" box of gene IX, the cap site (5′ end) and ribosomal RNA-binding site of gene-IX RNA, and the protein termination and polyadenylation signals at the 3′ end. Such extensive variations in transcription regulatory regions and RNA-splicing sites emphasize the utility of comparative sequence analysis in the study of eukaryotic gene expression, since the natural variants effectively provide a collection of viable mutations.

Regulation of Ad2 E2 by Products of E1

M. Rossini

Studies initiated at Temple University with R. Baserga concerned the induction of cellular DNA synthesis in Ad2-infected *ts*AF8 cells (Rossini, Weinmann, and Baserga, *Proc. Natl. Acad. Sci. 76:* 4441 [1979]), a G_1-specific, temperature-sensitive mutant derived from BHK-21 cells. Ad2 E1 and E2A were identified as the minimal requirement for the induction of cellular DNA synthesis in *ts*AF8 cells at the permissive temperature (Rossini, Jonak, and Baserga, *ICN-UCLA Symp. Mol. Cell. Biol. 18:* 317 [1980]; M. Rossini, G. Jonak, and R. Baserga, *J. Virol.*, in press). By direct infection and by microinjection of different combinations of adenoviral DNA fragments into quiescent (serum-depleted) cells, it was found that the expression of E2A is activated by the presence of E1A. This is analogous to the regulatory effect observed using adenoviruses deficient in expression of E1A, which fail to turn on E2A (Jones and Shenk, *Proc. Natl. Acad. Sci. 76:* 3365 [1979]; Berk et al., *Cell* **17:** 935 [1979]). These results demonstrate the possibility for dissecting the eukaryotic genome and placing into cells noncontiguous segments of DNA for the study of the regulatory effects of certain genes on the expression of others.

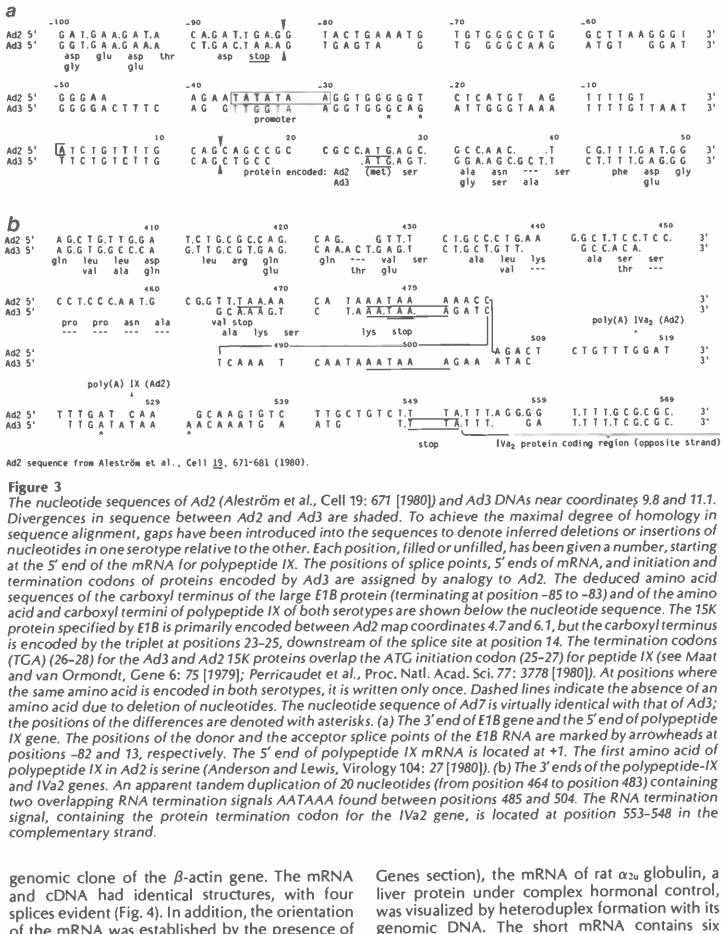
At present, microinjection techniques are being used to introduce into cell nuclei various plasmids containing adenoviral DNA segments corresponding to those of wild-type or mutagenized E1A (from Nigel Stow and David Solnick of the Tumor Virus section, and T. Shenk of SUNY, Stony Brook) and E2A, in different combinations. In particular, one such variant, mutated at coordinate 2.8 to resistance to *Sma* restriction endonuclease, behaves like the wild-type E1A plasmid in activating E2. In contrast, another mutant, which has a deletion at the *Xba* restriction site at coordinate 3.8, is unable to induce a detectable amount of the DNA-binding protein. More detailed characterizations of these and other E1A mutants are under way. Experiments using E2A clones containing only the early or the late promoter, or no promoter, are also in progress. These studies should localize the sequences in the E1A responsible for the activation of E2A and should ultimately define the target operator sequences and the roles of the early and late promoters for E2.

Mapping of Eukaryotic Genes

L.T. Chow

β-Actin gene from whole chicken embryo

In collaboration with Paul Thomas (Protein Synthesis section), Jim Feramisco (Cell Biochemistry section), and Steve Hughes (Eukaryotic Genes section), electron microscopy was used to map the mRNA and cDNA of chicken β-actin against a



Ad2 sequence from Aleström et al., Cell 19, 671-681 (1980).

Figure 3

The nucleotide sequences of Ad2 (Aleström et al., Cell 19: 671 [1980]) and Ad3 DNAs near coordinates 9.8 and 11.1. Divergences in sequence between Ad2 and Ad3 are shaded. To achieve the maximal degree of homology in sequence alignment, gaps have been introduced into the sequences to denote inferred deletions or insertions of nucleotides in one serotype relative to the other. Each position, filled or unfilled, has been given a number, starting at the 5' end of the mRNA for polypeptide IX. The positions of splice points, 5' ends of mRNA, and initiation and termination codons of proteins encoded by Ad3 are assigned by analogy to Ad2. The deduced amino acid sequences of the carboxyl terminus of the large E1B protein (terminating at position -85 to -83) and of the amino acid and carboxyl termini of polypeptide IX of both serotypes are shown below the nucleotide sequence. The 15K protein specified by E1B is primarily encoded between Ad2 map coordinates 4.7 and 6.1, but the carboxyl terminus is encoded by the triplet at positions 23-25, downstream of the splice site at position 14. The termination codons (TGA) (26-28) for the Ad3 and Ad2 15K proteins overlap the ATG initiation codon (25-27) for peptide IX (see Maat and van Ormondt, Gene 6: 75 [1979]; Perricaudet et al., Proc. Natl. Acad. Sci. 77: 3778 [1980]). At positions where the same amino acid is encoded in both serotypes, it is written only once. Dashed lines indicate the absence of an amino acid due to deletion of nucleotides. The nucleotide sequence of Ad7 is virtually identical with that of Ad3; the positions of the differences are denoted with asterisks. (a) The 3' end of E1B gene and the 5' end of polypeptide IX gene. The positions of the donor and the acceptor splice points of the E1B RNA are marked by arrowheads at positions -82 and 13, respectively. The 5' end of polypeptide IX mRNA is located at +1. The first amino acid of polypeptide IX in Ad2 is serine (Anderson and Lewis, Virology 104: 27 [1980]). (b) The 3' ends of the polypeptide-IX and IVa2 genes. An apparent tandem duplication of 20 nucleotides (from position 464 to position 483) containing two overlapping RNA termination signals AATAAA found between positions 485 and 504. The RNA termination signal, containing the protein termination codon for the IVa2 gene, is located at position 553-548 in the complementary strand.

genomic clone of the β-actin gene. The mRNA and cDNA had identical structures, with four splices evident (Fig. 4). In addition, the orientation of the mRNA was established by the presence of polyadenylate at the 3' end. Similar to most eukaryotic mRNAs, the longest uninterrupted sequence of the segmented RNA is located at the 3' end.

α_{2u} *Globulin gene from rat liver*

In collaboration with David Kurtz (Eukaryotic Genes section), the mRNA of rat α_{2u} globulin, a liver protein under complex hormonal control, was visualized by heteroduplex formation with its genomic DNA. The short mRNA contains six splices, with the longest uninterrupted segment at its 3' end (Fig. 5). Two segments of the cloned genomic DNA were found to be flanked by short inverted duplications, and the single-stranded DNA formed stem-loop structures. Part of the α_{2u} mRNA is located within one of the stem loops, and one of the repetitious sequences is situated within one of the intervening sequences.

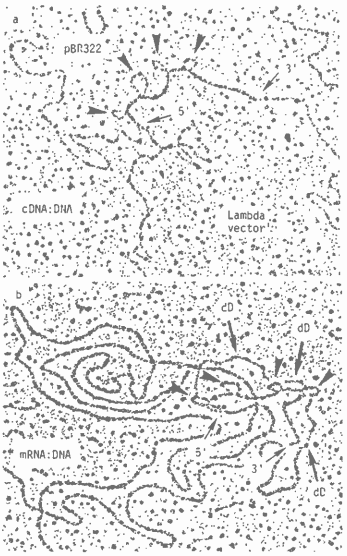


Figure 4
The structure of chicken β-actin mRNA. (a) A heteroduplex between cDNA cloned in pBR322 and the genomic DNA cloned in a λ Charon vector. (b) An R loop between poly(A)-selected mRNA and the genomic DNA clone. The 5' and 3' ends of the message sequences are indicated. Arrowheads point to the four DNA deletion loops corresponding to the sequences deleted in the spliced RNA.

Sequencing of the Insertion Sequence IS5

J. A. Engler, M. P. van Bree

Insertion sequences are short DNA segments found in the chromosomes of prokaryotes and which can translocate to other positions in the chromosome at low frequency. Insertion sequences can mediate inversion, deletion, and fusion of DNA segments, as well as disrupt normal expression of nearby genes. At least seven different insertion sequences are known. Each element consists of a unique region flanked by a short inverted repetition; they generate a duplication of up to 11 bp upon integration into a new DNA site.

In an earlier paper from this laboratory, Chow and Broker (*J. Bacteriol. 113:* 1427 [1978]) identified several examples of insertion sequence IS5. They concluded that IS5 was approximately 1.2 kb long and contained a short inverted duplication at its ends. These observations have now been confirmed in the nucleotide sequence. IS5 is 1195 bp long and has an inverted duplication of 16 nucleotides (with one mismatch) at each end (Fig. 6). IS5 has the shortest terminal inverted duplication of any of the seven insertion sequences. The largest potential region for encoding a polypeptide extends through approximately 90% of IS5 and could encode 338 amino acids. Upon insertion into the chromosome, IS5 makes a 4-bp duplication of the host DNA.

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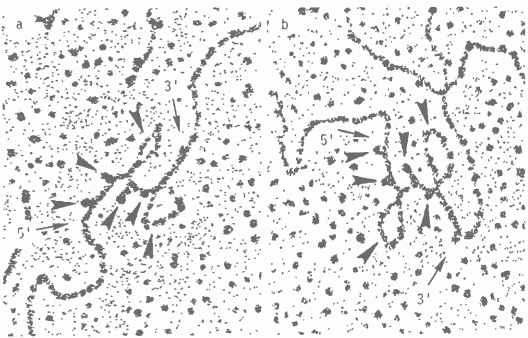

Figure 5
Heteroduplexes formed between genomic rat DNA cloned in a λ Charon vector and α_{2u} mRNA. The RNA preparation was total rat liver RNA. The 5' and 3' ends of the RNA are indicated. Arrowheads point to the six DNA deletion loops corresponding to the sequences deleted in the spliced RNA.

Figure 6

The nucleotide sequences of the sites of integration and of the ends of bacterial insertion element IS5. All sequences are shown in the 5'—3' direction. The four nucleotide pairs of host DNA duplicated upon insertion of IS5 are marked by light shading. The termini of the IS5 element that are inverted duplications of one another are indicated by dark shading. (a) The nucleotide sequences of two bacteriophage Mu sites prior to IS5 insertion. (b) The left-end junctions of two IS5 insertions cloned in plasmids pGP31 and pGP33 (van de Putte et al., Cold Spring Harbor Symp. Quant. Biol. 45: 347 [1981]). (c) The right-end junctions of three IS5 insertions in pGP31, pGP33, and pGM1 (Kamp et al., Cold Spring Harbor Symp. Quant. Biol. 43: 1159 [1979]). The first nucleotide of IS5 is labeled number 1, and the major open translation frame runs from smaller numbers to higher numbers. The single EcoRI cleavage site in IS5 is near the left end.

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PROTEIN SYNTHESIS

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During the last year the Protein Synthesis section has continued to focus its attention on the expression of adenoviral genes, with an increasing concentration on regulatory aspects. The adenoviruses are DNA viruses that can indulge in two types of interactions with human and other mammalian cells: They can multiply in permissive cells, giving rise to a new generation of progeny viruses; alternatively, they can form a more lasting liaison with nonpermissive cells, which then become transformed or tumorous. In the latter case, only a fraction of the genetic potential of the virus is expressed and no progeny virus results. The adenoviruses, therefore, pose questions in the areas of eukaryotic gene structure and regulation and of viral carcinogenesis. The topics to be addressed here fall under five headings: (1) control of gene expression during productive infection, (2) viral products in transformed cells, (3) viral DNA replication, (4) expression of adenoviral genes injected into frog oocytes, and (5) small viral RNAs.

The Early/Late Switch

The life cycle of DNA viruses has customarily been viewed as biphasic, with an early phase during which the stage is set for replication of the viral chromosome followed by a late phase in which DNA replication is achieved and the structural proteins of the virus particle are elaborated. Using a superinfection protocol, we have established, both for adenoviruses and for the monkey virus SV40, that the transition between the two phases is contingent upon DNA synthesis. Templates that have not undergone replication do not express their late genes (with the exception discussed below), even when present in cells containing genomes actively engaged in late gene expression. The *cis*-acting nature of this control renders it most probable that it takes place at the level of transcription, rather than at some subsequent stage such as processing, transport, or stabilization of the mRNA. Since the late promoter is active at early times (see below), we believe that it is transcriptional termination, rather than initiation, that is the point of regulation. What remains elusive, however, is the nature of the coupling between the processes of replication and transcription: We, and others, have looked for differences of various kinds between the early and late forms of the template, but as yet without success. The mechanism is likely to be important in regulatory switching elsewhere (during differentiation, for example), but all we can say at present is that some structural change in the template that is associated with DNA replication is required to permit RNA polymerase to read beyond a particular point.

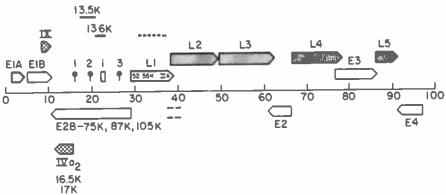
Regulation in the Early Phase

Work from this and other laboratories in the last few years has led to a recognition of the complexities of interactions between the products of individual viral genes during the early phase. The 72K DNA-binding protein appears to act as a repressor during later stages of the early phase, turning off expression of other early genes. In contrast, the early region 1A (E1A) gene, which is part of the transforming region, seems to function as an inducer, and under some circumstances it is absolutely required for transcription from the other early genes. In the course of experiments designed to identify the E1A products involved, we discovered that profound inhibition of protein synthesis interferes with the production of E1A mRNA. This unexpected result implies that E1A-gene activity is dependent on the synthesis of protein(s) of either host or viral origin. Hitherto, the E1A products were supposed to be the first to be expressed by the virus, which would seem to rule out the possibility that viral proteins are required for E1A expression. However, it transpired that the production of at least two viral mRNAs is immune to profound inhibition of protein synthesis; these RNAs encode the closely related polypeptide pair 52K,55K (polypeptides of 52,000 and 55,000 daltons) and another protein of 13,500 daltons (13.5K). We had previously shown that the former are, in fact, products of the first portion of the late gene block; the mRNA for the 13.5K protein has not been fully characterized, but its map position and strand orientation are consistent with an origin in the major late transcription unit (Fig. 1). Thus, we have the paradox that the only autonomous viral gene products stem from the late gene block. Whether these so-called "immediate-early" genes exert control over the E1A gene or other viral genes is presently under consideration.

Regulation of Late Genes

The mRNA for the 52K,55K protein appears to be the first (leftmost) member of the late gene family. Together with the mRNA for virion polypeptide IIIa, which overlaps its 3' portion, it constitutes the L1 block of late mRNAs (Figs. 1 and 2). Polypeptide IIIa, however, is a bona fide late protein, and neither it nor its mRNA is found prior to DNA replication. Thus, the late region

Figure 1
The locations and regulation of adenoviral gene blocks. The early regions, E1–E4, are transcribed prior to viral DNA replication. The newly described E2B region contains the gene for the terminal protein precursor. Products of the E1A gene seem to be involved in activating the other early genes. For the most part, the late gene families, L1–L5, are not expressed until DNA synthesis has commenced. An exception is the L1 mRNA encoding the 52K,55K protein pair, which, like that for the 13.5K protein, is "immediate-early"—requiring no prior viral protein synthesis. Polypeptides IX and IVa2 and the 16.5K and 17K proteins are "intermediate" in that they are produced to some extent both before and after replication. (Based on results from this and other laboratories.)



appears to be subject to two types of regulatory phenomena: (1) transcriptional and (2) processing. The transcriptional control, discussed above in relation to the early/late switch, now must be viewed in terms of attenuation of RNA chain elongation at or shortly after the 3′ end of the L1 block, such that at early times precursors to L2–L5 mRNAs are not made (Fig. 2). Processing control must be invoked to explain how it is that all of the sequences of the polypeptide-IIIa mRNA are contained within the mRNA for the 52K,55K polypeptide pair, and they presumably stem from the same precursor, yet only the former species is produced at early times.

One prediction of this scheme is that the polypeptide-IIIa mRNA, alone among the late species, should be made from unreplicated templates if, and only if, they are present in the milieu of a "late" nucleus. This hypothesis has been verified by superinfecting cells already in the late stage of an initial infection with an adenovirus that produces a distinguishable IIIa protein. Even when the replication of the second virus is blocked, its characteristic polypeptide-IIIa mRNA is detected, and at levels proportional to the superinfection multiplicity. No other late mRNAs of this virus are made—apart, of course, from that encoding the immediate-early 52K,55K protein.

Taken together, the data suggest that an

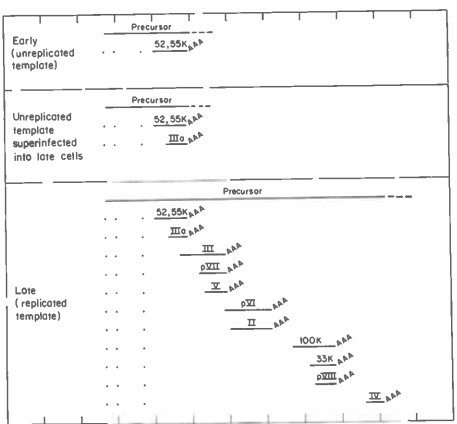


Figure 2
Regulation of late gene expression. Contrary to earlier views, the late promoter is active before DNA replication commences. Under these conditions, only one member of the L1 family of mRNAs (encoding the 52K,55K protein pair) appears. However, both this and the L1 mRNA for polypeptide IIIa are made from an unreplicated DNA template if it is introduced by superinfection into a cell that is already in the late phase and producing the full set of late species. (Data from Lewis and Mathews 1980; Thomas and Mathews 1980.)

adenovirus-specific factor(s) replaces or modulates the preexisting splicing activity of the host cell, thereby providing the apparatus necessary for the fabrication of the many late mRNAs from their common precursor. This change could well explain other phenomena in adenovirus-infected cells—the changing ratios of differently spliced versions of early mRNAs and the shut off of host protein synthesis, for example, which both seem to be linked to DNA replication in as yet undefined ways. Current experiments are directed toward identifying the viral products responsible for these alterations.

Viral Products in Transformed Cells

We have nearly completed a survey of some of the standard adenovirus-transformed cell lines, employing the hybridization-selection/cell-free translation technique, to catalog the mRNAs that they contain. The survey concentrated on adenovirus-type-2 (Ad2)-transformed rat cell lines, although we also included one adenovirus-type-5 (Ad5)-transformed human cell line (293 cells) that is in widespread laboratory use as a permissive host for viruses defective in regions E1A and E1B. The E1A and E1B genes give rise to six to eight early proteins in infected cells and represent the transforming region of the virus invariably expressed in tumor cells. The four largest E1A proteins (42K–58K) are made in all cell lines, but the smallest species (28K), which in infected cells makes a relatively late appearance, is not found. Both of the major E1B proteins (57K and 15K) are produced; again, the late species (22K) is not seen, and polypeptide IX, an "intermediate" product (Fig. 1), has only been observed in one cell line (F4). Some of the cells also contain and express other early regions—late regions, if present in the cells' DNA, are quiescent—and there are few surprises. One curiosity is that analysis of the structure of the integrated viral DNA and of the mRNAs in the F4 line, by Joe Sambrook and Shiu-Lok Hu (Tumor Virus section), had shown that some E1A mRNAs were fusions between E4 and E1A. Accordingly, some of the mRNA for the E4 11K protein hybridizes to E1A DNA (and, to a lesser extent, vice versa); however, the polypeptides translated from these chimeras appear to be authentic proteins. Some cell lines contain novel mRNA species in addition to the expected complement: The T_2C_4 line, for example, produces mRNAs from the newly discovered E2B region described below.

DNA Replication

One motive for the analysis of transformed-cell RNAs and proteins is to seek alternative sources of quantities of early proteins for functional studies, many of the most interesting proteins being rather scarce in productively infected cells. As part of a long-term goal to define the proteins involved in adenoviral DNA replication, we have begun to screen for monoclonal antibodies that are capable of inhibiting DNA replication in vitro. Starting with mice challenged with extracts of adenovirus-infected HeLa cells (and other preparations), we hope to obtain monoclonal antibody that directly inhibits the function of a replication protein, thus enabling identification of that protein and eventually of its function. Results obtained so far show that medium from wells containing hybridoma cells does not, in general, affect in vitro replication. Furthermore, monoclonal antibody against SV40 T antigen is without effect, whereas monoclonal antibody against the adenovirus 72K single-stranded-DNA–binding protein (provided by Dan Klessig and Ron McKay, Tumor Virus section) effectively blocks replication. We are currently screening hybridomas for inhibition of DNA replication as well as by immunofluorescence against infected and mock-infected HeLa cells.

An important advance in eukaryotic DNA replication is the development, by T. J. Kelly and his coworkers (Johns Hopkins University), of a soluble nuclear extract from adenovirus-infected HeLa cells that is able to initiate and complete one round of semiconservative synthesis (*Proc. Natl. Acad. Sci. 76:* 655 [1979]; *Proc. Natl. Acad. Sci. 77:* 5105 [1980]). The system uses exogenous adenoviral DNA as template and requires the presence of the covalently attached (55K) terminal protein on the 5′ termini of the template strands. Surprisingly, newly made DNA strands synthesized in vitro bear a covalently linked 80K protein that is probably a precursor to the virion 55K terminal protein.

During the past year we have improved the system to obtain more consistent results and enhanced synthesis and have demonstrated that nascent DNA strands synthesized in vitro also contain a protein covalently attached to the 5′ end of the DNA. Nuclear extracts prepared from cells infected with Ad2 also support the replication of DNA-protein complex prepared from the unrelated adenovirus type 7 (Ad7). Ad7 DNA has a longer inverted terminal repetition than does Ad2, and the replication origins of these different serotypes have only limited base-sequence homology. Complementation of this kind could be used to identify the regions of adenoviral DNA that are important for initiation of DNA replication.

The Terminal Protein Precursor

The gene encoding the terminal protein precursor, 87,000 daltons in our hands, has been identified by exploiting a virus mutant that specifies a conditionally defective protease. J. Weber and his colleagues (*Virology 76:* 709 [1977]; *Virology 80:* 83 [1979]) showed that under restric-

tive conditions Ad2*ts*1 fails to process the precursors of several virion structural proteins to their mature forms. At the same time, the virus contains the 87K form of the terminal protein. We have shown by peptide analysis, performed in collaboration with John Smart (Protein Chemistry section), that this protein is indistinguishable from the 87K protein translated in vitro from purified viral mRNA. The mRNA was mapped by molecular hybridization and found to anneal to a large tract of the genome between coordinates 11 and 29 and, in addition, to sequences between positions 37 and 41. The mRNAs encoding two other large proteins, 105K and 75K, also map in this region of the genome. All of these species are transcribed in a leftward direction, and they correspond in structure to a family of RNAs that has been observed by electron microscopy (in conjunction with Louise Chow, Electron Microscopy section). The RNAs have long "bodies," extending from coordinate 30, 26, or 23 to coordinate 11, linked to "leaders" mapped to positions 75, 68.5, and 39. The location of the third leader explains the selection of the mRNAs by hybridization to the DNA sequences between coordinates 37 and 41, and the first two leaders are also found in the early form of the mRNA encoding the E2 72K protein (Fig. 3). It is most likely, therefore, that they use the same promoter, and we propose the term "region E2B" for the gene encoding the 87K protein. It is a striking fact that two proteins that are intimately involved in DNA replication should also be closely related in their mode of expression.

The current view of adenoviral DNA replication is shown in Figure 4. Synthesis is initiated from either end of the linear DNA duplex by a complex between the terminal protein and dCTP. The reaction also requires the presence of terminal protein on the template molecule: Both the 87K form and the 55K form of the protein are effective here. DNA polymerase and the 72K DNA-binding protein permit growth of the new chain, displacing its parental counterpart, which is then free to act as template for second-strand synthesis. The cleavage of the 87K protein to the normal mature 55K form seems to occur in the virus particle, passing through a 62K intermediate stage, under the direction of the virus-specified proteolytic enzyme that is defective in Ad2*ts*1.

Expression of Adenoviral Genes in Frog Oocytes

Xenopus oocytes (immature and unfertilized eggs) afford singular advantages for studies of gene activity. Their large size makes it possible to inject DNA or RNA into either nucleus or cytoplasm and to detect the products of transcription or translation by standard biochemical techniques. When oocytes are injected intranuclearly with adenoviral DNA and labeled proteins are examined by immunoprecipitation with antisera against viral proteins, the activity of two viral genes can be detected: the genes for the E2 72K protein and for polypeptide IX.

The E2 gene is distinguished by the possession of two separate promoters, used differentially at early and late times, neither of which has been shown to function efficiently in vitro. In the oocyte, the 72K protein is the main immunoprecipitable virus-specific product, amounting to 1–3% of the labeled oocyte protein. Viral DNA cleaved within the structural gene, or between the gene and the late promoter, is inactive in 72K protein synthesis. On the other hand, DNA cleaved between the early (distal) and late (proximal) promoters is fully active, implying that the early promoter is dispensable (and possibly inactive) in oocytes and that the late E2 promoter is probably functioning. An isolated fragment of adenoviral DNA containing the E2 region, cloned in pBR322, is also capable of efficient synthesis of normal 72K protein. Two other polypeptides, with molecular weights of 48,000 and 43,500, are immunoprecipitated from oocytes injected with adenoviral DNA or the cloned E2 region. Tryptic peptide maps of these proteins have been constructed in collaboration with John Smart (Protein Chemistry section). The 48K polypeptide contains all the internal methionine-containing tryptic peptides of the 72K protein,

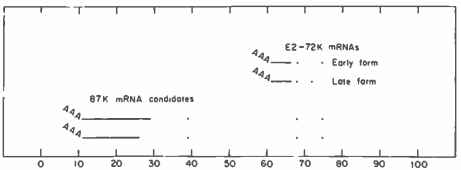


Figure 3
Region-E2 products. At least three proteins have been assigned to the E2B region. One or both of the RNA species illustrated probably encodes the 87K terminal protein precursor. There is an additional E2B RNA with a messenger body extending between coordinates 23 and 11 (not shown). The E2B RNAs share leaders with E2A RNAs, encoding the 72K DNA-binding protein, and they are likely to be regulated coordinately. (Reprinted, with permission, from Stillman et al. 1981.)

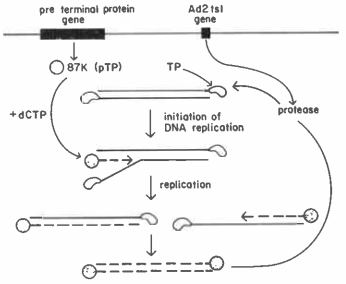


Figure 4
Model of adenoviral DNA synthesis. The upper line represents the adenoviral chromosome, showing the genes for the terminal protein precursor and the protease (Hassel and Weber, J. Virol. 28: 671 [1978]). The precursor (stippled circles) *seems to function in the initiation of new DNA strands* (dashed lines) *and is normally cleaved by the protease in a later maturational event. A resident terminal protein, either the 87K precursor or the 55K mature form (kidney-bean-shaped), is also needed at the DNA terminus for faithful replication to occur. (Modified from Stillman et al. 1981; Rekosh et al.,* Cell 11: *283 [1977]; Challberg et al.,* Proc. Natl. Acad. Sci. *77: 5105 [1980].)*

and it is probably the proteolytic degradation product of the 72K protein. The 43.5K polypeptide lacks one of the internal methionine-containing peptides. Its production in oocytes is independent of the promoter that directs the synthesis of the 72K and 48K polypeptides, suggesting that it is the product of a different region-E2 mRNA. The observation that it is also produced by translation of adenoviral mRNA from infected HeLa cells indicates that it is not an anomaly caused by the transcription of adenoviral DNA in a foreign environment.

The synthesis of polypeptide IX, an intermediate product in infected cells, occurs much less efficiently than does 72K protein synthesis, despite the fact that its mRNA is unspliced and therefore might be less demanding to produce. It is also made when cloned viral DNA fragments are injected. We are presently analyzing a series of deletion derivatives of the polypeptide-IX and region-E2 plasmids for their activity in oocytes, with a view to examining several of the stages in the pathway of gene expression: the initiation and termination of mRNA synthesis, polyadenylation of the 3' end and splicing of the mRNA, and its transport to the cytoplasm and its translation there. An analysis of the subcellular localization and structure of the mRNA synthesized by non–protein-synthesizing mutant plasmids should provide insights into the interrelationships between the various steps in mRNA processing and translation.

Small Viral RNAs

Adenovirus specifies the production of two small (160 nucleotide) RNAs, the VA RNAs, which are synthesized in copious amounts during infection but do not code for proteins. Both species of VA RNA, VA RNA_I and VA RNA_{II}, bind to late adenoviral mRNA or to DNA clones thereof, apparently across splice junctions. This may imply that these RNAs play a role during the splicing process, bringing together the two arms of an impending splice point in a precursor molecule, as has also been suggested for the small nuclear U1 RNA of uninfected cells (Lerner et al., *Nature 283:* 220 [1980]; Rogers and Wall, *Proc. Natl. Acad. Sci. 77:* 1877 [1980]). Detailed analysis of the mRNA:VA RNA interactions is in progress.

To assess the range of variability permissible in the VA RNAs of the group-C adenoviruses (serotypes 1, 2, 5, and 6), we have compared them by RNA fingerprinting. In addition, we have attempted to generate variant forms by selecting versions of the Ad2 VA RNA_I gene that lack the *Bam*HI restriction site which it contains (in collaboration with Terri Grodzicker of the Tumor Virus section). The naturally occurring strains contain VA RNA_I in one of two forms (one found in Ad2 and Ad6 and the other in Ad1 and Ad5) that differ by two nucleotides. There are three sites of variation in VA RNA_{II}, the Ad1, Ad2, and Ad5 forms, each differing from Ad6 VA RNA_{II} at one of the positions. Of the selected variants, one possesses a 4-base duplication within the *Bam*HI cleavage site, whereas the other two have acquired sequence changes rendering their VA RNA_I genes indistinguishable from those of Ad5. Not only are these changes relatively minor in themselves, but also their impact on the probable secondary structure of the RNAs is slight. Both the VA RNAs can adopt very stable base-paired hairpinlike conformations with some unpaired loops; the sequence alterations almost all lie within unpaired regions and so would not be expected to disrupt the structures significantly. Thus, the range of variation among the VA RNAs of the group-C adenoviruses is rather slight and certainly far less than the differences between the VA RNA_I and VA RNA_{II} species.

Sequences from other serotype groups are not yet available, but fingerprints suggest that they are not very closely related. Among the group-C adenoviruses, the VA RNA data would indicate that Ad6 is closer to a prototype strain than are the other serotypes. Taking into account infor-

mation from M. Sleigh and Joe Sambrook (Tumor Virus section) on the distribution of restriction enzyme sites in the four canonical group-C strains, we have been able to make numerical estimates of the relationships. These show that Ad1, Ad2, and Ad5 are almost equally removed from the "average" group-C virus, whereas Ad6 is much closer to the generalized prototype and also to Ad2.

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PROTEIN CHEMISTRY

J. Smart, *G. Binns, P. Chow, K. Drickamer, H. Engeser, M. Hallaran*

The Protein Chemistry section came into existence at the beginning of 1980. During January, February, and March the laboratory was completely renovated and equipped for protein-sequence work. The work in this laboratory is divided into two main areas: (1) studies on the structure and function of the transforming proteins of tumor viruses (adenovirus type 2 [Ad2], simian virus 40 [SV40], and the avian sarcoma viruses) and (2) structural work on receptors for glycoprotein endocytosis.

Hepatic Lectins

K. Drickamer

Removal of terminal carbohydrate residues from serum glycoproteins results in the rapid clearance of the glycoproteins from the circulation. The effect is mediated by liver cells, which specifically recognize the partially degraded glycoproteins via a receptor at their surfaces. The receptor, by virtue of its carbohydrate-binding ability, is known as an hepatic lectin. The receptor is the first component in the cellular mechanism for endocytosis of the glycoproteins; after binding to the receptor, the ligand moves to the lysosomes via coated pits and vesicles. This process is thus an example of the phenomenon of receptor-mediated endocytosis. Our work is aimed at elucidating the structure and function of the hepatic lectins in this process. The specific areas currently being studied are (1) the sequence of hepatic lectins (to form the basis for studying their function), (2) the essential residues in the glycoprotein-binding site, (3) the arrangement of the lectins in the membrane, and (4) the mechanism of synthesis of the lectins.

Sequence of chicken hepatic lectin

Most of the past year has been devoted to sequence analysis; this involved establishing a working protein-sequencing facility, which is now in operation. The major accomplishment has been the determination of the complete amino acid sequence of the chicken hepatic lectin (CHL). The results are shown in Figure 1. The sequence reveals several interesting features. Of particular interest is the stretch of uncharged, mostly hydrophobic residues in positions 24 to 48. The location of carbohydrate attachment is at position 67; this indicates that sequences in this region are probably exposed at the cell surface, which is in accord with the general finding that carbohydrate in plasma membranes faces the cell exterior. The hydrophobic nature of the amino acids in positions 24 to 48 is highly reminiscent of hydrophobic stretches in other cellular and viral proteins that span membranes. If CHL does, in fact, span the hepatocyte plasma membrane, the amino terminal would be on the cytoplasm side if the carbohydrate-attachment site is on the extracellular side. It is interesting to note that this proposed orientation of CHL would be the opposite of the orientation of some other membrane proteins, such as erythrocyte glycophorin and lymphocyte histocompatibility antigens. Current suggestions, based on the "signal hypothesis," about the synthesis of transmembrane proteins would not appear to provide any ready explanation for how a CHL polypeptide becomes inserted in the membrane.

Carbohydrate-binding site

Two approaches are being used to study the carbohydrate-binding site of CHL. In the first, various affinity labeling reagents analogous to the normal binding substrate (*N*-acetylglucosamine) are being tested for their ability to compete with glycoprotein ligands for binding to CHL. The most promising results so far are with the reagent *N*-(3-nitro-4-azidophenyl)-glucosamine, which competes with agalactoorosomucoid at concentrations very similar to free *N*-acetylglucosamine (K_D approximately 1.5 mM). This reagent can be photoactivated to generate a very reactive nitrene that may serve to label the binding site specifically. A second approach to study the essential residues of CHL employs group-specific reagents to modify certain amino acid side groups. To date, the most interesting results involve sulfhydryl groups. With the alkylating reagent *N*-ethylmaleimide, rapid inactivation of binding activity occurs only under conditions that result in decreased ligand binding (no divalent cation). We are now studying this conformation-sensitive reaction to determine the effect of various alkylating reagents and to identify the responsible cysteine residue(s).

Vectorial labeling studies

Vectorial labeling techniques adapted from erythrocyte membrane studies are being used to identify which portions of CHL are on each side of the membrane. Two membrane systems seem most amenable to these experiments. The first is the outer surface of intact hepatocytes, and the second is the outer surface of isolated coated vesicles. These two labeling schemes should

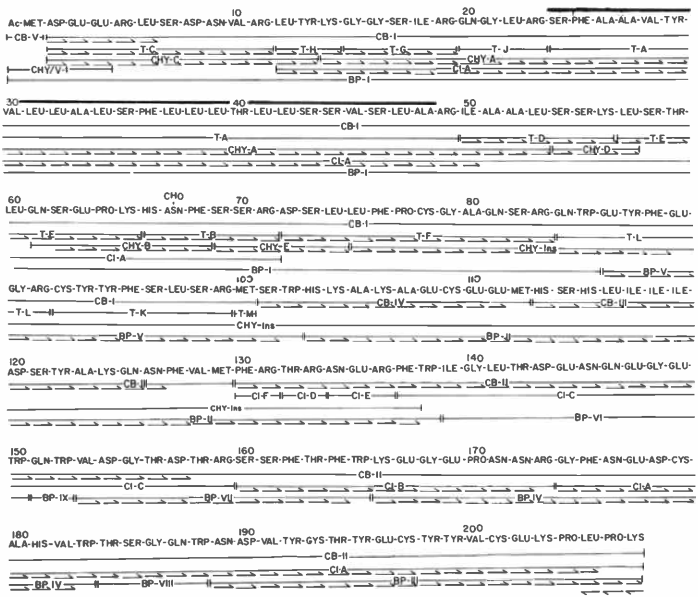

Figure 1

Complete amino acid sequence of CHL. Various fragments used to establish the amino acid sequence are indicated; leftward arrows indicate residues established by carboxypeptidase digestion, and rightward arrows indicate residues determined by automated sequential Edman degradations. Hydrophobic stretch discussed in text is indicated by bold line.

identify portions of CHL on either side of the membrane. The experiments have progressed to the point of identifying CHL in cells suitable for surface-labeling experiments (11-day-old embryonic liver cells) and in coated vesicles prepared from adult chicken liver.

Liver cells from 11-day-old chicken embryos have been cultured for 1 to 2 days and then labeled with lactoperoxidase and radioactive iodine. Labeled CHL can be extracted from these cells, indicating that the protein is indeed expressed at the cell surface. Current work is aimed at identifying the portions of CHL that have been labeled.

Synthesis of CHL

As indicated above, the proposed orientation of CHL raises questions about how this protein is inserted into the hepatocyte membrane. Our current hypothesis is that many membrane proteins, such as CHL, are probably *not* synthesized with "signal sequences." This is being tested by in vitro translation of chicken liver mRNA; the necessary antibody has also been prepared, and translations are now being undertaken. This experiment is analogous to one that we have recently performed to demonstrate that synthesis of the rat serum protein α_{2u} globulin does involve a signal sequence.

SV40 Small T Antigen

H. Engeser

SV40 small T antigen is a tumor antigen that has been implicated in some of the transforming properties of the virus. SV40-infected cells produce the protein in only limited quantities, and thus, even though possible in principle, biochemical studies on the protein have been sparce. Recent advances in genetic engineering techniques have made it possible to construct bacterial

plasmids that contain the genetic information for this protein, and strains of *Escherichia coli* carrying such plasmids have been found to express the protein in reasonable quantities (Roberts et al., *Proc. Natl. Acad. Sci. 76:* 5596 [1979]). Therefore, we are purifying SV40 small T antigen from this bacterial source in order to obtain quantities sufficient for subsequent biochemical and cell biological studies. In this regard, production of monoclonal antibodies against small T antigen is considered a primary research interest.

Ad2 Early Proteins

J. E. Smart

Early during lytic infection by Ad2, four regions of the viral genome are transcribed into RNA. These RNAs encode proteins known to be involved in the initiation and maintenance of transformation, as well as other proteins that directly or indirectly affect DNA replication and the regulatory processes necessary for the switch from early to late gene expression. Although a great deal of information now exists about the structures and nucleotide sequences of many of these RNAs (see Electron Microscopy and Nucleic Acid Chemistry sections), relatively little is known about the polypeptides that are derived from these regions. Because the synthesis of these early region mRNAs often involves complex splicing events, it is not trivial to identify which of the observed polypeptides is translated from a given mRNA. In vitro studies (see report of Protein Synthesis section) have helped considerably, but it has proved essential to obtain additional data by the further analysis of the polypeptides themselves in order to establish the detailed relationships. The main initial objective of the Protein Chemistry section during its first year has been to establish which of the theoretically possible polypeptides are actually present during the early phase of Ad2 infection and to assign these polypeptides to their respective mRNAs. The main approach that we have used to analyze the Ad2 early proteins has involved the characterization of peptides produced by selective proteolysis and/or chemical cleavages of the polypeptides labeled both in vivo and in vitro with various amino acids ([^{35}S]methionine, [^{35}S]cysteine, [^{3}H]leucine, etc.). The resulting peptides are then separated by high-performance liquid chromatography (HPLC) on reverse-phase or cation-exchange columns and analyzed individually by sequential Edman degradation to locate the positions of the individual labeled amino acids. By correlating this partial amino acid sequence information with the DNA sequence data and mRNA splicing data, the coding location of these polypeptides can be assigned unambiguously. This approach also permits deduction and confirmation of the complete amino acid sequence of these proteins.

Region E1A: Detailed example

Several lines of evidence suggest that the left end of the Ad2 genome (early region 1 [E1]) codes for the functions that are responsible for the initiation and maintenance of transformation. This region is divided into regions E1A and E1B, and at least a portion of each is required for complete transformation. Previous work has shown that six metabolically labeled E1A proteins are obtained from Ad2-infected HeLa cells (Harter and Lewis, *J. Virol. 26:* 736 [1978]). Three of the six proteins focus at about pH 6.0 and exhibit apparent molecular weights of about 50,000, 46,000, and 42,000, whereas the remaining three proteins focus at about pH 5.9 and exhibit molecular weights of about 46,000, 42,000, and 38,000. In a recent study (Smart et al. 1981), we have shown that the three E1A proteins that focus at about pH 6.0 contain two major [^{35}S]methionine-containing tryptic peptides that do not occur in the three E1A proteins focusing at about pH 5.9. Sequential Edman degradation of these peptides shows that one of them contains methionine at residue numbers 8 and 14, and the other contains a methionine at residue number 4. Comparison with the amino acid sequence predicted from the DNA sequence from the leftmost 4.5% of the genomes of Ad5 and Ad2 shows that these peptides are derived from genetic information between 2.6 and 3.0 map units, which is contained in the 1.1-kb E1A mRNA but which is spliced out of the 0.9-kb E1A mRNA. Similarly, we have shown that the E1A 58,000- and 48,000-dalton in vitro translation products contain the peptides encoded between 2.6 and 3.0 map units and thus are derived from the 1.1-kb E1A mRNA. The 54,000-, 42,000-, and 28,000-dalton E1A in vitro translation products do not include these two methionine-containing peptides, confirming that they, as well as the three in vivo E1A proteins that focus at about pH 5.9, are encoded by shorter E1A mRNAs from which this information has been removed. The conclusions from this work are summarized in Figure 2.

Similar analyses have been completed for region E2A and are at various stages of completion for regions E1B, E3, and E4. Recently, a new early region, designated E2B, has been found to be transcribed from the *l* strand and includes sequences between coordinates 30.0 and 11.0, with leaders mapping further to the right (see Protein Synthesis section for further details). We have shown that the 55K terminal protein, which is covalently attached to the 5′ termini of Ad2 DNA, is encoded by this region (Stillman et al. 1981). Three distinct spliced E2B mRNAs have been identified, and we are presently analyzing the tryptic peptides of the terminal protein and its 87K precursor to establish their precise coding locations.

One important outcome of these studies will be a growing catalog of the tryptic (and other)

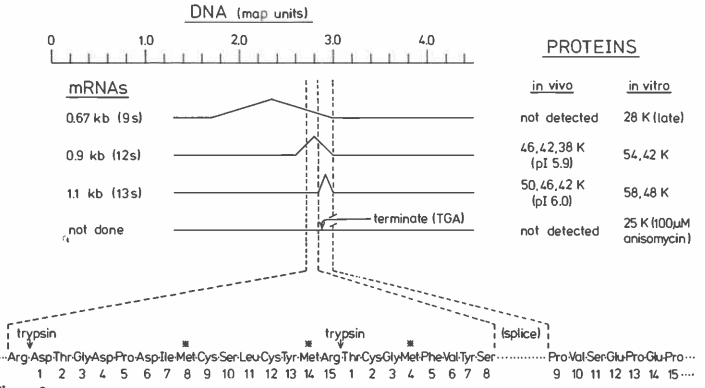

Figure 2

Region E1A: DNA, mRNAs, and proteins. Predicted locations and amino acid sequences of the methionine-containing tryptic peptides that are unique to the proteins encoded by the 1.1-kb (13S) mRNA and possibly mRNAs from region E1A.

peptides of the various Ad2 early proteins, which can be used diagnostically for the unambiguous identification of the proteins or subfragments of them. This will allow fragments of the proteins to be mapped easily and should prove of value in defining (1) the antigenic domains recognized by monoclonal antibodies against the Ad2 early proteins, (2) domains of intra- and intermolecular interactions within and among viral and host-cell components, and (3) domains of ligand binding (e.g., ATP, NADP, and cAMP).

Transformation-specific Gene Products of Distinct Avian Sarcoma Viruses Have Homologous Protein Kinase Target Sites

J. E. Smart

Transformation of cells by Rous sarcoma virus (RSV) is initiated and maintained by the production of a virus-encoded 60,000-dalton transforming protein, pp60^{src}. One of the functions of pp60^{src} is to catalyze the transfer of phosphate from ATP to acceptor tyrosine(s) of various polypeptides. Furthermore, when ATP is added to the immunoprecipitated transformation-specific proteins of all three classes of avian sarcoma viruses (classified on the basis of homology between the transformation-specific nucleotide sequences and their polypeptide products), some of the phosphate is transferred to the transformation-specific proteins themselves. The transforming activity of these proteins implies that they may play an important role in metabolic regulation. In fact, by analogy with other protein kinases, the modification of a number of different enzymes and/or nonenzymic proteins via phosphorylation provides an attractive model for the pleotropic effects of the transformation event. In the case of cAMP-dependent protein kinase, it has been shown that the amino acid sequences at the phosphorylation sites in physiologically significant protein substrates fall into two categories: (1) -Lys-Arg-X-X-Ser-X- and (2) -Arg-Arg-X-Ser-X-, in which X stands for any amino acid (except it should be noted that the amino acid residues immediately adjacent on either side of the serine to be phosphorylated usually have hydrophobic side chains). Accordingly, it is of interest to inquire into the unique structural features that enable certain proteins to serve as substrates for tyrosine kinases.

Czernilofsky et al. (*Nature 287:* 198 [1980]) have determined the nucleotide sequence of RSV *src* and have used these data to deduce a possible amino acid sequence for pp60^{src}. We have confirmed that the predicted reading frame is one that is employed for pp60^{src} over most, if not all, of its length by showing that the positions of the methionine residues in the tryptic peptides of [^{35}S]methionine-labeled pp60^{src} correspond to those predicted from the DNA sequence (J. Smart, H. Oppermann, A. Czernilofsky, and J. M. Bishop, in prep.). Using the same approach on in vivo ^{32}P-labeled pp60^{src}, we have shown that the phosphotyrosine-containing tryptic peptide has the follow-

ing sequence: -Leu-Ile-Glu-Asp-Asn-Glu-Tyr(P)-Thr-Ala-Arg- (J. Smart, H. Oppermann, J.M. Bishop, A. Purchio, and R. Erikson, unpubl.). The same phosphotyrosine-containing tryptic peptide is observed when immunoprecipitated $pp60^{src}$ is phosphorylated in vitro by incubation with [γ-^{32}P]ATP.

Avian sarcoma viruses rarely occur in nature. One school of thought as to their evolution has recently been reviewed by Weinberg (*Cell 22:* 643 [1980]). He points out that in an animal acutely infected with a replication-competent virus, it is likely that a large number of viral replication cycles lead to a variety of chimeric viral genomes, each carrying a different cellular gene that has been expropriated from the host genome by a recombination event. We know only about the rare recombinant carrying a useful (from the point of view of the virus) cellular sequence and expressing an advantageous phenotype—in the present case, tumorigenicity. This trait allows preferential amplification of this virus (since the cells that it has infected grow better) and creates a macroscopically scorable phenotype (a tumor). Thus, it has been shown that normal cells contain and express (in much lower amounts) a gene that is homologous to the transforming gene of RSV. We have recently shown that the immunoprecipitated normal cellular homolog of RSV $pp60^{src}$ is phosphorylated in vitro at a tyrosine that is contained in a tryptic peptide that is identical to the phosphotyrosine-containing tryptic peptide of RSV $pp60^{src}$.

Although the class-III avian sarcoma viruses contain transformation-specific genes that are apparently different from that of RSV *src* (class I), their immunoprecipitated transformation-specific gene products still show an associated tyrosine kinase activity. In spite of the fact that the class-III viruses transform cells by a mechanism that is presumably different (at least initially) from that employed by the class-I viruses, we have found that their transformation-specific gene products are phosphorylated in vitro at a tyrosine that is contained in a tryptic peptide that is again identical to the phosphotyrosine-containing tryptic peptide of RSV $pp60^{src}$. These data, as well as preliminary data on the substrates of other tyrosine kinases, suggest that the occurrence of acidic amino acids adjacent to the phosphoacceptor tyrosine may be a general property of the target sites for tyrosine-specific protein kinases. The observation of the apparent total conservation of these target sites in the otherwise distinct class-I and class-III avian sarcoma viruses supports the hypothesis that such sites may play a central role in regulating the function(s) of the viral transforming proteins, as well as their normal cellular homologs.

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Movable Genetic Elements

Unstable phenotypes and variegated phenotypes, ascribed to "unstable genes," have intrigued geneticists for more than half a century. A meticulous study of "variegation" in maize by Barbara McClintock at Cold Spring Harbor led her to propose many interesting ideas relating gene expression to DNA rearrangements. Her work has shown remarkable intellectual durability. It seems that in many cases unstable phenotypes result from high-frequency DNA rearrangements. Perhaps within the next few years many of these interesting genetic mysteries will be explained in molecular terms. We hope that the work done by various groups in our section will contribute to a general understanding of the principles underlying genetic flexibility.

A highlight of the year was the 45th Cold Spring Harbor Symposium entitled Movable Genetic Elements. A myriad of DNA rearrangements in bacteria, fungi, protozoa, plants, and animals came under discussion, resulting in a very exciting Symposium volume, which has recently been published.

As can be seen from the progress reports that follow, our studies on DNA rearrangements span prokaryotes, lower eukaryotes, and higher eukaryotes. Our section received a boost with the arrival of the groups of Fred Heffron and Maggie So. These groups are examining in great detail the structure and function of Tn*3* and some other bacterial transposons. Other groups in our section continue to study the transposition of the mating-type genes in yeast, transposition of bacteriophage Mu DNA and flip-flop of the G segment of Mu, and the functional organization of the herpesvirus thymidine kinase gene. Our general focus is on the mechanisms by which DNA rearrangements occur and may affect gene expression.

INSERTION ELEMENTS AND PLASMIDS

A. I. Bukhari, *G. Chaconas, M. S. DuBow, D. Evans, M. F. George, R. M. Harshey, G. McGuinness, L. Mintz, N. Sarvetnick*

Transposition of bacteriophage Mu DNA, inversion of the G segment of Mu, and Mu-induced modification of host DNA are highly interesting biological phenomena and they continue to arouse the curiosity of molecular geneticists. We understand many things about these phenomena, and yet there are many intriguing questions left unanswered. Our focus during the past year was on the mechanism of Mu DNA transposition. Mu rearranges the host DNA during its lytic cycle; thus, the *Escherichia coli* genome appears to undergo cataclysmic changes within 30 minutes after Mu transposition-replication functions are induced. These changes are evidently related to the process of Mu transposition. We have sought to dissect this process by examining the interaction of plasmids containing Mu or "mini-Mu's" with the host chromosome (see Annual Report 1979). We have also tried to find intermediates involved in Mu transposition using electron microscopy techniques. It now seems clear that Mu DNA and host DNA intimately interact during transposition and that one consequence of such interaction is the replication of the Mu genome. Two questions arise logically: (1) What prepares Mu DNA and host DNA for such an interaction? and (2) How does Mu integrate in molecular terms? A third question, "Is this process monolithic or can it take different forms?" has arisen from observations discussed below.

Mu DNA Transposition Can Occur by More Than One Pathway

For many years Mu biologists have assumed that transposition of bacteriophage Mu DNA occurs by a single mechanism and that this process could be activated by infection with phage Mu or by induction of a Mu prophage. Our recent findings indicate that this assumption may be incorrect; the end products of Mu DNA transposition events occurring during lysogenization are different from those found as a result of lytic induction of a Mu prophage. Previous studies from our laboratory, as well as those by others, have indicated that during lysogenization a single copy of Mu DNA is inserted at the host target site. Deletions in the host DNA do not usually occur, and a duplication of 5 bp of the host DNA is found at the insertion site, as has been reported for a number of other transposons. We have probed the transposition process during the lytic cycle using Mu or mini-Mu prophages. We did not recover any simple Mu insertions emanating from the prophages; instead, replicon fusions were found as the end products of transposition. The types of experiments that have led us to these conclusions are as follows:

1. The fate of a 9-kb plasmid, pSC101, carrying a Mu prophage was monitored biochemically following prophage induction. It was found that by 33 minutes after temperature shift-up all free forms of the plasmid disappeared and were found associated with the host DNA. Analysis of plasmid-host DNA association by restriction endonuclease cleavage revealed that the pSC101 and *E. coli* chromosomal DNA had been fused. At the junctions of these fused replicons were copies of Mu DNA oriented as direct repeats, as shown in Figure 1. This type of structure, also known as a "cointegrate," has been observed with a variety of different transposons and apparently represents the end products of Mu DNA transposition from a Mu prophage carried on a plasmid.
2. Using genetically marked mini-Mu prophages carried on pSC101, we monitored the transposition of these mini-Mu molecules into an F′ episome and found that virtually every mini-Mu insertion carried the plasmid DNA sequences along, as judged by the persistence of the tetracycline-resistance (Tc^r) phenotype that is encoded by pSC101. Further analysis of these transposition end products also demonstrated that they were cointegrate structures as described above.
3. Attempts were made to recover simple Mu insertions in an F′ episome following induction of a Mu prophage in the host chromosome. At least 95% of the Mu insertions into F′ generated this way carried extensive deletions or insertions of host DNA. Insertion of Mu DNA in the F′ factor during lysogenization, however, resulted in simple Mu DNA insertions.

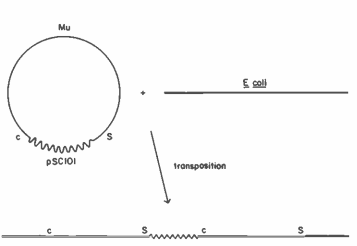

Figure 1
Transposition of pSC101::Mu to generate a replicon fusion or "cointegrate."

Our findings show, therefore, that Mu DNA transposition can give two different types of end products and that the transposition pathway utilized is dependent on the Mu developmental cycle. During lysogenization, simple Mu insertions are formed; following prophage induction, replicon fusions predominate.

A Model of Mu DNA Transposition

A characteristic DNA structure found during Mu growth is a circle with a tail. Both the circles and their tails vary in length. It seems compelling that these structures arise as a result of a Mu prophage trying to insert itself at another site on the host DNA. One way to visualize this process is as shown in Figure 2.

Protein-mediated Changes in Mu DNA Conformation

Several years ago we found, upon infection of Mu lysogenic cells, a form of Mu DNA distinct from the linear form. We have examined this form electron microscopically and have found that it consists of "open" circles and a "supercoiled" form of Mu DNA. These forms are not covalently closed circles, because phenol extraction converts both forms to linear DNA. Thus, a protein appears to mediate supercoiling of a linear Mu DNA molecule. The nature of this protein-DNA interaction is not clear.

Maturation of Mu DNA

During the lytic cycle, Mu DNA undergoes transposition to many different sites on host DNA. Near the end of the growth cycle, Mu DNA is cut and packaged into phage heads in such a manner that heterogeneous host sequences are attached to both ends. Examination of 22 amber mutants of Mu has revealed that genes *E* and *I* are responsible for the maturation of Mu DNA.

We have also examined the lengths of the host sequences attached to the left end of Mu DNA by labeling the 5′ end with ^{32}P, cutting the DNA at a site 7 bp away from the left end, and then running the DNA on a sequencing gel to size the fragments. The minimum size of the host sequences attached to the left end of the mature bacteriophage Mu DNA is 56 bp; host sequences longer than 144 bp are rare. The host sequences do not show a continuum from 56 bp to 144 bp but, rather, are packaged in discrete blocks. The first such block is 56–61 bp, the second block is 67–72 bp, and so on. Thus, in each block the length can vary from 5 bp to 6 bp, and there is a space of 5 bp between each block. It seems that Mu DNA is being measured in units of helical turns for packaging.

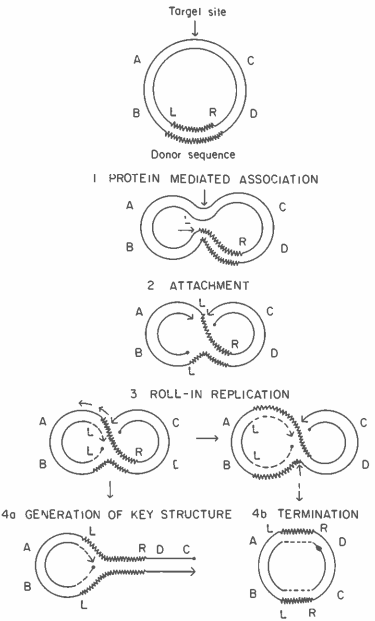

Figure 2
A model of transposition (within the same replicon). The transposable element is shown by jagged lines. The arrowheads indicate free 3′-hydroxyl ends of DNA, and the dots indicate 5′-phosphate ends. The initial cleavage points are shown by arrows. The newly replicated strands are shown by broken lines. Polarities indicated are completely arbitrary and may well be reversed. The DNA ends are presumed to be held together by proteins. Upon disruption of the protein complex, a circular structure with a tail would be generated (4a). The length of the circle would depend on the distance of the target site from the initial location of the element, and so also the length of the tail attached to the circle. Upon completion of replication, when the distal end of the element arrives at the replication fork, the 3′ end of the parental strand is nicked and ligated to the free 5′ phosphate of the target DNA. Upon sealing of all the relevant strands, a transposition accompanied by inversion (of C and D markers in the figure) would result (4b).

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MOLECULAR GENETICS

D. Zipser, *D. Kwoh, L. Lipsich, L. Rodgers*

If the work of our section in 1980 were to be given a title, we would call it "the use of genetics in a test tube to study control of gene expression." This is because virtually all of our projects have depended very heavily on the use of in vitro recombinant DNA technology to generate mutations within precisely defined areas of various cloned DNAs, followed by the use of restriction endonucleases and DNA ligase to rearrange these mutations in fashions appropriate for the studies we are conducting. These techniques, which have now become quite standard in the molecular genetics laboratory, are formally equivalent to classical genetic manipulations. They are, however, much more powerful because they are rapid and enable us to focus attention on the sequences of interest. In addition, the mutations that we create are of precisely identified kinds at carefully chosen locations. What is more, the products can be analyzed down to the nucleotide sequence level. Although most of this could be accomplished in bacteriophages and bacteria by classical genetic techniques, it was virtually impossible to do similar things with animal cells by classical genetics. The improvements in speed and efficiency by going from in vivo to in vitro genetic technology are at least as great as the improvements in speed and efficiency gained from going from animals such as fruit flies to bacteriophages like λ and T4.

The two gene-control systems on which we have been working are those that we have been interested in for several years. One comes from bacteriophage Mu and is called the G-inversion system. The other is the induction of cell-resistant herpes thymidine kinase (TK) by infecting herpesvirus. In the G-loop system, we have long known that the arrangement of DNA is responsible for gene control. As a result of manipulations performed this year, we now have good reason to hypothesize that control at the DNA level is also at least partially responsible for TK induction.

Identification of the *gin* Protein of Bacteriophage Mu

D. Kwoh, D. Zipser

The *gin*-gene product of bacteriophage Mu catalyzes a specific intramolecular recombination event leading to the inversion of a 3000bp segment of Mu DNA. In addition to the inversion of this segment, called G, *gin* also inverts a segment in P1 that is similar to the G segment of Mu and a region of the *Salmonella* chromosome controlling flagella formation. The G segment of Mu codes for proteins that determine the host-range specificity. Different proteins are produced depending on the orientation of the G segment. The *gin* gene maps outside and to the right of the G segment on the Mu genome. Previously, we had cloned the G segment and *gin* region of Mu into a multicopy plasmid. These plasmids express *gin*, but the amount of *gin* produced is too low to allow identification of the *gin* protein in the presence of the other proteins made. This year we subcloned into a plasmid, with a strong promoter, a small fragment containing the *gin* gene and constructed *gin*$^-$ mutations by in vitro linker insertion. Using these mutations, we have identified the *gin* protein on SDS-polyacrylamide gels.

Studies on G inversion have shown that the rate of G inversion in both the lytic and lysogenic life cycles of phage Mu is extremely low. We have shown that the level of *gin* protein affects the rate of G inversion. One reason for the low levels of *gin* could be a weak promoter. To overcome this, we decided to subclone *gin* next to a strong promoter contained on a multicopy plasmid.

To obtain a plasmid vector, we started with a derivative of pBR322 carrying 95 bp of the *lac* operator-promoter (*lacO-P*) region and deleted the DNA segment containing the genes encoding tetracycline, as shown in Figure 1. As a source of *gin*, we used an *Eco*RI linker insertion mutant of a previously constructed G *gin* plasmid. As shown in Figure 1, this plasmid carries the entire G segment as well as the *gin* gene. The G segment of this plasmid inverts, so DNA preparations of it contain equal amounts of both the G(+) and G(−) forms. Fragments containing the *gin* gene were obtained from this DNA by first cleaving the plasmid with *Hpa*I, which cuts near the right end (the end closest to *gin*) of the G segment, and then with *Eco*RI, which cuts to the other side of *gin*. Two *gin*-containing fragments and several others are produced. The *gin* fragments contain opposite ends of the G segment but they are otherwise identical. By addition of *Eco*RI linkers, four fragments (labeled I–IV in Fig. 1) are obtained that can be inserted at the *Eco*RI site of the vector and produce viable plasmids. We eventually isolated four plasmids differing in *gin* orientation with respect to the *lac* promoter and the end of G attached (Fig. 1). All four subclones retain the *lacO-P* region, as determined by their ability to induce β-galactosidase in transformants. Also, all four subclones are Gin$^+$, as determined by their ability to invert the G segment of a Mu Gin$^-$ prophage, a very sensitive, but not quantitative, biological assay for *gin*.

To analyze the proteins produced, minicells containing the vector plasmid and the four Gin$^+$ recombinant plasmids were prepared. Two proteins with molecular weights of 27,000 and 21,500 are seen in minicells carrying plasmids that have the G(+) *gin* and G(−) *gin* fragments oriented with

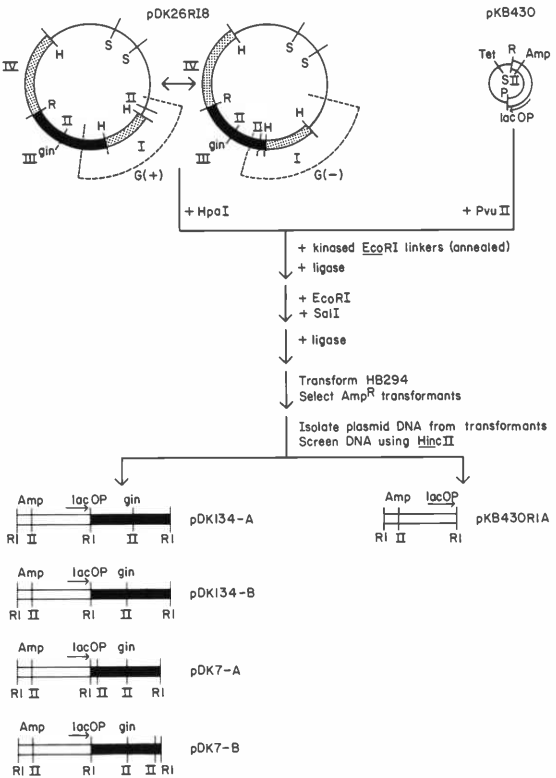

Figure 1
Scheme for subcloning the Mu gin *gene next to the* lac *promoter of pKB430.*

the G end adjacent to the *lacO-P*. These proteins are not present when the *gin* fragments are present in the opposite orientation, and they differ in size from proteins detected previously in minicells containing Gin$^+$ plasmids. These two proteins were considered candidates for the *gin* protein.

To determine whether either the 21.5K or 27K protein was *gin*, we inserted *Xho*I linkers into a *Hin*cII site within *gin*. We isolated three *Xho*I Gin$^-$ insertion mutants of both the G(+) *gin* plasmid and the G(−) *gin* plasmid. The proteins produced in minicells carrying the G(−) mutant plasmids were compared with those produced by their Gin$^+$ parents. The 21.5K protein is missing in minicells of the Gin$^-$ derivatives, and in each case a protein with a different mobility is detected. From these results, we concluded that the 21.5K protein produced by the G(+) and G(−) plasmids is the product of the Mu *gin* gene.

Proteins within the G Loop of Bacteriophage Mu

D. Kwoh, D. Zipser

Bacteriophage Mu has two distinct bacterial host ranges. Which of these two sets of bacteria will be infected depends on the orientation of the G-

loop region on the phage during the time phage growth is occurring within a bacteria. For example, if a Mu phage is Gin$^-$ so that it cannot invert its G loop, then when *Escherichia coli* lysogens carrying phage with the G loop in the minus configuration are induced, they produce so-called G(–) phage that can no longer infect *E. coli*. These G(–) phage, however, can infect bacteria from the alternate host range. G(+) phage can infect *E. coli* but not the other host range. By the use of bacterial strains carrying plasmids in which the G loop has been cloned in one or the other of the two possible orientations, we have been able to demonstrate that phage that are genetically G(–) can be wrapped in G(+) coats which enable them to infect *E. coli* at high efficiency. Thus, for example, when a bacteria lysogenic for a Gin$^-$ G(–) Mu and carrying a Gin$^-$ G(+) plasmid is induced, the progeny phage will plate efficiently and form plaques on host bacteria carrying a Gin$^-$ G(+) plasmid but not on bacteria carrying a Gin$^-$ G(–) plasmid. This result is consistent with the long-held view that the proteins produced by the G loop determine host range largely by determining the ability of Mu to properly adsorb or inject its DNA into one or another host. It is in fact known that one of the genes mapping in the G region, the *S* gene, is responsible for producing a protein that is part of the virion, almost certainly a tail-fiber-type protein, called S, that is responsible for proper phage adsorption and injection. It has been hypothesized by several people that an alternate of S, called S′, would be produced in the G(–) configuration and would be the protein required for adsorption to the alternate host range. This year we took plasmids that we had constructed previously, containing the G loop frozen in each of the two possible orientations, and generated a series of restriction enzyme linker insertion mutations at known restriction sites in order to help in the identification of this set of proteins that is altered when the G loop is inverted. When these linker insertion mutations were examined for the proteins they make, using the extracts of labeled minicells that carry the respective plasmids, we were able to identify the two alternate forms of the S protein, S and S′, together with alternate forms of at least one other protein, probably the product of the *U* gene, known to map within G. This result confirms the conjectures postulating different sets of adsorption proteins made by the different orientations of the G loop.

Induction of Cell-resident Herpesvirus TK by Infecting HSV-1

L. Lipsich, D. Zipser

When L cells carrying a herpes simplex virus type 1 (HSV-1) *tk* gene that has been introduced by DNA-mediated cell transformation are infected with Tk$^-$ HSV-1 virus, there is a significant increase in the level of herpes-specific TK activity. This contrasts with a drop in the level of cellular TK activity seen following HSV-1 infection of cells that carry the normal cellular *tk* gene. It is known from extensive work with herpesvirus that expression of herpes TK requires prior expression of certain viral functions. Our working hypothesis about the induction phenomena has been that these herpesvirus functions recognize some feature of the cell-resident herpes *tk* gene or its products to allow induction to occur after herpesvirus infection. The fact that herpesvirus TK is expressed in L cells without herpes infection indicates that the cellular RNA polymerase and other cellular expression systems can also recognize herpes *tk*. To study the mechanism of expression of herpes TK by both the cellular and the viral systems, we have constructed a library of various kinds of mutations in a pBR322-cloned version of herpes TK. Then by transforming Tk$^-$ L cells with members of this library, we have been able to determine the effect of various mutations on the expression of herpes TK either by the cellular or by the herpes system. One set of mutations involved deleting sequences from the 5′ untranscribed region of the cloned herpes *tk* gene. This work was greatly facilitated by knowledge of the sequence of the herpes *tk* gene and by much work on its expression that has been done in other laboratories. We found a deletion that removed most of the 5′ untranscribed region, which was no longer able to transform LTk$^-$ to LTk$^+$ under conditions where a single copy of DNA entered the cells. However, where many copies could be incorporated, Tk$^+$ L cells could be isolated. These cells had 10 to 30 copies of the cloned herpes *tk* gene. Their level of TK expression was from 5 to 20 times lower than would normally be expected from a single copy of the clone but was sufficient to allow the cells to grow in HAT medium. Our interpretation of this result is that these clones use a residual promoter, probably located in the cloning vehicle, to eke out a very low level of TK expression. When L cells carrying this deletion plasmid in multiple copies were challenged by infection with Tk$^-$ HSV-1, they were not inducible; i.e., the level of TK activity did not go up. This indicated to us that, in addition to removing the normal promoter sequences, the deletion also removed sequences required for recognition of the herpes *tk* gene by superinfecting virus. Since a deletion about 200 bp shorter than that previously discussed had little or no effect on induction, we conclude that a sequence required for the recognition of cell-resident herpes TK by superinfecting HSV-1 is located in the 5′ untranslated region of the herpesvirus clone. Sequence analysis indicated that the deletion that inactivated induction terminated 8 nucleotides before the cap site of herpes TK mRNA. This experiment is of extreme importance in our study because it indicates that induction is due, at least in part, to interaction between some

herpes function or herpes-modified function and the chromosomal DNA of the mouse L cell carrying the herpes *tk* gene.

To refine these results and more closely map the regions involved in herpes TK induction, we have enlarged our mutation library in two ways. First, we have generated a series of ten additional deletions all ending in the 200-nucleotide 5′ untranslated sequence between the end of the shortest deletion that had an effect on induction and the longest deletion that did not. These deletions could be concentrated in the region of interest by opening the cloned herpes *tk*-gene plasmid at an appropriate restriction enzyme site and then chewing back the DNA with the nuclease *Bal*31 for very short periods of time. This enzyme cuts DNA in both the 3′ and 5′ directions. After *Bal*31 treatment, ragged ends were filled in with DNA polymerase, an appropriate restriction enzyme linker piece of DNA was ligated on, and the treated *tk* gene was recloned in a different vehicle plasmid. The endpoints of these deletions have been mapped, and we are in the process of determining their effect on induction.

The second approach to the creation of mutations involved making a library of restriction enzyme linker insertion mutations in the control regions of herpes *tk*. The hope here is to be able to find insertions that affect induction without destroying the promoter sequences required for expression. Such an insertion mutation library has been made and has produced eight insertions in the 5′ untranscribed region and the 5′ untranslated region of the herpes gene sequences. We are in the process of identifying the exact site of each insertion and of analyzing their effects on TK levels and TK induction in L cells transformed with each of the insertion mutations.

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YEAST GENETICS

J. Hicks, A. Klar, J. Strathern, *J. Abraham, D. Barczik, S. Ismail, J. Ivy, C. McGill, J. McIndoo, K. Nasmyth, J. Wood*

For the Yeast Genetics section, this past year marked the beginning of several new aspects of our research on the mechanism of gene regulation and cell differentiation. The cloning and structural analysis in 1979 of the mating-type genes, which verified the cassette model for gene switching, have added a more biochemical emphasis to our genetic work. It is now theoretically possible to isolate the chemical components that cause "jumping genes" to jump, and it has become the job of the molecular geneticist to purify these components and reconstruct the system in the test tube. It is appropriate, then, that in the summer of 1980 construction began on an addition to Davenport Laboratory that will contain a new biochemistry laboratory and associated facilities plus much-needed office space. The combined complex has been renamed Delbrück Laboratory in honor of Professor Max Delbrück, who first taught the bacteriophage course in the main building and whose Nobel-prize-winning work in phage genetics was performed there. The Yeast Genetics section will reside in the new complex on a year-round basis and will share the old laboratory with the genetics courses in the summer. The complex is due to be completed in June 1981. Thus, in addition to a number of firsts, 1980 marked a very significant "last" for us—the last time we will move out of the building to make room for the summer courses.

The year 1980 also saw the arrival of the first additions to our scientific staff since the section was formed. Dr. Kim Nasmyth, who previously worked on the yeast mating-type genes at the University of Washington, came as the first Robertson Research Fellow at Cold Spring Harbor. Also arriving at the end of the year were two postdoctoral fellows from the University of California, Dr. Judy Abraham from Berkeley and Dr. John Ivy from the San Diego campus.

Another highlight of 1980 was the winter meeting on Plant Molecular Biology held at the Banbury Center. This meeting marked the initiation of efforts to explore research in molecular genetics in plants. The enthusiasm generated at the meeting led to our first attempts to culture plant cell lines in the laboratory, and we hope this will lead to an active plant research group at Cold Spring Harbor.

Research Summary

As described in last year's report, we were able to isolate the mating-type cassettes using molecular cloning techniques. Physical analysis showed that cassettes at each of two cryptic loci (*HML* and *HMR*) and the expressed locus (*MAT*) contain regions of sequence homology flanking a sequence that determines the "sex" of the cassette (**a** or α). This physical structure and the arrangement of the genes on chromosome III are shown in Figure 1.

Our work in 1980 centered on the mechanics

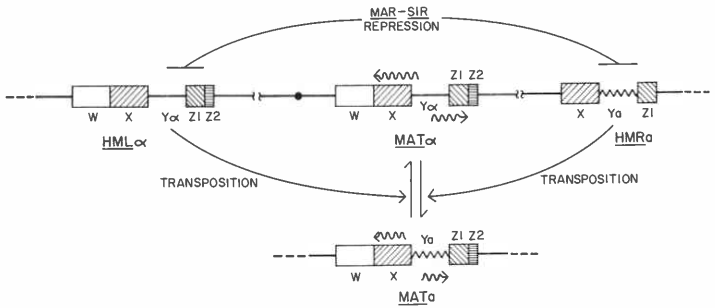

Figure 1
Schematic diagram of the structures of the mating-type cassettes on chromosome III. Boxes represent regions of homology at more than one site. Hatched regions are homologous to other regions with the same pattern. Wavy lines represent RNA transcripts. Homothallic switching results in the replacement of the Y sequences at MAT *with* Ya *or* Yα *sequences from* HMR *or* HML.

of gene regulation in the expressed and unexpressed cassettes and has led to the notion that the position of the cassette in the chromosome determines its expression and its role in switching. Several examples of this "position effect" are described below.

Control of Transcription

Although most yeast strains maintain either **a** or α cassettes at three loci (*HML*, *HMR*, and *MAT*), only the cassette at the *MAT* locus is normally expressed. The genetic information at the *HML* and *HMR* loci apparently is kept silent by the combined action of several unlinked genes known collectively as the *MAR/SIR* functions. We infer from this arrangement that some site of negative control exists near the *HML* and *HMR* cassettes but not at *MAT*. Since the DNA sequence of an α or **a** cassette is constant whether it resides at *MAT*, *HML*, or *HMR*, it follows that the site that determines expression must lie outside of the cassette itself.

That *HML* and *HMR* might maintain a control site distinct from that of *MAT* would not in itself be surprising were it not for the peculiar arrangement of the transcription units within the cassette. Electron microscopy of RNA-DNA hybrids, along with gel blotting analysis of mRNAs isolated from **a**, α, and **a**/α strains, has shown that each cassette can give rise to two classes of RNAs transcribed divergently from the interior of the cassette outward (Klar et al., 1981; Nasmyth et al., *Nature 289* [1981]). These transcription patterns for the three cell types are shown in Figure 2. In normal (*MAR*$^+$) cells, only the cassette at *MAT* is transcribed. In *mar*$^-$ mutants, however, all three cassettes are transcribed at about the same level. Thus, as in most bacterial regulatory systems previously studied, gene regulation of the mating-type cassettes is at the level of mRNA transcription. Contrary to the lessons learned in bacteria, however, the initiation of transcription in this case appears to be far away (between 700 and 1400 bp) from the possible sites of control outside of the cassette. Control sites are not likely to be found inside the cassettes because they have identical DNA sequences whether expressed at *MAT* or silent at the *HM* loci. This paradox is illustrated in Figure 1 and has led us to propose that long-range forces must be available for regulation of eukaryotic genes that can act over thousands of base pairs.

Chromatin Structure and Nucleosomal Phasing

Even though a cassette contains all of the machinery and genetic information necessary for full expression, the position of that cassette on the chromosome can dictate whether or not it is actually expressed. A possible source of this "position effect" may reside in the complex DNA-protein configuration of eukaryotic genes known as chromatin. Eukaryotic DNA in chromosomes is packaged along with proteins into units called nucleosomes. A number of nucleosome units are necessary for the packaging of a single gene. It is possible that regulatory molecules binding to a DNA-protein complex in a chromosome could stabilize a particular three-dimensional structure of the DNA sequences that is necessary for efficient gene expression and implies that we must not think of a gene simply as a linear array of genetic information. If a particular DNA sequence (a control site) caused a singularity in the nucleosome series so that flanking nucleosomes were forced into a specific arrangement, then control could be exerted at some distance from that site. That is, DNA sequences responsible for initiation of transcription might be more or less accessible to RNA polymerase, depending on their position in the nucleosome. Alternatively, in a particular arrangement of nucleosomes, two or more non-adjacent sequences might be brought into juxtaposition, thus allowing a control protein to interact with both simultaneously. Any other arrangement of nucleosomes would put the sequences out of register and prevent the interaction.

The existence of nucleosomal phasing at specific genetic loci has recently been demonstrated in other organisms. Using similar methods, Kim Nasmyth has shown that nucleosomal phasing exists at both silent and active mating-type genes. The phasing pattern is affected by defects in the *MAR/SIR* genes that are known to affect transcription of the silent genes. It is still unclear whether the change in phasing pattern reflects the cause or the effect of changes in transcriptional activity; however, the possibility that chromatin structure might provide the basis for eukaryotic gene regulation is a tantalizing prospect.

Control of Direction of Transposition: A Second Positional Effect

MAT interconversion occurs by transposition of copies of the silent cassettes at *HML* and *HMR* to *MAT*. The silent loci remain unaltered; i.e., they themselves do not switch. In light of the fact that the DNA sequences of the *HM* loci and the corresponding *MAT* alleles are identical, an important question is raised: Why is the cassette switched when situated at *MAT* but not when situated at the *HM* loci?

There is an important difference between MAT and the *HM* loci. The cassette at *MAT* is expressed, whereas those situated at *HM* loci are silent. We wondered whether the *HM* loci could switch in strains where they are allowed to express. We imagine that the mechanism that keeps the *HM* loci silent may also be operative for determining switchability of a particular cassette.

The distribution of mating-type switches in the

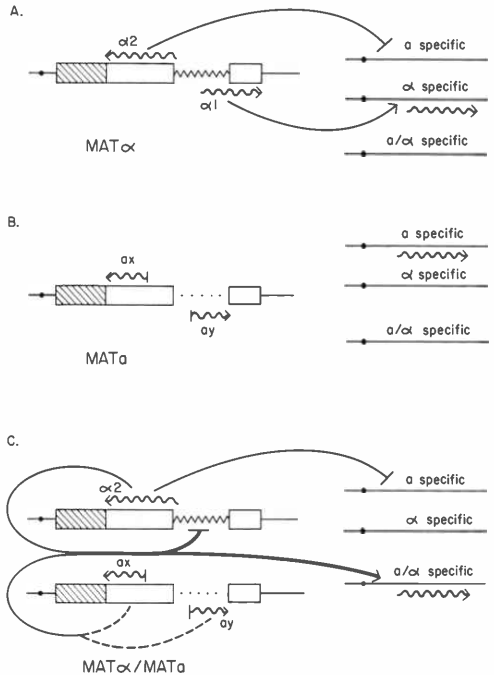

Figure 2
Schematic diagram of the transcription patterns (wavy arrows) of the MAT locus in normal MATa MATα *and* MATa/MATα *cells and the roles of the gene products in the regulation of cell type. Arrows represent putative positive control functions. Flat lines represent negative control. (Data from Strathern et al., 1981.)*

pedigree of a homothallic cell can be established because the original cell (mother) can be distinguished and physically separated from the bud cell, and the mating type of a cell can be reliably assayed by its response to α factor. The patterns are strikingly nonrandom (Fig. 3A) in *MAR*$^+$ cells. Three "rules" are required to describe the patterns observed (Hicks and Herskowitz, *Genetics 83:* 245 [1976]; Strathern and Herskowitz, *Cell 17:* 371 [1979]): (1) Both cells produced by a given division are always the same mating type. (2) Cells that have not previously divided (buds and spores) rarely or never switch mating type in the next division. (3) Cells that have previously divided (mothers) give rise to a pair of cells that have switched mating type in 70–90% of the divisions. The asymmetric division of the ability to switch between the mother and the bud can be conceptually related to restriction of developmental potential with the early divisions of embryogenesis in higher eukaryotes.

Now, let us compare the pattern of switching in a cell lineage in *mar1* strains. Figure 3 shows a switching pedigree of a *mar1* cell in which changes from the α to the a phenotype reflect changes at *HML* from the α to an a cassette. Whereas such switches are extremely rare in *MAR*$^+$ cells, this *mar1* pedigree demonstrates efficient switching. In addition, the rules for switching are frequently violated. Consider Figure 3b in which a daughter cell at the four-cell stage produced one switched cell and one unswitched cell. Figure 3c shows an example where the daughter, but not the mother, produces switched cells at the four-cell stage.

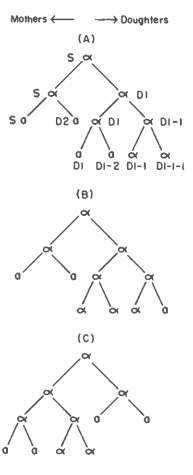

Figure 3

Pedigree analysis of switching from MATα *to* MAT**a**. *A pedigree of a zygotic cell constructed by mating* HMLα mat**a**$^-$ hmr**a**$^-$ *mar1* ho *to* hml**a** mat**a**$^-$ hmr**a** *mar1* HO. *Daughter cells are drawn to the right, and mother cells to the left, at each cell division. (S) Spore cell; (D1) the spore's first daughter; (D2) the spore's second daughter; (D1-1) the first daughter of cell D1, and so on.* α *and* **a** *cells were distinguished by resistance and sensitivity, respectively, to* α *factor as described in the text.*

Thus, three rules for switching are violated in *mar*$^-$ cells: both mothers and buds can switch, silent loci can be switched, and cells are not restrained to switch in pairs. Similar results were obtained for the switching of *HMR*. At present, we cannot determine whether the *MAR* function(s) controls the ability of switching of the *HM* loci by controlling their state of expression or whether *MAR* itself dictates the direction of transposition. The equally challenging question remains unanswered as to why the rules for switching are violated in *mar1* strains. It is clear, however, that the mechanism that controls transcription at the silent loci appears also to provide discrimination between donor and recipient cassettes and dictates the pattern of cassette switching.

Directedness of Mating-type Switching: The Type-I, Type-II Experiment

Strains with the common cassette arrangement, which places α on the left (*HML*α) and **a** on the right (*HMR***a**), called type-II strains, switch at a high frequency of up to 86%. In contrast, we have determined that so-called type-I strains, which have the *HM* alleles reversed (*HML***a** and *HMR*α), switch at a frequency of less than 10%. What is the basis of this disparity between type-I and type-II strains in switching efficiency?

One hypothesis is that the mechanism that directs, for example, a *MAT***a** cell to switch to *MAT*α in fact only directs the *MAT***a** cell to preferentially use *HML* regardless of the latter's genetic content. Likewise, *MAT*α cells might preferentially transport copies of the *HMR* information into *MAT*. By this hypothesis, the type-I (*HML***a** *HMR*α) strains would predominantly switch **a** to **a** and α to α, events that would go unnoticed. The 10% switching that does occur could reflect the error limits of the system and could represent cases where the α cells choose *HML* and the **a** cells choose *HMR* as donors. In contrast, the type-II (*HML*α *HMR***a**) cells switch efficiently because each (or most) of the switches should occur to the opposite mating type.

We tested the left-donor and right-donor positional effect by studying the switching of a strain *HML*α *MAT***a** *HMR*α, which contains different α information at left and right cassettes. α's can be genetically distinguished after substitution into *MAT*. We observed that over 90% of the switches of *MAT***a** to *MAT*α result from transporting genetic information from *HML*, with the balance apparently resulting from transport from *HMR*. We are presently investigating whether in type-I strains the homologous, i.e., **a**→**a** and α to α, switches occur and also what is the molecular explanation for this bias.

Switching Intermediates

Yet another manifestation of positional control has appeared in experiments designed to identify molecular forms representing intermediate steps in the switching process. Because the consequence of switching is the formation of **a**/α diploid cells in which switching is shut off, normal homothallic strains cannot be grown preparatively in an actively switching state. However, certain genetic arrangements can be constructed in which mating is prevented and switching occurs continuously. For example, if all three loci are occupied by a cassettes, switching occurs, but the cells remain **a** and mating does not occur.

Using these continuously switching strains, we have observed that a substantial portion of the DNA at *MAT* (up to 5%) is cleaved at a unique site, as shown in Figure 4. This cleavage is not observed in nonswitching strains. Interestingly, this site has been mapped to a region of *MAT*, Z1, that is identical with *HML* and *HMR*. Yet, we do not observe this cleavage at the *HM* loci. We have not yet determined whether the *MAR/SIR* functions play a role in this example of positional effect.

The presence of a cut at *MAT* that is not found in *HML* or *HMR* may be functionally related to *MAT*'s role as a recipient during the switching reaction. Such a cut could generate a recombinagenic structure involved in the DNA substitution

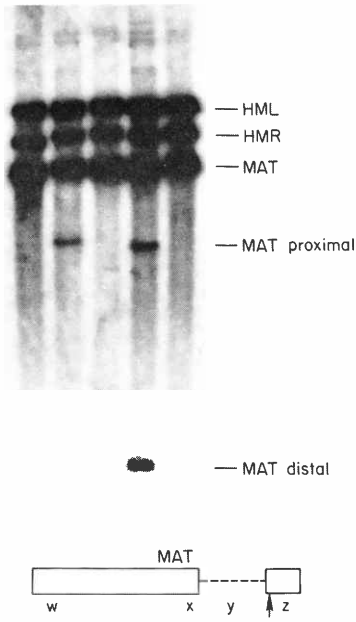

Figure 4
Southern blots of restriction digests of DNA isolated from HO HMLα MATα HMRα *(track 2) and* HO hml**a** MAT**a** HMR**a** *(track 4) strains showing an extra cleavage site not found in strains that are not switching (tracks 1, 3, and 5).*

process during switching. Alternatively, this switching-associated cut in the Z1 region of *MAT* could reflect an activity required to resolve a switching intermediate composed of the exiting *MAT* DNA and the incoming cassette. The existence of this cut suggests that particular sites within the regions of homology between *MAT* and the *HM* loci have defined roles in initiating and completing the process. Furthermore, this cut defines a candidate for such a site.

Perspectives

We have recently demonstrated that the *MAR/SIR* products will regulate the expression of *HML* or *HMR* when these loci are on replicating plasmids in yeast. Furthermore, *MAT* on a yeast plasmid is a recipient for switching, and *HML* or *HMR* on a yeast plasmid acts as a donor. These observations allow us to mutagenize the *HM* cloned DNAs and identify sites involved in the positional effect and sites required for donating cassettes. Mutagenesis of the *MAT* clones will allow both the identification of sites required as a recipient for switching and the analysis of sites required for the proper transcription of *MAT*. Finally, the observation that these cloned DNA fragments perform their various normal biological functions suggests that they are appropriate substrates for the in vitro analysis of the components involved in *MAR/SIR* control and the mechanics of the cassette mechanism.

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Plant Molecular Biology Workshop, February 29–March 2

ORGANIZER: J.B. Hicks

The Cold Spring Harbor staff was brought up to date on the current state of molecular research in plants at a workshop at Banbury Center. This meeting brought together the leaders in the field of plant genetics and molecular biology and promoted the interaction of these scientists with members of the Cold Spring Harbor Laboratory staff. Research presentations were made by the visiting plant biologists, and group discussions were held that focused on possible methods of further exploiting molecular approaches in the study of plant genetics and development. Additional discussions centered on the state of funding for plant research in the United States and the ways in which expanded funding might be directed toward increased scientific participation in this area. Representatives of the major Federal agencies funding plant research (National Science Foundation; Department of Energy; Department of Agriculture) described the funding programs now available and those that will be expanded in the future. The workshop was funded jointly by the National Science Foundation programs in Developmental Biology and Genetics and by the Department of Energy.

PARTICIPANTS/PRESENTATIONS

Day, Peter, Plant Breeding Institute, Cambridge, England: Plant breeding and pathology.

Walbot, Virginia, Washington University, St. Louis, Missouri: Plant morphogenesis.

Freeling, Michael, University of California, Berkeley: Induction of alcohol dehydrogenase and other anaerobic proteins in maize; Accessibility of plant genetic markers for molecular cloning.

Dixon, Elizabeth, Rockefeller University, New York, New York: Molecular biology of viroids.

Sheperd, James, Kansas State University, Manhatten: Genetic variation in potato plants regenerated from cultured protoplasts and its utility for plant breeding.

Meins, Frederick, University of Illinois, Chicago: Directed, heritable changes in tobacco cell culture and regenerated plants.

Levings, C.S., North Carolina State University, Greensboro: The maize mitochondrial genome—Plasmids associated with male sterility.

Flavell, Richard, Plant Breeding Institute, Cambridge, England: Genome organization in cereals; Evolutionary consequences of movable genetic elements; DNA cloning in plants.

Fedoroff, Nina, Carnegie Institution of Washington, Baltimore, Maryland: Controlling elements in maize.

Burr, Ben, Brookhaven Laboratory, Upton, New York: The *shrunken* locus in maize; Cloning a gene which responds to controlling elements.

Burr, Frances, Brookhaven Laboratory, Upton, New York: Zein—Maize storage protein.

Bogorad, L., Harvard University, Cambridge, Massachusetts: Genetic organization and cloning of chloroplast DNA.

Ausubel, Fred, Harvard University, Cambridge, Massachusetts: Genetic studies of nitrogen fixation.

van Montagu, Marc, Laboratory for Genetics, Ghent, Belgium: Interaction of bacterial plasmids and plant DNA in *A. tumifaciens* (crown gall).

Giles, Kenneth, Worcester Polytechnic Institute, Massachusetts: Introduction of plasmid DNA into plant protoplasts by liposome fusion.

McClintock, Barbara, Cold Spring Harbor Laboratory, New York: Summary remarks—Untapped phenomena in plants.

OTHER PARTICIPANTS

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MOLECULAR APPROACHES TO MICROBIAL PATHOGENICITY AND VIRULENCE

M. So, *D. Kwoh, C. H. Lee, K. Messina, T. Meyer and D. Mlawer*

The emphasis in this laboratory is on factors that influence the pathogenicity and virulence of microorganisms. In particular, we are interested in examining the genetic nature of these factors (how they are expressed, how they are disseminated) and in manipulating these genes biochemically in the hopes of finding a solution to the disease process. Enterotoxigenic *E. coli* (ETEC) are responsible, in part, for the syndromes known as infantile diarrhea and "Traveller's Diarrhea." The disease affects both man and agriculturally important animals and is particularly severe in the newborn. It can come about only if the bacteria produce (1) colonization factor (CF), a cell-surface antigen that initiates the disease process by allowing the invading cells to colonize the intestines of the host, and (2) heat-labile (LT) or heat-stable (ST) toxins, which actually elicit the clinical symptoms of diarrhea.

Two groups of heat-stable toxins are produced by ETEC. One, STA or STI, affects only newborn (suckling) animals and the other, STB or STII, affects only the adult (weaned) of the species. Both are low-molecular-weight and not antigenic unless coupled to a carrier protein. We have characterized the gene coding for one of the STI toxins and found it is on a transposable segment of DNA bounded by inverted repeats of IS*1* (Tn*1681*). We have deduced an amino acid sequence from the DNA sequence of the STI gene. A comparison of this deduced amino acid sequence with the amino acid sequence of another STI toxin obtained by R. Giannella has shown that the two sequences are identical at the C'-terminal end except for two substitutions. In collaboration with R. Giannella, using one STI DNA sequence as probe in Southern hybridizations and his anti-STI antibodies in radioimmunoassays of ST-producing clinical isolates, we have shown that the STI toxins are not a homogeneous group, but do share some common sequences, and these regions of homology probably lie in the C'-terminal region of the protein. We are now in the process of characterizing the nature of the STII gene.

The colonization-factor antigens (CFAs) are both species- and tissue-specific. Recently, we have started to examine the genes encoding the CFAs specific to humans (in collaboration with Dolores and Doyle Evans, University of Texas, Houston). There are two, and possibly more, antigenically different CFAs. It is our intention to study the relatedness of these genes, to isolate and mutate them in vitro and in vivo to examine the attachment functions of their proteins, and to examine their evolutionary behavior in vivo and in vitro in response to environmental (antigenic) pressures.

Neisseria gonorrhea also have interesting cell-surface antigens that function as virulence factors. We have also started to study the genetics of this organism, concentrating on the regulatory mechanisms responsible for the expression and antigenic diversity of these factors.

MOLECULAR MECHANISM OF TRANSPOSITION

F. Heffron, *R Kostriken, T. Meyer, C. Morita, R. Bialt, J. Celenza*

Transposable elements are discrete DNA sequences that can transpose from one location on a DNA molecule to another. In prokaryotes, transposons frequently carry genes, such as for antibiotic resistance, that are advantageous to the bacterial cell. Such transposons have revolutionized bacterial genetics, where they are now universally used to produce mutations and simplify strain construction.

Our laboratory is dissecting the molecular mechanism of bacterial transposition. Tn*3*, one of the earliest transposons identified, encodes resistance to penicillin. In the last few years we have identified three genes and three sites that are essential to Tn*3* transposition and are encoded within the transposon itself. Tn*3* encodes a transposase that is essential for its transposition and that is negatively regulated by a repressor. On the basis of our genetic studies, it appears that transposition is a two-step process. The first step involves formation of cointegrates or fusions containing the donor DNA molecule linked to the recipient via a direct repeat of the transposon. In the second step, the fusions are resolved. Accordingly, the transposon contains two types of functions. Formation of the cointegrate requires the two ends of the transposon and the transposase. We have demonstrated that the resolution step requires the repressor (described above) and an additional internal site. Thus, the repressor/resolvase is a bifunctional protein (Kostriken et al., *Proc. Natl. Acad. Sci.* [1981], in press). Resolution of cointegrates takes place through a unique internal site (the IRS) within an A+T-rich sequence located between the transposase and the resolvase. We have constructed an overproducer of the resolvase and are using this to purify this protein and examine its in vitro binding and recombination activities (R. Kostriken, T. Meyer, and F. Heffron, in prep.). By studying these intermediates, we hope to understand how the recombination takes place. In the next few years the major emphasis of our work will be to understand the complex way in which transposition is regulated and to establish an in vitro system in which the complete or partial transposition reactions take place. The transposition frequency is controlled by the expression of a transposase negatively regulated by a repressor, yet there are many molecules in which Tn*3* will not integrate. One of the Tn*3*-encoded regulatory systems acts in *cis* to prevent insertion of Tn*3* into a molecule with a resident Tn*3*. To establish an in vitro transposition system, it will be necessary to understand this complex regulation.

Vertebrate Gene Structure and Expression

Until the advent of recombinant DNA, genes of higher organization were foreclosed from effective study, and those molecular biologists who wished to focus on higher-cell DNA were for the most part restricted to work on the DNA viruses of higher cells. As a consequence, SV40, polyoma, and adenovirus DNAs have, for over a decade, been intensively examined and are now among the best studied of all nucleic acids. It was thus not at all surprising that RNA splicing was first observed through their study, and the great scientific benefits that have arisen through the focusing of tumor virus DNA have already been universally acclaimed.

The arrival of the recombinant DNA procedures, together with the marvelous new methodologies for sequencing DNA, now provides the opportunity for studying equally successfully the genomes of higher cells. Given our already major commitment to tumor virus research, it seemed obvious that as soon as expanded resources became available, we should also focus on the DNAs of their respective host cells. Toward this end, we have created the new research division of Vertebrate Gene Structure and Expression. Major support for it will come in the next several years from a Program Project Grant from the National Cancer Institute that commenced April 1, 1981. This new division occupies space in both Demerec and James Labs and was initially set up with four sections: Mammalian Cell Genetics, Hormonal Control of Gene Expression, Genes for Major Structural Proteins, and Structure and Expression of Polypeptide Hormone Genes.

Our Mammalian Cell Genetics section, headed by Mike Wigler, was until now connected to both our Tumor Virus and Movable Genetic Elements Divisions. It will now belong exclusively to the Vertebrate Gene Structure and Function Division. Heading the Hormonal Control section is Dave Kurtz, who came here to be part of Mike Wigler's research team. Dave has been promoted to the Senior Staff Investigator level, and will soon have his own lab facilities in which to run an expanded group focusing on how steroid hormones control gene expression. Heading our section on Genes for Structural Proteins is Steve Hughes, who initially moved here to be part of our Tumor Virus Division. Newly arrived to head up our efforts on Polypeptide Hormones is John Fiddes, who came to us from the University of California at San Francisco, where he postdoced for several years with Howard Goodman, after doing his Ph.D. in Fred Sanger's Lab at Cambridge. John has already been joined by Nikos Vamvakopoulos to actively pursue research on the genes coding for several human polypeptide hormones.

MAMMALIAN CELL GENETICS

M. Wigler, M. Perucho, *K. Shimizu, J. Kwoh, M. Goldfarb, D. Levy, C. Fraser, C. Lama*

In our second year at Cold Spring Harbor, we have continued the explorations of gene expression in cultured mammalian cells made possible by techniques of gene cloning and gene transfer, have consolidated insights and developments of the previous year, and have opened up new areas for experimentation. We have been joined by Mitchell Goldfarb, a postdoctoral fellow from R. Weinberg's lab at MIT; Jesse Kwoh, formerly with the Molecular Genetics section at Cold Spring Harbor; and Kenji Shimizu, a microbial geneticist and biochemist from the University of Kyushu, Japan. In addition, Manuel Perucho has been given a staff appointment.

The Physical State of Exogenous DNA in Transformed Cells

M. Perucho, M. Wigler

The genetic content of cultured mammalian cells may be altered—a process classically referred to as transformation—by exposure of cells to DNA as a calcium phosphate precipitate. Transformants are most readily identified by their acquisition of a new phenotype. Previously, we established that under certain conditions cells will stably incorporate up to 4000 kb of exogenous DNA. An extensive series of experiments demonstrating the genetic linkage of acquired phenotypes led us to postulate that transformed cells assemble a structure of up to 4000 kb comprised primarily, if not exclusively, of foreign DNA and that most, if not all, incorporated DNA persists in transformants in one such structure, which we have called a "pekelasome." This hypothesis was confirmed by hybridization analysis of transforming elements after their molecular cloning in prokaryote hosts. Thus, foreign DNA incorporated by mammalian cells undergoes a relatively efficient intermolecular ligation, a process as yet without parallel in microorganisms. We do not yet know whether this process is random or whether it favors certain sequences or even certain molecular configurations. Our initial studies, performed with a Tk^- (thymidine-kinase-deficient) murine cell, LTk^-, have now been further confirmed in that cell and also extensively confirmed in a Tk^- rat cell line, Rat-2, derived by Bill Topp (Tumor Virus section). Other workers have also confirmed our initial observations and have, in addition, demonstrated that foreign DNA is usually integrated into a host chromosome as a single unit. This organization of foreign DNA in transformants may be an important factor to consider in the analysis of expression of newly acquired genes. Moreover, the phenotypic linkage between previously unlinked, newly acquired markers provides a very strong criterion for true gene-transfer events, a point to which we shall return when discussing the isolation of tumor genes.

The Expression of Genes in Transformed Cells

M. Perucho, M. Wigler

Previously, we studied the expression of the simian virus 40 (SV40) early region when cotransferred into LTk^- cells using *tk* as the selectable marker. Following expression of the early region by indirect immunofluorescent staining for T antigen, we (in collaboration with D. Lane, D. Hanahan, and M. Botchan) established that cloned populations of cells expressed T antigen in a heterogeneous pattern (Hanahan et al., *Cell 21:* 127 [1980]). Only a portion of the population were found expressing at any one time, and yet it was impossible to clone out a pure nonexpressing subpopulation. We concluded that expression of the early region was switching off and on during the progression of individual cell lineages. The molecular basis for this switching is uncertain, as is its generality. However, in 1974, Risser and Pollack observed similar patterns of expression in unselected populations of 3T3 cells infected with SV40 (*Virology 59:* 477 [1974]). Moreover, we have observed certain "ambivalent" phenotypes in the expression of the endogenous *tk* gene in avian and rodent cells, which are consistent with rapid switching (see below). Evidence from blot hybridization suggests that switching is not due to large-scale sequence rearrangements, and so other mechanisms are now beginning to be studied. The following major questions can be asked: Is switching peculiar to SV40? Does it occur coordinately to several independently integrated transcription units? Is the constitutively switched-on state (which occurs in a minor proportion of independent transformants) due to a host mutation or to an SV40 mutation, or is it due to a mutation at all? Is switching correlated with changes in DNA methylation patterns?

In a manner similar to that employed in our SV40 studies, both the cloned chicken and herpes *tk* genes were introduced into doubly mutant mouse LTk^-Aprt^- cells using *aprt* as the selection system. As judged by the ability of cotransformants to grow in *tk*-selection (HAT) medium, all cell populations that contained either the herpes *tk* 3.4-kb *Bam*HI fragment or the 2.25-kb *Eco*RI/*Hin*dIII fragment of chicken *tk* were capable of growing in HAT. Thus, these genes are completely encoded on their respective fragments. Work in progress indicates that the pattern of expression of the chicken *tk* gene in mouse cells resembles the

expression of mouse *tk* in so far as levels of the gene product are greatly reduced in stationary cell cultures. If this regulation is at the level of transcription, it may be possible to identify the control region for modulation of expression. Neither in the case of the herpes *tk* gene nor that of the chicken *tk* gene have we studied expression at the single cell level as we did for SV40, and so our results are not comparable. Preparations for such studies, based on the construction of chimeric genes, are in progress. Future studies include the construction of chimera between selectable genes and genes under differential control in order to apply the techniques of mutation and selection to obtain information about the mechanisms of cellular differentiation.

Characterization of the Chicken *tk* Gene

K. Kwoh, M. Goldfarb, M. Perucho, M. Wigler

As described in last year's report, we have isolated the chicken *tk* gene by the technique of plasmid rescue. The gene has been cloned both as a 2.25-kb *Eco*RI/*Hin*dIII fragment in pBR322 and as a 15-kb clone in the λ vector Charon 4A. As described in the previous section, we believe that the 2.25-kb fragment is complete—encoding information for its own transcription and regulation. Our aim is to provide a complete anatomical description of this gene, and to this end we are sequencing it, have made an extensive collection in vitro of pseudo-random linker (*Xho*I) insertion mutants, and have a preliminary characterization of the transcription unit—its size, splicing pattern, and orientation.

Characterization of Tk Mutants in Cultured Chicken Cells

M. Wigler

We have obtained a permanent chicken cell line courtesy of G. Shutz and T. Graf (University of Heidelberg, Germany). Called 249, it is derived from an MC29-induced hepatoma in chickens. The availability of this line and the cloned chicken *tk* gene has made possible a molecular investigation of mutations that arise at the *tk* locus after selection of cells resistant to killing by BrdU. 249 cells plate in medium containing BrdU at about 5 colonies per 10^8 cells plated. It is possible to select mutants in that way or by gradually selecting cells resistant to ever-increasing BrdU concentrations. We have used both approaches to obtain 10 independent BrdU-resistant mutants. All the mutants that have been analyzed to date appear to have a normal blot hybridization pattern for *tk* but one of reduced intensity relative to the 249 parent, suggesting that one step to BrdU resistance has been deletion of all *tk* sequences from one locus. A similar deletion of the remaining *tk* locus has not occurred in any line and is presumably lethal. The second step appears to differ in various mutants, since there are at least two distinct phenotypes: mutants that cannot grow in HAT medium (plating efficiencies less than 10^{-6}) and mutants that grow in HAT medium with high efficiency. The latter class appears analogous to the cells that demonstrated heterogeneous expression of the SV40 early region as discussed earlier, in that efforts to clone a pure population have so far failed. Work is currently directed at determining whether the ambivalent phenotype does represent rapid switching and, if so, whether this phenotype can be regarded as due to a mutation at the DNA sequence level.

Isolation of Human Tumor Genes

M. Perucho, M. Goldfarb, K. Shimizu, M. Wigler

Recent work of R. Weinberg's laboratory (MIT) and G. Cooper (Sidney Farber Cancer Institute) has demonstrated the feasibility of transforming normal-growth-controlled rodent cells to the tumor phenotype using DNA from chemically transformed or virally transformed cells as donor. These experiments, together with our ability to clone the active gene by plasmid rescue, raise the possibility of a genetic analysis of tumorigenesis. We have begun screening DNAs from a variety of cells derived from various human tumors for their ability to serve as donors of the tumor phenotype using NIH-3T3 cells as the normal recipient. Several human cell lines have been identified that are excellent donors of the tumor phenotype, and we are now attempting to isolate these genes for further study. The techniques we will employ involve plasmid rescue, which we used to isolate the chicken *tk* gene, and a new approach utilizing a bacterial tRNA suppressor gene as the prokaryotic factor. The latter will complement a λ packaging phage containing an amber mutation in the lysis function. Only recombinant phage will be able to grow on wild-type hosts.

Inheritance and Function of DNA Methylation

D. Levy, M. Perucho, M. Wigler

The DNAs of higher eukaryotes contain a low proportion of modified cytosine residues (5-methylcytosine [5-MeC]), whose function has been the subject of much speculation and interest. Most of the 5-MeC residues occur in the simple palindromic sequence

5′ CpG
3′ GpC

and this sequence is also found as part of the recognition site of several restriction enzymes that will not cleave methylated recognition sequences. By using such "CpG" enzymes, many workers

have investigated the distribution of methylation within specific gene sequences in specific tissues. To a first approximation, methylation patterns are not random: There appears to be a master pattern, present in germ cells, of which the somatic cells partake, the exact variation in methylation pattern differing between tissues but being characteristic for particular tissues. This distribution is consistent with the idea that methylation patterns are inherited by somatic cells. Moreover, there is a rough inverse correlation between degree of methylation and expression, consistent with the idea that methylation patterns might function in some way to control specific expression patterns in differentiated tissues. We have decided to explore these questions using the methods of gene transfer. We purified M-*Hpa*II, the methylase from *Haemophilus parainfluenza*e that methylates the internal cytosines of the sequence

5′ CpCpGpG
3′ GpGpCpC

a subset of potentially methylatable sites in higher vertebrates. Using M-*Hpa*II, we demonstrated that methylation patterns established on cloned DNA in vitro are replicated in vivo when that DNA is introduced into living cells. In other words, methylation is its own genetic determinant. We estimate that the fidelity of inheritance is at least 95% per site per cell generation. Moreover, we observed that the methylated herpes or chicken *tk* genes transform cells less efficiently than unmethylated genes, indicating that there is a causal relationship between expression and methylation.

Publications

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HORMONAL CONTROL OF GENE EXPRESSION

D. T. Kurtz, *D. Bishop, C. Nicodemus, A. Baxter*

The hormonal control of the synthesis of specific proteins has proved to be a fruitful model system for the study of differential gene expression in higher eukaryotes. The response of specific genes to hormonal stimuli is tissue-specific, and, within a given cell type, the modulation involves only a few specific markers. A general model for the mechanism of action of steroid hormones has evolved on the basis of experiments done in a large number of model systems: The steroid hormone enters the cell and binds to a cytoplasmic receptor protein. The steroid-hormone receptor enters the nucleus, wherein it interacts with the genetic material, resulting in the appearance or disappearance of a few select mRNAs in the cytoplasm. The events that occur after the localization of the steroid-receptor complex in the nucleus, leading to this modulation of cytoplasmic mRNA levels, remain a mystery. The well-accepted models of prokaryotic gene regulation have led some to speculate that hormonal induction may be the result of a simple repressor-operator–type mechanism in which the steroid-receptor complex itself binds near the target gene. However, until recently, it was virtually impossible to test even the rudiments of such a model. The recent advances in recombinant DNA technology and the ability to transform mammalian cells with defined DNA segments now allow us to approach some fundamental questions concerning the molecular mechanism of action of steroid hormones.

Rat α_{2u} Globulin

Several years ago a protein was discovered in the urine of male rats that was absent from the urine of females. This protein, with a molecular weight of 20,000, was designated α_{2u} globulin. This protein was first thought to be a prostatic secretion, but it was later found to be synthesized in the liver of mature male rats (wherein it represents 1% of hepatic protein synthesis), from which it was secreted into the serum and then excreted in the urine. The function of this protein remains unclear, but it appears to be necessary to maintain normal spermatogenesis in male rats. The synthesis of α_{2u} globulin is under extremely complex hormonal control in vivo. Glucocorticoids, androgens, thyroid hormone, and growth hormone are necessary to maintain a normal level of α_{2u}-globulin synthesis, and estrogens administered to male rats strongly repress the synthesis of this protein. No α_{2u}-globulin synthesis occurs in adult female rats, but it can be induced in females by ovariectomy followed by androgen or glucocorticoid administration.

α_{2u} Globulin Is Encoded by a Multigene Family

The cDNA for α_{2u} globulin was cloned in *Escherichia coli*, using a high-efficiency "double-linker" technique for cloning cDNAs. This cloned α_{2u}-globulin cDNA was used as a probe in Southern hybridization analysis of rat genomic DNA cleaved with several restriction enzymes that do not cleave the cDNA. An extremely complex pattern of bands appeared, suggesting the presence of multiple α_{2u}-globulin genes. This was confirmed by DNA-excess solution hybridization using cDNAs for α_{2u} globulin and albumin, which is known to be a single-copy gene in the rat. This analysis indicated that there are 18–20 α_{2u}-globulin genes per haploid genome. In situ hybridization to rat metaphase chromosomes, using α_{2u}-globulin cDNA labeled with ^{125}I, indicates that most, and perhaps all, α_{2u}-globulin genes are clustered on the long arm of chromosome 5. (The finding that α_{2u} globulin may be encoded by several genes is not surprising in that the corresponding protein in the mouse, designated MUP, is encoded by approximately 20 genes.) The finding that α_{2u} globulin(s) may be encoded by a multigene family immediately raises the possibility that the complexity of the hormonal modulation, i.e., the large number of hormones that affect α_{2u}-globulin synthesis, may be the result of the individual α_{2u}-globulin genes responding to different hormones.

α_{2u}-Globulin Gene Structure

The α_{2u}-globulin cDNA was used to screen a library of rat genomes cloned in the λ vector Charon 4A. Six independent clones were obtained that contained the entire α_{2u}-globulin gene, and these were analyzed by a combination of restriction enzyme mapping and electron microscopy of RNA:DNA heteroduplexes.

It was found that the six genes had most restriction enzyme sites in common and, in fact, shared sites that were in the 5′ and 3′ flanking regions. However, some heterogeneity was found: Two of the genes were found to contain a portion (~ 400 bp) at the extreme 3′ end that did not hybridize to the cloned α_{2u}-globulin cDNA, although these two variant genes seemed to be identical with the other four in the 5′ and middle portions of the mRNA-coding portions and in the intron regions. It is possible that these represent "pseudogenes" that are never transcribed or are transcribed in another tissue rather than liver.

Electron microscopic analysis revealed that the α_{2u}-globulin genes are interrupted by six intervening sequences. The length of the gene was estimated to be approximately 3.5 kb. (This corresponds to the length of the largest α_{2u}-globulin RNA precursor found in rat liver nuclear RNA.) It was also found that there is a sequence (50–100 bp) in the third intron of the α_{2u}-globulin gene that has a complementary inverted sequence approximately 4 kb from the 5′ end of the gene. This is visible as a stem-loop structure under the electron microscope. The intron-exon pattern for

all of the α_{2u}-globulin genes analyzed was identical, and the inverted repeat was found in every case.

Expression of Rat α_{2u} Globulin in Mouse Cells

To define the DNA sequences in or around the α_{2u}-globulin gene that are responsible for its hormonal modulation, two of the α_{2u}-globulin genes were cotransformed into mouse LTk$^-$ cells, using the herpes simplex virus (HSV) thymidine kinase (*tk*) gene as a vector. (These experiments were done using total λ DNA containing the α_{2u}-globulin gene and 3–6 kb of flanking rat DNA on either side.) Several Tk$^+$ clones were isolated and were found to have incorporated from three to ten copies of the α_{2u}-globulin gene.

When these clones were induced with dexamethasone, it was found that in more than 50% of the clones, α_{2u}-globulin mRNA and protein were produced in response to these hormones and that the protein produced was secreted into the medium. The induced level of α_{2u}-globulin mRNA was estimated to be ~ 200 copies/cell, approximately one-tenth the level in a normal adult male rat.

This indicates that the information necessary for hormonal response is contained in the DNA fragment used for transfer. Site-specific mutagenesis and deletions of specific regions in and around the α_{2u}-globulin genes, followed by transfer into L cells, should help define the DNA sequences responsible for this hormonal modulation.

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GENES FOR THE MAJOR STRUCTURAL PROTEINS: Organization and Control of Expression

S. H. Hughes, *A. Barros, J. Feramisco, J. Sorge, G. P. Thomas*

We began studying avian sarcoma virus (ASV) both because it has distinctive properties as a virus and because it is a good model system for studying gene expression in higher eukaryotes. We believe that the unique properties of ASV should allow us to prepare a new class of eukaryotic vectors that could efficiently reintroduce a variety of cellular genes into avian cells. To use the virus successfully as a vector, however, its life cycle must be well understood. A very useful property of ASV is the stable integration of a DNA copy of the viral genome into the host chromosomes, and we have just completed an analysis of the structure of the integrated provirus of ASV.

The Structure of Integrated ASV DNA

As an obligate step in their life cycle, retroviruses insert a DNA copy of their genome into host DNA. This recombination event is known to be relatively specific with respect to the site in viral DNA and relatively nonspecific with respect to the site in the host DNA. The retroviral genome is RNA, and an essential step that must occur before integration is the copying of the RNA genome into DNA by the viral enzyme RNA-dependent DNA polymerase. The first stable intermediate, a double-stranded linear DNA molecule, is made in the cytoplasm of infected cells. This viral DNA, which has a full-length minus (–) strand and segmented plus (+) strands, is slightly larger than the RNA from which it is derived. Sequences from both the 3′ and 5′ ends of the genomic RNA are found at each end of linear viral DNA (see Fig. 1). These sequences form a direct repeat at the termini of the linear DNA (called the LTR), which varies in size from one species of retrovirus to another. The LTR of ASV is 330 bp long. Each repeat has the structure U_3RU_5, where U_3 is a unique sequence from the 3′ end of viral RNA and U_5 is a unique sequence from the 5′ end of viral RNA. The R sequences are repeated at both the 5′ and 3′ ends of viral RNA (see Fig. 1). In the case of ASV, U_3 is 230 bp long, R is 21 bp long, and U_5 is 80 bp long. The linear viral DNA migrates from the cytoplasm of an infected cell to the nucleus, where it is converted to two major forms of circular molecules. The smaller circle has a single LTR and presumably is formed by homologous recombination between the LTRs. The larger circle presumably is formed by a ligation event that brings together the ends of the linear viral DNA. The nature of this event is unknown, partly because the exact termini of the linear DNA have not been defined. It is not known whether the linear form or one of the circular forms of DNA is the direct antecedent of the integrated provirus.

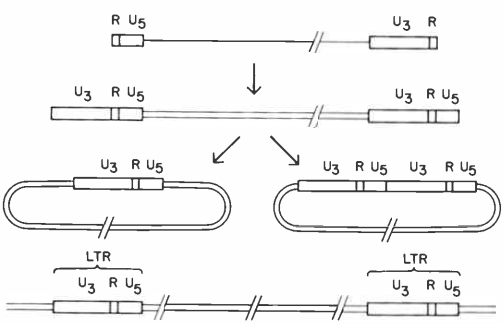

Figure 1
The stable intermediates in the replication of the ASV genome. Virion RNA (top) is copied by the virion RNA-dependent DNA polymerase to yield a linear DNA molecule. This linear DNA is the precursor of both the large and small circular forms of viral DNA. It is not known which of these three unintegrated viral DNAs is the direct antecedent of the integrated provirus (bottom). Cellular RNA polymerase copies the provirus into RNA, which completes the virus life cycle. Adapted from Shank et al., Cell 15: *1473 [1978] and Hughes et al.,* Cell 15: *1397 [1978].)*

The structure of the integrated provirus is superficially similar to the unintegrated linear ASV DNA, with cellular DNA attached to the ends of the LTR. The structures of viral RNA, the three forms of unintegrated viral DNA, and the provirus are given in Figure 1.

To learn more about ASV integration, we have cloned segments of DNA that include the junctions between viral and host DNA, and we have compared these to the unaltered host sequences into which the viral DNA integrated. These clones were derived from the DNA of the transformed line ASV NRK-2 (Hughes et al., *Cell 15:* 1397 [1978]), which contains a single complete ASV provirus.

The ends of the integrated provirus are flanked by a 6-bp repeat of cellular DNA. This repeat apparently is created by the integration event, since this 6-bp sequence is present only once in the host DNA into which the provirus integrated (see Fig. 2). The provirus appears to have lost 2 bp from the right end and either 1 or 2 bp from the left end, compared with the circular forms of unintegrated ASV DNA. The last base in the 6-bp repeat could be of either cellular or viral origin, since the same base (A) is found at the corresponding sites in the viral and cellular sequences (see Fig. 2).

A survey of eight other independent ASV-transformed rat cell lines demonstrates, in agreement with our earlier results, that the ASV proviruses are found in different segments of rat-cell DNA. We have also sequenced a junction between viral and host-cell DNA from a second

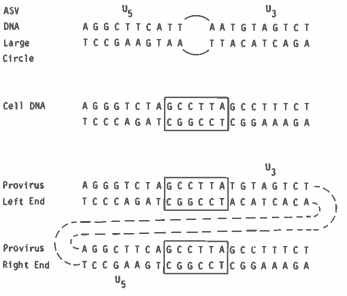

Figure 2

Sequences of unintegrated viral DNA, the integration site in the host genome, and at the ends of the integrated provirus. The sequence of the joint between U_3 and U_5 in the large circular form of viral DNA is that given by Swanstrom et al. (1981) for the SR-A strain of ASV. The integration site in the host genome and the junctions between host DNA and viral DNA at both ends of the provirus are all derived from the line ASV NRK-2, which contains a single complete provirus (Hughes et al., Cell *15: 1397 [1978]).*

ASV-transformed rat cell line (ASV NRK-4) and have found that there are no obvious similarities between the two integration sites, although the host sequences to the right of both proviruses are rich in A-T base pairs. Integration of ASV therefore is similar, as far as we know, to the integration of the other retroviruses, murine sarcoma virus (MSV), spleen necrosis virus (SNV), and mouse mammary tumor virus (MMTV), which lose 2 bp at each end during integration and are flanked by repeats of cellular DNA of 4 bp, 5 bp, or 6 bp (Dhar et al., *Proc. Natl. Acad. Sci. 77:* 3937 [1980]; Shimotohno et al., *Nature 285:* 550 [1980]; Majors and Varmus, *Nature* [1981], in press). Furthermore, the integration of MSV and MMTV create the repeat of cellular DNA (Majors and Varmus, 1981; G. Vande Woude, pers. comm.); i.e., there is only one copy of the 4-bp or 6-bp repeat in cellular DNA before the provirus integrated. The product of integrative recombination of retroviral DNA also seems to resemble closely the product of integrative recombination of a bacterial or yeast transposon, the bacterial virus Mu, and the *Drosophila* movable elements such as *copia.* This suggests that the mechanisms of integration of these diverse DNA molecules are likely to be related.

Construction of ASV Vectors

Certain retroviruses are natural recombinants that have picked up a gene (or genes) originally encoded by the host, and, at least in some cases, the level of both the mRNA and protein produced from the incorporated gene are profoundly enhanced. We have chosen to modify one of the naturally occurring retrovirus recombinants—ASV. The DNA genome of this helper-independent virus is approximately 10,000 bp long and is known to encode four genes. Three of the genes, *gag*, *pol*, and *env*, are required for virus replication. The fourth gene, *src*, is derived from a cellular gene c-*src*. *src* is responsible for the induction of sarcomas in the animal and the transformation of fibroblasts in culture. The *src* gene is lost from ASV at high frequency, to yield a nonsarcomagenic transformation-defective (*td*) virus that replicates at least as well as the ASV parent. From a cloned ASV genome (kindly provided by W. DeLorbe of the Bishop-Varmus Lab, University of California, San Francisco), we have produced a series of derivatives that superficially resemble a naturally occurring *td* virus. By appropriate manipulations, *src* has been removed, and a unique restriction site (*Cla*I) has been inserted. This allows us to cleave the DNA specifically and to substitute a variety of other genes for *src*. The choice of *Cla*I is advantageous, since ASV has no *Cla*I sites; in addition, the products of a *Cla*I digestion can be ligated directly to the products of a *Taq*I digestion without the addition of linkers.

What are the potential uses of these cloning vectors? They should provide a stable association between host DNA and a gene to be introduced. The parental virus infects cultured cells efficiently; it should be possible with recombinants to infect every cell in a dish. The ASV vectors have been designed to be helper-independent, which would eliminate problems of overgrowth by helper viruses.

Since the viral genome is RNA, a question arises: What will happen if a fragment containing intervening sequences (as opposed to cDNA) is introduced into a retrovirus vector? Will the intervening sequences be removed or not? Retroviral RNA is apparently unique in that approximately equal amounts of spliced and unspliced RNAs are found in the cytoplasm of infected cells. Genomic retroviral RNA is obviously unspliced; of the messages, at least *env* and *src* are spliced. One could imagine that the expression of viral functions (such as coating a portion of the viral RNA with viral proteins) somehow abrogates normal splicing for at least a portion of the viral RNA. In this case, a cellular gene that would normally produce a spliced message might, if introduced into a retrovirus, produce unspliced or partially spliced RNAs. Alternatively (and we favor this alternative), retroviruses may not contain sites that are spliced efficiently. If this is the case, it may be possible, after introducing into a retrovirus vector a cellular gene with intervening sequences, to allow the retrovirus to complete its life cycle and to recover, by cloning, a copy of the gene with all of its intervening sequences precisely removed.

Finally, it should be possible to use ASV derivatives to search for genes that, when amplified, have transforming potential. The retroviruses that are highly oncogenic all appear to have picked up a cellular gene. Considering all known retroviruses, there are approximately a dozen known cellular genes with transforming potential. Apparently, high levels of expression of these genes can transform certain cell types. We do not know how retroviruses acquired cellular genes, nor do we know whether the set of acquired genes that have been found in the naturally occurring retroviruses represent most (or all) of the potential transforming genes. We propose to "shotgun" the chicken genome into the ASV vector (which will lack the transforming *src* gene) and to screen for new transforming viruses in tissue culture.

Isolation and Identification of Genes for Major Structural Proteins

We have devised a protocol for directly screening genomic libraries for highly expressed genes and for identifying the genes encoded by individual recombinants. A highly labeled ($\sim 10^9$ cpm/μg) copy of any RNA or mixture of RNAs can be prepared with reverse transcriptase, using as primers oligonucleotides derived from calf thymus DNA. λ recombinants that carry genes corresponding to the major RNAs in the fraction used to prepare the labeled cDNA hybridize strongly and are readily identified. To determine which gene(s) is carried by an individual λ recombinant, the λ recombinant is used to purify, by hybridization selection, the homologous mRNA. This mRNA is translated in vitro, and the resulting protein products were analyzed by one-dimensional and two-dimensional gels and/or by immunoprecipitation with specific antibodies. We have chosen for study the set of genes that encode the cytoskeletal proteins of nonmuscle cells and the related proteins that make up the contractile apparatus of muscle cells. The clones will be used (1) for comparative studies of gene structure, (2) for studies of the relationships between gene organization and development/differentiation, (3) to examine the relationship between transformation and gene expression, and (4) to study effects of the presence of inappropriate homologs of cytoskeletal proteins (i.e., the actins) on cell structure and motility. The system has several inherent advantages: (1) The muscle-specific proteins exhibit coordinate regulation during chick myoblast differentiation. This differentiation takes place in culture, and the differentiation in culture can be specifically inhibited by the expression of the ASV *src* gene. (2) The proteins and mRNAs for both the cytoskeleton and muscle proteins are present in large amounts, which facilitates cloning and analysis. (3) There is a major commitment to the study of the protein products of these genes by the Cell Biology section. These proteins have been extensively studied both here and elsewhere, and we have access to antibodies (in many cases hybridoma antisera) specific for many of these proteins, which will permit us to characterize, immunologically, cells into which we have introduced cloned genes. (4) In contrast to the relatively large amounts of information available about the proteins, very little is known about the genes themselves, their structure, chromosome location, or what controls transcription in either normal or transformed cells.

We have isolated individual clones that encode actin, tubulin, and 100-Å filament genes. We are currently concentrating on the physical characterization of clones that contain a complete copy of the chicken β-actin gene, which is expressed in nonmuscle cells. The gene contains four intervening sequences, and we are now sequencing the region around the 5' end of the gene. Although there is considerable work still to be done in isolating and characterizing the genes, we expect to begin experiments soon in which we reintroduce cloned genes back into various differentiated and transformed cells. The cloned genes will be reintroduced by either microinjection or by the use of the ASV vector. We plan to monitor expression at the protein level; and since many of the proteins are ubiquitous, we will, in some cases, be forced to make recombinants that will produce hybrid proteins whose expression we can conveniently monitor immunologically. Our long-term goal is both to understand how individual cell types are able to selectively express only one of a series of genes that encode closely related proteins (e.g., striated-muscle cells express one of approximately six actin genes) and to ask what are the functional differences between these closely related proteins (e.g., What are the effects on a nonmuscle cell if it is forced to make large amounts of muscle actin?).

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THE STRUCTURE AND EXPRESSION OF POLYPEPTIDE HORMONE GENES

J. C. Fiddes, *N. C. Vamvakopoulos, W. R. Boorstein*

Over the past few years the development of molecular cloning technology has allowed the structure and expression of several polypeptide hormone genes to be studied in considerable detail. The genes that encode polypeptide hormones are particularly interesting because they frequently comprise multigene families. For example, the growth-hormone family consists of three polypeptides, growth hormone itself, placental lactogen, and prolactin; the insulin-related peptides are encoded by another multigene family. In addition to these intrinsic structural features, the factors that control the expression of polypeptide hormone genes are important because of the wide range of complex physiological functions that are under hormonal control.

The aim of this newly established group at Cold Spring Harbor, which only started working in November, is to study two distinct groups of polypeptide hormones. These are the glycoprotein hormones made in the pituitary and the placenta and the releasing factors made in the hypothalamus. Although these two groups of hormones are not structurally related, their physiological roles are very closely linked since the hypothalamic factors are responsible for the release of the glycoprotein hormones from the pituitary.

The Glycoprotein Hormones

There are four glycoprotein hormones in the human: chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). Chorionic gonadotropin is produced by the placenta; the other three are made in the pituitary. All four hormones are dimeric, consisting of dissimilar α and β subunits. The α subunit appears to be common to all four hormones, whereas the β subunits are unique and confer biological specificity on each hormone. The amino acid sequences of the β subunits show that they are related to each other and are therefore encoded by a multigene family. A distant relationship has also been proposed between the common α subunit and the β subunits.

Previously, we have isolated and sequenced full-length cDNA clones for the α and β subunits of the placental hormone CG. The cDNA for the α subunit of CG has now been used as a hybridization probe to isolate and characterize chromosomal gene sequences that code for the α subunit.

The cloned α-subunit gene encompasses a total of 9.4 kb of DNA and contains three intervening sequences whose precise locations have been established by restriction enzyme mapping and by DNA sequencing. One of the intervening sequences is located in the 5′ untranslated region, generating a leader sequence that is separated from the rest of the gene by 6.4 kb. The other two intervening sequences are 1.7 and 0.4 kb long and are located within codon number 6, between codons 67 and 68, respectively. Restriction endonuclease digestion of total human DNA suggests that the common α subunit is encoded by a single gene that is identical to the cloned copy. This gene is therefore expressed in the pituitary for synthesis of LH, FSH, and TSH and in the placenta for synthesis of CG. Similar-size transcripts of the α-subunit gene have been detected in both tissues. A marked correlation between malignancy and the production of chorionic gonadotropin has been reported. Preliminary characterization of this observation, using the cloned cDNAs as hybridization probes, shows that several transformed cell lines, including, for example, HeLa, which is derived from a human cervical cancer, do, in fact, produce either α- or β-subunit mRNAs that apparently are of the same length as in normal tissue.

The organization of the β-subunit genes is considerably more complex than that of the single α-subunit gene. Hybridization of the β CG cDNA to restriction-endonuclease-digested human DNA gives a complex pattern consistent with there being several copies of the gene. Two chromosomal isolates have been obtained and have been characterized by DNA sequencing, restriction enzyme mapping, and electron microscopy. These two segments of cloned chromosomal DNA contain four or five β-subunit genes and account for about one half of the total number of hybridizing fragments observed in total human DNA. It appears that all of the copies of the gene for β CG are physically linked on the chromosome.

The overall organization of each of these genes seems to be the same in that they all have one intervening sequence within the signal peptide and one within the coding region. The locations of the 5′ ends of these genes have not yet been established. The coding regions for each of the β CG genes apparently are the same, but small differences of sequence are found in the intervening sequences. It is not known which of these genes is actively transcribed.

In at least one case, and probably in more, two apparently identical β-subunit genes are arranged in pairs with convergent transcription. When viewed under the electron microscope, this pair of genes forms a "snap back" structure with a loop. No information is as yet available on the chromosomal organization of the genes for the β subunits of LH, FSH, or TSH.

The Hypothalamic Releasing Factors

The glycoprotein hormones are stored within secretory granules in the pituitary. The release of these hormones from the granules is directly controlled by factors synthesized in the hypothalamus. The decapeptide, gonadotropin releasing hormone (gnRH), controls the release of both LH and FSH, and the tripeptide TRH (TSH releasing hormone) controls the release of TSH. Due to the extremely low levels of these peptides, no information is available concerning their biosynthesis. It is not known, for example, whether the releasing factors are initially synthesized as larger precursors that are subsequently processed by proteolytic cleavage, as in the case of pro-opiomelanocortin.

In collaboration with A. Markham (ICI, England) and J. Roberts and M. Evinger (Columbia University), we are attempting to isolate cDNA clones for the putative precursor to gnRH. To do this, we are making use of synthetic oligonucleotides to prime specific cDNA synthesis from hypothalamic RNA. The sequences of these oligonucleotides are deduced from the known amino acid sequence of gnRH.

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CELL BIOLOGY

The Cell Biology group continued to probe into the functional and spatial organization of the cytoplasm of mammalian cells in culture. Even though the amount of information about the composition and distribution of cytoplasmic components is rapidly increasing among cell biologists, the understanding of their function is still at best sketchy. Nevertheless, there is a general feeling that cell biologists are at the brink of understanding the cytoplasmic organization of this giant, self-replicating, multienzyme complex which we call a cell. We hope that we have contributed this past year to furthering this understanding.

Newcomers to our group were Fumio Matsumura from the National Insitute for Basic Biology, Okazaki, and Steve Norris, who replaced Carter Burwell as programmer of the PDP 11. Birgitte Lane left us after one year of postdoctoral training and is continuing her successful work on cytokeratins at the University College, London.

A terrible fire destroyed the entire biochemistry laboratory at 4 A.M. on March 30th. It caused severe smoke damage in almost every other room of the McClintock building. Nevertheless, restoration proceeded swiftly, and we were back in operation in less than one month's time. We are very grateful to the crew of the Buildings and Grounds Department, who responded quickly, cleared out the rubble, and restored the building by efforts far beyond the call of duty.

Detailed reports of published work by the group are given below.

G. Albrecht-Buehler, *S. Blose, A. Bushnell, A. Calasso, T. Lukralle, M. Schwartz, M. Szadkowski*

The Autonomous Movements of Cytoplasmic Fragments

G. Albrecht-Buehler

Most cell biologists in the past and many today believe that animal-cell movement is random. Regardless of how complex a machine, if it acts randomly, at least one of its mechanisms must be intrinsically indeterminate. Tissue-cell migration involves complex extension and ruffling of the cell's leading edge, the appropriate sequence of making and breaking attachments to the substrate, and the retraction of the ever-narrowing tail at the rear of the cell. Consequently, at least one or another of these crucial mechanisms is ultimately caused by random thermal fluctuations of the cytoplasm or by a similarly stochastic event, if cell movement is to be random. (In the machines of human technology, such random causes can occur accidentally as "bad electrical contacts" or "loose screws" or deliberately as built-in "random number generators.") It seems safe to assume that moving cells do not carry around "bad electrical contacts" or "loose screws" all the time. A billion years of development of cells should have greatly reduced the occurrence of such faults in the design. Therefore, the hypothetical randomizer of cell movement should be considered a deliberate feature of the construction of the cell's motile machinery.

How so? Animal-cell movement is crucial for embryonic development, wound healing, and for the immunocompetent cells to reach and attack invading microorganisms. In each case, the cells arrive at certain goals with accuracy. Even the destructive aspect of cell migration, namely, the metastasis of malignant cells, shows amazing accuracy in the way cancer cells reach target organs. Why then should the mechanisms of cell motility need a built-in randomizer if the cells are to reach their goals accurately? Are there not enough unforeseeable disturbances along the way of migrating cells?

Therefore, the question of control of movement is central to my interest. If there is control, we have to conclude that cells are able to collect and process data about their spatial environment and do not merely react chemically to their chemical environment. Data-processing capacity in single tissue cells would open entirely new avenues of thinking and research. In particular, malignancy and aging may be approached with the thought in mind that possible changes of molecular components are causes or consequences of changes in the "programs" of data-processing cell centers. One experimental result of this past year seems to be of particular importance to this question.

A moving cell appears to contain smaller units, each able to move autonomously. I suggest these units be called "microplasts." One can prepare them experimentally by treating the cells with the drug cytochalasin B, which causes the cells to retract their cytoplasm, leaving many thin strands of cytoplasm still spanning the distance to the former cell periphery ("arborization"). After the cells are washed away from the substrate, their peripheral attachment points are left behind on the substrate and heal at the breakage points. They can be removed from the substrate in order to be plated on new ones. Once plated on new substrates, the former attachment areas of the cells, now isolated from the cell, show a surprising spectrum of cellular movement. They are able to flatten; ruffle (Fig. 1A); bleb; wave filopodia; retract from the contact with another cell; organize microfilaments, microtubules, and intermediate filaments (Fig. 1B); and respond to drugs that interfere with cellular movement, such as colchicine and cytochalasin B. All these expressions of motility are usually only observed in intact cells, but they are found in those portions of the cytoplasm that consist of about 2% of the cell volume. The only known living fragments of cytoplasm that are smaller than microplasts are blood platelets. They are about 25% of the microplast volume and are able to express very limited movement. In other words, microplasts may be the smallest units of cytoplasm that can autonomously express the entire spectrum of cell movement. In a sense, one may consider them to be elements of amoeboid movement ("amoeboid movement" is meant to indicate cell movement involving complex shape changes, as opposed to the longitudinal contraction of a muscle cell or locomotion by beating cilia and flagella). It is important to note that microplasts cannot migrate, and whatever type of movement they express, they seem to continue it indefinitely in a stereotypical way.

Considering the problem of control of cell movement, the existence and properties of microplasts have far-reaching implications:

1. Since cells contain cytoplasmic fragments that move autonomously, there must be communication between parts that attunes them to one another. Without such communication, each part would engage in its own autonomous movements, thus causing paralysis, tremor, or fibrillation of the entire cell.
2. Such communication would still be insufficient to allow the cell to migrate, because each portion is incapable of displacement. Consequently, there must be a mechanism that coordinates the actions of the constituent

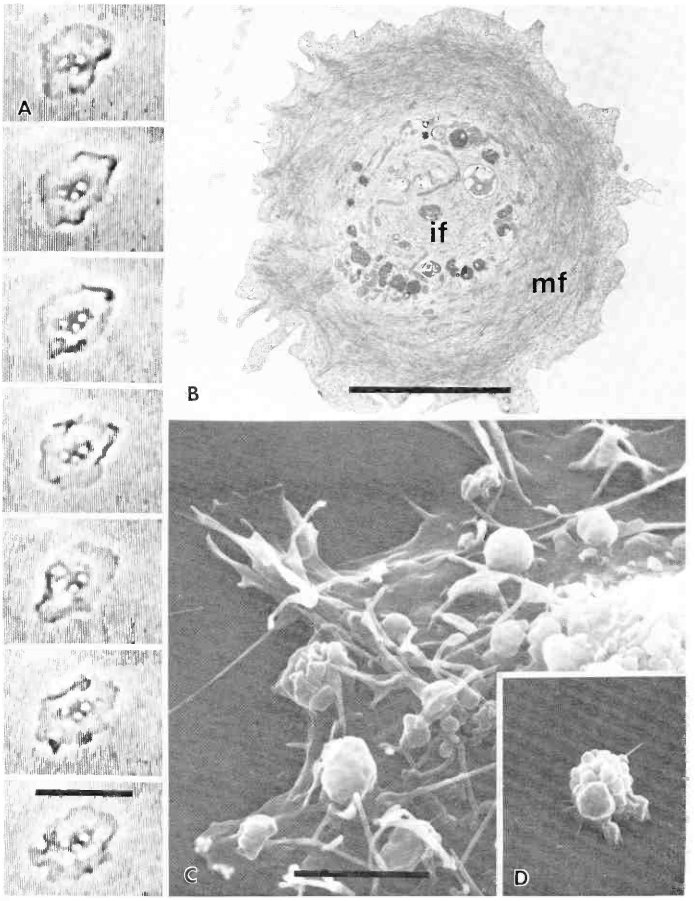


Figure 1

Aspects of microplasts. Bar indicates 1 μm. (A) Sequence of a ruffling microplast taken from a time-lapse video recording. The microplast ruffles all around its perimeter; it is essentially an isolated ruffle. (For size comparison, see C.) (B) Typical ultrastructure of microplasts from human fibroblasts. A circular arrangement of microfilaments (mf) surrounds a core of intermediate filaments (if) which are intermingled with vesicles and organelles. Microtubules (not shown) loop freely through the interior of microplasts despite the absence of centrioles. (C,D) Size comparison between the edge of a 3T3 cell (C) and one of the smallest blebbing microplasts (D) we found.

cytoplasmic portions by appropriate strategies. The extension of the leading cell edge followed by the retraction of its trailing tail and the making and breaking of substrate contacts in the proper time sequences, which are necessary for cell migration, cannot arise from communication alone. Therefore, one has to postulate the existence of a strategy-issuing

mechanism in cells, i.e., a control center of movement.
3. There seem to exist long-lived determinants for each type of cell movement within microplasts, as suggested by the stereotypical repetition of their movement.

Therefore, the existence of microplasts points to the existence of one or more communicating cellular control centers of movement. Whatever this control center may turn out to be—one may consider centrioles as suitable candidates—its existence calls for a revision of the concept of random movement and also more seriously poses the question of whether important expressions of aging and cancer lie in alterations of such a control center of cells.

The Use of Monoclonal Antibodies to Probe the Common Antigenic Sites between Tropomyosin and the Vimentin-type of 10-nm Filaments in Nonmuscle Cells

S. Blose and J. Lin

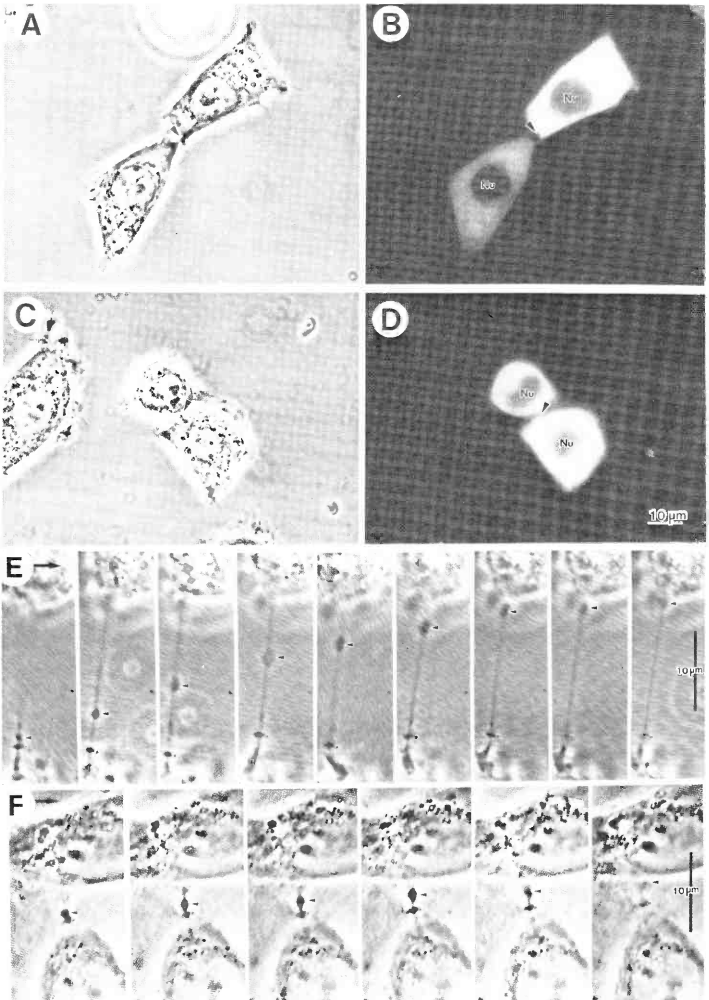
Several studies have suggested an association between tropomyosin and 10-nm filaments (Uehara et al., *J. Cell Biol. 50:* 484 [1971]; Blose and Chacko, *J. Cell Biol. 70:* 459 [1976]; Lazarides, in *Cell Motility*, p. 347, Cold Spring Harbor Laboratory, NY [1976]). To investigate this possibility, monoclonal antibodies were prepared against gizzard tropomyosin according to the methods of Köhler and Milstein (*Nature 256:* 495 [1975]). Supernates from the cloning wells were screened by immunofluorescence to locate clones that stained cells with the tropomyosin pattern and/or the 10-nm-filament pattern. A clone was found, LCK-16, that exhibited both staining patterns in the same gerbil fibroma cells (IMR-33). Both staining patterns were blocked by preabsorbing the LCK-16 antibody with either purified vimentin (from IMR-33 cells) or gizzard tropomyosin. Furthermore, if the IMR-33 cells were extracted with 0.1% Triton X-100, 1 M KCl, 2 mM DTT in 10 mM Na/PO_4 at pH 7.5, the tropomyosin staining pattern was abolished but not the 10-nm-filament pattern. Tropomyosin and vimentin were then purified from ^{35}S-labeled IMR-33 cells. Binding of LCK-16 antibody to both of these proteins could be demonstrated by immunoprecipitation. Studies in progress are directed at (1) determining the kinetics of LCK-16 antibody binding to both molecules to determine whether they have equivalent affinities and (2), determining, by proteolytic cleavage and immunologic methods, the peptides that share common antigenic sites.

Maintenance of Cytoplasmic Continuity between Daughter Cells Long after Mitosis

S. Blose and E. Schulze

Daughter HeLa cells and IMR-33 (gerbil fibroma) cells connected by a fine cytoplasmic bridge, containing the midbody, were unilaterally microinjected with small fluorescent molecules to determine whether cytoplasmic continuity is maintained. Sodium fluorescein (SF; $M_r = 376.3$) and fluorescein isothiocyanate-conjugated goat IgG (FITC-IgG; $M_r = 148{,}000$) were pressure-microinjected (Feramisco, *Proc. Natl. Acad. Sci. 76:* 3967 [1979]), and Lucifer Yellow CH (LY; $M_r = 457.3$) was injected by iontophoresis with constant-current hyperpolarizing pulses (Stewart, *Cell 14:* 741 [1978]). Cells were observed immediately after microinjection. In each case, the fluorescent molecules were introduced into one of the two daughter cells connected by the cytoplasmic bridge. Cells injected with SF and LY showed diffuse fluorescence equally distributed between daughter cells. Cells injected with the larger molecule, FITC-IgG (Fig. 2A–D), showed diffuse fluorescence unequally distributed (higher in the injected daughter cell) together with nuclear exclusion of FITC-IgG. It could be argued that pressure microinjection may force the dye through a diaphragm created by the midbody, but the iontophoretic injection of LY is a less-invasive technique and gives the same results. The fact that FITC-IgG was excluded from the nucleus of injected cells shows that a larger-M_r protein can cross through the cytoplasmic bridge and was not contaminated with unbound fluorochrome. On the basis of video-tape, time-lapse studies, in HeLa cells the cytoplasmic bridge between two daughter cells is maintained for an average time of 4.25 hours (range 1–16.8 hr) and in IMR-33 cells, for an average time of 2.5 hours (range 1.25–3.6 hr). There is considerable movement of this bridge (Fig. 2E,F), as observed by oscillations in the position of the midbody. Peristaltic undulations of the membrane and vacuole movement (Fig. 2E,F), reminiscent of slow axoplasmic flow (Pomerat et al., in *The Neuron*, p. 119. Elsevier, NY [1967]), were frequently observed. These results indicate that postmitotic HeLa and IMR-33 cells maintain cytoplasmic continuity, through the cytoplasmic bridge, to small molecules. This continuity can remain for

Figure 2
Phase (A and C) and fluorescence (B and D) micrographs of living daughter HeLa cells unilaterally microinjected with FITC-IgG. Unequal diffuse fluorescence (more intense in the injected cell) was observed in the cell pairs together with nuclear (Nu) exclusion of the molecules. Arrowheads indicate the position of the midbody and cytoplasmic bridge. Sequences observed with video-tape, time-lapse microscopy (E and F) of the cytoplasmic bridge revealed movements of vacuoles (arrowheads) and oscillation of the midbody(*). The vacuole in E moved an average velocity of 1.54 μm/min, and the vacuole in F moved 1.22 μm/min. Sequences in E are 1.16 min apart, and the sequences in F are 24 sec apart.


A
B
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Nu
10 μm
E
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a considerable length of time, extending into G_1 and even S or G_2. Passive diffusion might be augmented by the dynamic movement of the cytoplasmic bridge to facilitate transport of molecules between daughter cells.

E. Schulze (University of California at Berkeley), was a participant in the Undergraduate Summer Research Program.

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CELL BIOCHEMISTRY

K. Burridge, *J. Feramisco, J. Lin, L. McCullough, B. McLaughin, S. Queally*

Living cells display an impressive range of movements that involve both the internal organelles and the cell surface, the latter frequently leading to cell locomotion. It is the objective of this group to understand the function and organization of the structural proteins involved in generating these movements.

α-Actinin

K. Burridge, J. Feramisco

During the past year, we have continued our studies on the protein α-actinin. This protein was first identified in the Z lines of skeletal muscle but has since been recognized as an integral component of the bundles of microfilaments that are prominent in many cultured cell types. Because of its location at the Z line in muscle, attention has for some time been focused on its possible role in the attachment of actin filaments to the Z line. Likewise in nonmuscle systems, a role was proposed for α-actinin in the attachment of actin filaments to membranes. In last year's report, we described experiments that we had done which suggested that such a role was unlikely. One of the most interesting properties of α-actinin is its ability to cross-link actin filaments. When α-actinin is mixed with filamentous actin in vitro, it will cause marked gelation of the actin. This property of gelation is particularly interesting, since crude extracts of various nonmuscle cells and tissues will also form gels under appropriate conditions. Analysis of these gels shows actin to be the major constituent, but a number of other proteins are also seen and some have been identified as actin cross-linking proteins or gelation factors. A protein with a similar molecular weight to that of α-actinin has been identified in these gelled extracts, and we hope to resolve whether it is, indeed, α-actinin. One of the characteristics of this gelation of crude cell extracts is that gel formation is inhibited by low calcium concentrations. We are currently investigating whether the gelation of actin by α-actinin is also sensitive to calcium.

We not only are examining the gelation activity of muscle α-actinin, but also have been studying nonmuscle α-actinin in this respect. We have purified α-actinin from HeLa cells and are examining the properties of this isolated nonmuscle α-actinin in detail. In our previous work, we identified the α-actinin in HeLa cells as a tight doublet of bands on SDS gels, and now we have been able to separate these two forms chromatographically. It appears unlikely that one is a proteolytic breakdown product of the other, since both types can be identified as products of cell-free translations of mRNA (in collaboration with Paul Thomas, Protein Synthesis section). The properties of the two forms appear very similar; both show binding to actin that is competed by tropomyosin at 37°C and both will cause actin gelation. The significance of two forms of nonmuscle α-actinin within a single cell remains to be resolved.

Vinculin (Focin)

J. Feramisco, K. Burridge

Last year we described our work on a new smooth-muscle protein that has a molecular weight of 130,000. This protein was first described by Geiger (*Cell 18:* 193 [1979]), who showed that it was also found in fibroblasts at the ends of the actin microfilament bundles where these terminate at adhesion plaques. We confirmed Geiger's finding and also showed that the protein was found in close association with the cell-surface protein fibronectin, another region of interaction between actin filaments and the membrane. On account of its prominent focal distribution, as seen by immunofluorescence, we suggested the name "focin" for this protein. Geiger and his colleagues, however, have adopted a different name for this protein, "vinculin," derived from the Latin *vinculum*, meaning a link in a chain, with the implication that this protein has a role in linking actin to the membrane (Geiger et al., *Proc. Natl. Acad. Sci. 77:* 4127 [1980]). We have adopted this name for the protein, although we question whether it does have a linking function. Examining this possible function, we looked for the presence of vinculin in isolated HeLa plasma membranes, but we were able to detect little or no vinculin in these preparations, even though they were rich in associated actin. It may be, therefore, that vinculin functions not in the attachment of individual filaments, but in the organization of whole bundles of filaments at attachment sites. Such bundles are absent from the HeLa membranes we have analyzed. The HeLa cells are rich in vinculin, and, as with the α-actinin, we have purified vinculin from these cells. Jockusch and Isenberg (*Cold Spring Harbor Symp. Quant. Biol.* [1981], in press) have found that vinculin from smooth muscle causes a decrease in the viscosity of F-actin, which they interpret as the formation of large bundles of actin induced by vinculin. By in vitro biochemical analysis, we have also found that vinculin causes a decrease in the viscosity of F-actin. However, we believe that this is due to the binding of vinculin to the end(s) of the filaments and blockage of the elongation reaction of actin. Interestingly, we have found that the vinculin from HeLa cells requires Ca^{++} to exhibit this activity. Knowing what vinculin interacts with might give us a clue as to its function, and so we are

currently using a variety of techniques to try to identify the cellular components that interact with this protein. Likewise, the function of vinculin may be revealed by the microinjection into live cells of antibodies against vinculin so as to block its function. The recent successes with antibodies against components of intermediate filaments (see below) encourage us in this direction.

A New Muscle Protein

J. Feramisco, G.P. Thomas

As part of our continuing efforts to understand the organization of the cytoskeleton and its components, we have been investigating some of the minor proteins that copurify with known cytoskeleton components. In the course of fractionating smooth muscle, we recognized a hitherto undescribed protein with a molecular weight of 152,000 (152K), which has proved to be of particular interest because of its restricted tissue distribution and its relationship to vinculin.

We have shown that the 152K protein and vinculin are very closely related: Peptide mapping indicates that these two proteins share extensive structural homology, although as yet we have not determined in what respect(s) they differ. Immunologically, the two proteins are indistinguishable, using polyclonal sera, because of extensive cross-reactivity: Rabbit antiserum raised against either one of the purified proteins recognizes both in immunofluorescence and immunoprecipitation assays.

The 152K protein appears to be restricted to muscle tissue (mainly smooth muscle), whereas vinculin seems to be ubiquitous. This result is obtained when either 152K or vinculin antiserum is used. Immunoprecipitation of the cell-free translation products of mRNA derived from different tissues not only supports the results obtained by fluorescence and precipitation, but also argues against a precursor-product relationship between 152K and vinculin. Vinculin, but not 152K protein, is programmed with fibroblast mRNA; however, both are synthesized in response to smooth-muscle mRNA and to a mixture of fibroblast and smooth-muscle mRNA preparations.

The discovery of the 152K protein emphasizes the need for a thorough characterization and determination of the antigens present in different tissues when employing antibodies characterized in one tissue as probes for an antigen in other tissues. Thus, in smooth-muscle cells, anti-vinculin serum will recognize both 152K protein and vinculin and will indicate coincident localization, whereas in truth there may be distinct intracellular distributions of the two proteins, a problem that may be important in the work of Geiger et al. (*Proc. Natl. Acad. Sci. 77:* 4127 [1980]) which examined vinculin in smooth muscle. Since they appear to be separate gene products (either of the same gene via differential processing or of related genes), it is attractive to hypothesize that the restricted tissue distribution of the 152K protein indicates distinct roles for these two related proteins in the organization of the cytoplasm and that expression of the gene(s) is strictly regulated in different tissues reflecting these different roles.

A Regulatory Element of Nonmuscle Stress Fibers

J. Feramisco, K. Burridge

The contractile cycle of skeletal muscle is regulated by the influx of calcium. Calcium ions bind to troponin, which permits the active interaction between myosin and actin, leading to ATP hydrolysis and contraction. Initially, it was anticipated that both smooth-muscle and nonmuscle cells would employ the same mode of regulating actomyosin interaction. Troponin, however, appears to be absent from these cells, and, instead, a Ca^{++}-regulated protein kinase has been found that will phosphorylate the 20K myosin light chain. In vitro this has two effects on myosin. First, this leads to a stimulation of the actin-activated ATPase of myosin, an effect that is functionally similar to the effect of Ca^{++} on troponin in skeletal muscle. Second, this phosphorylation induces the aggregation of myosin molecules into short bipolar filaments. It will be important in our understanding of the regulation of nonmuscle contractile events to know whether the myosin light chain kinase exerts these effects on myosin in living cells. As a first step toward answering this question, we have asked where the light-chain kinase is localized in nonmuscle cells and whether this location is such that it could regulate the interaction of actin and myosin in these cells.

In collaboration with P. de Lanerolle and R. S. Adelstein (NIH), we have used immunofluorescence microscopy to study the localization of the light-chain kinase. This has revealed that the most prominent location of this enzyme in several cell types (chick fibroblasts, human fibroblasts, and gerbil fibroma cells) is along the stress fibers or bundles of actin microfilaments. In some cell types (e.g., the gerbil fibroma cells), this enzyme displays a regular periodic distribution along the stress fibers (see Fig. 1). By double antibody staining techniques, we have shown that this distribution closely corresponds to the distribution of both myosin and tropomyosin but alternates with the distribution of α-actinin. This close association with both actin and myosin in nonmuscle cells suggests that it is localized such that it could regulate their interaction and also regulate the formation of myosin filaments in live cells.

Microinjection of Fluorescently Labeled Proteins into Living Cells

J. Feramisco

Our interests in the molecular mechanisms of cell movement have led us to look at the dynamic

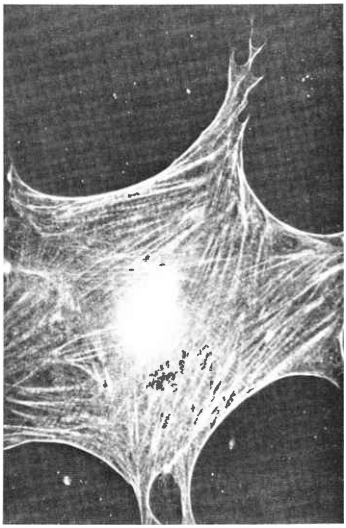

Figure 1
Fluorescent micrograph of a gerbil fibroma cell stained with myosin light-chain kinase antibodies.

changes of several of the actin-stress fiber components within the living fibroblast. As described in last year's report, we have been involved in the development of the experimental approach extended from Taylor and Wang's work (*Proc. Natl. Acad. Sci. 75:* 857 [1978]), in which purified structural proteins of the cells, labeled with fluorescent dyes to render them visible to the eye with a microscope, are microinjected into living, motile fibroblasts, using the injection technique of Graessmann (*Proc. Natl. Acad. Sci. 73:* 366 [1976]; Feramisco, *Proc. Natl. Acad. Sci. 76:* 3967 [1979]). Similar experiments with a different component of cells were done independently by Kreis et al. (*Proc. Natl. Acad. Sci. 76:* 3814 [1979]) in Switzerland. We have documented that for fluorescently labeled α-actinin and vinculin (focin), the proteins are assembled into the appropriate supramolecular structures within the living cells within 1–2 hours after injection (Feramisco and Blose 1980; Burridge and Feramisco 1980a).

Working in collaboration with C. Keith and M. Shelanski (New York University), we have been able to apply this experimental approach to a second filamentous system in cells: microtubules (Keith et al., 1981). Microtubules have been implicated in many cellular events, including mitosis, certain types of prey-catching, and organelle movements; thus, the ability to observe the dynamics of these filaments during these cellular events should prove to be useful in establishing the functions of the microtubules.

Detailed recordings of the distributions of these important components of the actin filamentous network and the microtubular network have yet to be made; work in our laboratory and further collaborative efforts with Keith and Shelanski, who are setting up a sophisticated time-lapse system for this purpose, are under way to carry out these experiments.

Monoclonal Antibodies to Cytoskeletal Proteins

J. Lin

Monoclonal antibodies against cytoskeletal components have the potential not only for improving the immunofluorescent localization of specific proteins within cells, but also for analyzing the functional sites of specific proteins and identifying the previously unidentified proteins. Furthermore, they can be used together with microinjection techniques to investigate the physiological roles of specific proteins.

We have prepared several monoclonal antibodies against a variety of cytoskeletal components by fusing myeloma cells with the spleen cells of mice immunized with crude cytoskeletal preparations from chicken gizzard. This strategy was designed with the aim of generating monoclonal antibodies not only against known cytoskeletal proteins, but also against previously unidentified components. Such fusions have given rise to monoclonal antibodies directed against known structural proteins, such as actin, filamin, and fibronectin, and against new components that we are now characterizing. The monoclonal antibody (JLA20) against actin shows wide species and cell-type cross-reactivity, binding to both muscle and nonmuscle actin types. JLA20 may become useful as a standardized reagent for cell biology. Three different monoclonal antibodies (JLA8, JLA10, and JLA17) were raised against filamin. These antibodies interact with different tryptic fragments of the protein.

We have also prepared two interesting monoclonal antibodies, JLB1 and JLB7, from fusion experiments using a total myofibril fraction as the antigen and screening the resulting hybrids on fibroblasts. This screening method would detect only those hybrids that produced antibodies reacting with antigenic determinants shared in common between the myofibrils and fibroblasts. Monoclonal antibody JLB1 recognizes an antigen that is distributed in the M-line region and on either side of the Z line of myofibrils, whereas monoclonal antibody JLB7 reacts with an antigen located at the M-line region of myofibrils. Both

JLB1 and JLB7 antibodies decorate the typical intermediate filaments of a variety of cultured cells. Immunoprecipitation, using chicken embryo fibroblasts, has revealed protein bands at 210K and 95K, and these have been identified as the antigens recognized by JLB1 and JLB7 antibodies, respectively. The biochemical properties of these intermediate-filament-associated proteins remain to be determined. Starger et al. (*J. Cell Biol. 78:* 93 [1978]) have found a minor protein component with a molecular weight between 250,000 and 350,000 that copurified with the intermediate-filament preparations from BHK-21 cells. Also, Granger and Lazarides (*Cell 22:* 727 [1980]) have found a protein, synemin (230K), of the intermediate filaments from smooth muscle. The larger antigen (210K) recognized by JLB1 antibody may correspond to these other proteins.

Three monoclonal antibodies (LCK16, JLH2, and JLF15) have been obtained from fusion experiments using purified tropomyosin as the antigen. Figure 2 shows the periodic stress-fiber staining pattern and the polygonal network staining pattern on gerbil fibroma cells by monoclonal antibody JLF15. Surprisingly, when cells were stained with monoclonal antibody LCK16, the intermediate-filament staining pattern, in addition to the stress-fiber staining pattern, was also observed. This may suggest that both vimentin, a major protein of fibroblastic intermediate filaments, and tropomyosin share a common antigenic site.

The intracellular localization of vinculin (focin) to adhesion plaques and the tips of microfilament bundles led to the suggestion that it may be one of the components involved in actin microfilament–membrane interactions (Geiger, *Cell 18:* 193 [1979]; Burridge and Feramisco, *Cell 19:* 587 [1980]). To study its function, we have made monoclonal antibodies directed against vinculin. It is hoped that some of these antibodies will block functional sites of vinculin, and then we can introduce the antibody into cells by microinjection to assess the in vivo function of this protein (see Microinjection of Monoclonal Antibodies into Living Cells).

Because our group is interested in the protein α-actinin, we have also prepared monoclonal antibodies against this molecule purified from chicken gizzard. Preliminary screening by indirect immunofluorescence shows many clones producing antibodies that can decorate the stress fibers of chicken embryo fibroblasts. These antibodies will be powerful reagents and may provide a useful tool to study in vivo function of α-actinin in muscle and nonmuscle cells.

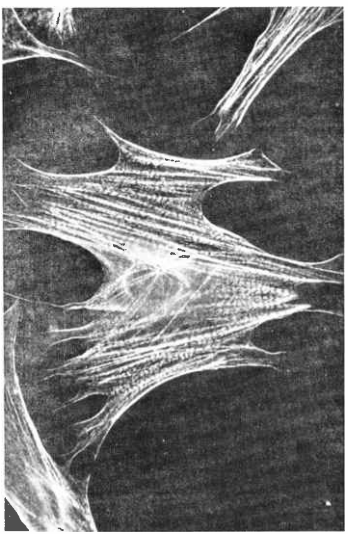

Figure 2
Fluorescent micrograph of gerbil fibroma cells stained with monoclonal antibody JLF15 against tropomyosin.

Microinjection of Monoclonal Antibodies into Living Cells

J. Feramisco, J. Lin

Complementing our experiments on the in vitro biochemical analysis of the structural proteins and their dynamic behavior in vivo, we have embarked on another approach to determine the physiological functions of those proteins in cell movement and organization. The basic idea is to introduce into living cells by microinjection highly specific monoclonal antibodies against selected intracellular components and to identify the cellular defects caused by complexing the target antigen with the antibody. Our first experiments of this type deal with the intermediate-filament network of fibroblasts (Lin and Feramisco, 1981).

Monoclonal antibodies that recognize minor components of the intermediate filaments (Lin, 1981) were introduced into living fibroblasts by microinjection. Five minutes after injection of the JLB7 antibody, virtually all of the intermediate filaments of the cells were found to be aggregated into tight bundles near the nucleus (see Fig. 3). In contrast, injection of the JLB1 antibody caused little or no aggregation of the intermediate filaments. Electron microscopy showed that the perinuclear bundles that formed after injection of the JLB7 antibody each consisted of ten or more intermediate filaments apparently cross-linked together. Double-label immunofluorescence showed

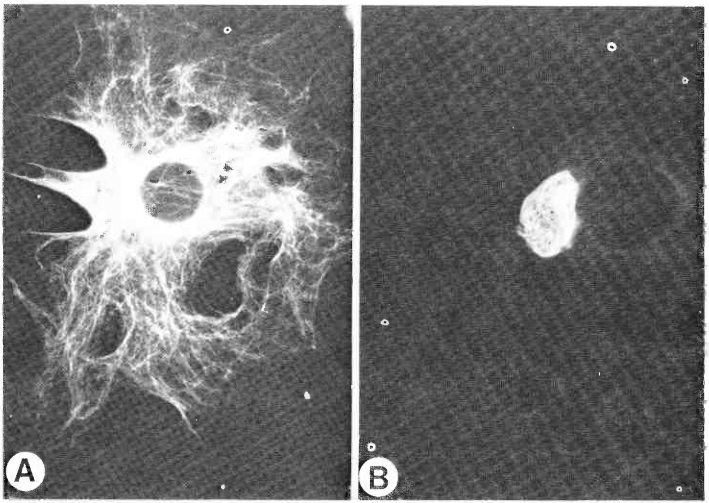

Figure 3
Fluorescent micrographs of gerbil fibroma cells stained (A) or microinjected (B) with monoclonal antibody JLB7 against 95K protein.

that virtually all of the vimentin-containing intermediate filaments in these cells were redistributed to the nuclear region and would remain there for at least 24 hours. The distributions of the actin microfilaments and microtubules were seemingly undisturbed following microinjection. No obvious changes in cell morphology or cell behavior were apparent in the injected cells; they displayed a flat appearance; showed a polarity; were able to bleb and ruffle; and even appeared to show the normal saltatory movements of intracellular vesicles, granules, and mitochondria, suggesting that the intermediate filaments are not involved in these activities. Further experiments to determine what defects, if any, are present in these cells lacking a normal intermediate-filament distribution are under way.

Molecular Biology of the Structural Proteins

S. Hughes, G. P. Thomas, J. Feramisco

As work is progressing in our analysis of the biochemical and functional properties of the proteins involved in cell motility, we have begun a major effort toward the analysis of structure, organization, and expression of the genes encoding the structural proteins. By using cDNAs made against the total mRNA of chicken embryos as probes, we have selected several hundred genomic clones from a bacteriophage λ library of the chicken genome. We are in the process of identifying the proteins encoded by the clones through the selective hybridization of mRNA to the genomic DNA clones and cell-free translation of the mRNA. Two-dimensional gel electrophoresis is used to aid in the identification of the proteins (in collaboration with Jim Garrels, Quest-2D Gel Laboratory). The first structural protein gene to be analyzed in this manner is that of the β-actin gene; we have obtained an extensive restriction map, intron-exon structure by R-looping (in collaboration with Louise Chow and Tom Broker, Electron Microscopy section), and a portion of the nucleotide sequence. Studies on the structure, organization, and expression of this gene and several others are continuing. (For more detailed information, see Eukaryotic Gene Structure and Function section in this Annual Report.)

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QUEST-2D GEL LABORATORY

J. Garrels, *F. Matsumura, T. Kelly, C. Burwell, S. Norris, J. Leibold, D. Gibson*

Our specialized laboratory for two-dimensional gel electrophoresis and computer analysis of proteins is now operating routinely. The types of specially designed equipment in our new facility were described in last year's report. During the past year, we have refined and further developed our equipment, improved some of the electrophoretic procedures, carried out control experiments to test the reproducibility of our system, and further developed our computer software. We are well into several projects to study the patterns of protein synthesis and phosphorylation in human, rat, and mouse cells, and collaborative work has been done with various other sections of the laboratory.

The Computerized Laboratory

The gel lab now operates at a steady-state rate of 20 large (22 × 22 cm) two-dimensional gels per day. Two trained technicians, Tom Kelly and Jessica Leibold, carry out the daily operation of the gel lab, as well as other duties such as sample preparation and cell culture. The use of a microcomputer to monitor and control various aspects of gel processing has been very successful in terms of greater convenience and reproducibility. For example, each slab gel can be controlled at any (programmable) wattage, the actual current and voltage to each gel is automatically recorded throughout the run, and the gels are automatically shut off when the ion front reaches the bottom. Improved procedural aspects include a much-simplified slab-casting system, a simpler method for counting TCA-precipitable radioactivity in samples, and a fast method for preparation of calibration strips (strips of acrylamide containing known amounts of radioactive protein that are used to calibrate the response of each film).

We have designed the gel laboratory for the routine production of many standardized gels each week, knowing that such a facility would be essential for our own work and anticipating that many others would also find it useful. As can be seen from the work described below, this has certainly been the case. The running of this lab at full capacity means the production of several thousand samples, over 5000 gels, and over 10,000 films per year. The record-keeping necessary to handle this load is immense. We have therefore taken the time to develop a powerful computerized data-management system. The system is based on intelligent laboratory terminals that can display many different forms for entry of data. The various investigators using this system enter the description of their experiments and samples on one of the forms. After receiving the sample numbers from the computer, the investigator leaves his samples in numbered racks in a freezer. To have his samples run, he merely fills out a simple gel-request form on which he specifies the types of gels to be run, the cpm to be loaded, and the desired exposure levels. The computer then calculates the dilutions and loads of the samples to be applied to each gel, and it automatically schedules the exposures. The lab technicians use forms compiled by the computer to know what gels to run each day, what gels to expose to film, and which films to develop each day. Other forms are used to record the exact composition, lot numbers, and quality control data for each solution that is prepared for gel electrophoresis. For each gel that is run, we can easily look up the batch numbers and quality control data for each reagent that was used. The system has been highly successful and is being extended to record-keeping for cell culture and for other laboratory data-management applications.

Studies of Human Disease

We have used our high-resolution gel system to analyze numerous human fibroblast lines in the study of two genetic diseases, cystic fibrosis and Huntington's chorea. The latter study is being done in collaboration with I. Fand (State University of New York at Stony Brook). The lines have each been labeled with [^{35}S]methionine to study rates of protein synthesis and with [^{33}P]-phosphate to study protein phosphorylation. Several thousand proteins have been detected and are being carefully compared in the hope that a difference indicative of the basic genetic defect can be detected. Differences have been found between the lines, but most appear to be normal polymorphisms in the population. Because so many proteins are being examined, it is necessary to look for a protein that is present in many lines that have the genetic defect, but not in any normal lines. This is to exclude polymorphism that could otherwise correlate with the genetic defect only by chance. No consistent differences between the normal and mutant fibroblasts have yet been discovered by visual inspection of the patterns; however, the examination of more lines and subcellular fractions is necessary. We have not yet subjected our data to detailed computer analysis, which will be necessary to find quantitative differences, especially among the minor proteins. A secondary outcome of this study will be a much better estimate of the degree of polymorphism in the proteins of normal unrelated individuals.

Cytoskeletal Proteins

With the arrival of Fumio Matsumura, a new postdoctoral fellow from Japan, we have begun to

map the major and minor proteins of the cytoskeleton of rat fibroblasts and myoblasts. The objectives are, first, to use two-dimensional gel electrophoresis to better define the minor components of the cytoskeleton and, second, to identify the regulatory proteins. By examination of the synthesis and phosphorylation of cytoskeletal proteins at times of massive cytoskeletal reorganization, such as mitosis, respreading after trypsinization, or myogenesis, we hope to detect changes in the critical regulatory proteins. Phosphorylation is known to regulate nonmuscle contractile systems, and phosphorylation has been implicated in cytoskeletal alterations in transformed cells. Therefore, knowledge of the phosphoproteins of the cytoskeleton and of the changes of phosphorylation in different states of cytoskeletal organization will be important.

We have found that metabolic labeling with [^{33}P]phosphate, followed by two-dimensional gel analysis, can give rise to sharp patterns of hundreds of phosphoproteins, in contrast to the result when ^{32}P is used, which gives much more diffuse patterns. We have already mapped the phosphoproteins of L6 myoblasts and myotubes, and differences of phosphorylation in Rat-1 cells have been detected during spreading. More studies will tell us how these changes correlate with other cytoskeletal states and how the metabolism of these proteins differs in other cell types. Efforts will be made to purify the most interesting proteins for further characterization.

Transformation by SV40

A series of experiments has been initiated leading to a detailed characterization of rat fibroblasts before and after transformation by simian virus 40 (SV40). We have obtained many clones of the Rat-1 cell line from Bill Topp (Tumor Virus section), and these have been analyzed for heterogeneity. Very little difference has been found between the clones, although significant differences are apparent when dividing and stationary-phase cells are compared. Further controls to determine the effect of serum lots and types are being pursued. The demonstration of consistent patterns of protein synthesis, with only small quantitative differences, between clones indicates that normal and transformed clones of Rat-1 cells can be meaningfully compared.

Collaborative Work

In collaborative work with Steve Hughes (Eukaryotic Genes section), Paul Thomas (Protein Synthesis section), and Jim Feramisco (Cell Biochemistry section), we have examined the products of translations directed by mRNA selected from cloned chick DNA fragments. These experiments have led to the identification of cloned genes for several of the actins, tubulin, vimentin, and other yet unknown proteins. In collaborative work with Jim Lewis and Mike Mathews (Protein Synthesis section), we have identified most of the adenovirus-specific proteins from infected cells and from in vitro translations directed by adenovirus-specific RNA. With Lee Silver (Visiting Scientist from Sloan-Kettering), we have examined the proteins of mouse cells that carry the mutant *t* locus. Consistent with theories that these mutants are in fact altered over a substantial portion of chromosome 17, we have found numerous protein differences (such as small shifts of charge and molecular weight) that correlate strictly with the presence or absence of the mutant *t* chromosome.

The full power of the two-dimensional gel system relies on detailed computer analysis of our protein patterns. Our development of image-processing programs has been carried out in the past year by Carter Burwell and Steven Norris. A complete set of programs for scanning the films, for detection of spots, for combining long and short exposures of each gel, for matching each new pattern to standards, and for integration of the detected spots has been developed. Since the image-analysis process is time-consuming, we are presently writing supervisory programs that will automatically run the merging, matching, and integration programs in the proper sequence overnight. With the completion of program testing and debugging, we will begin to match each of our new patterns to standard patterns and to store the quantitative data in a set of permanent data files. Data in this standardized format can be used for immediate comparison of related cell lines and cell fractions, and it will be available in our computer files for comparison to data obtained in subsequent experiments.

Publications

Garrels, J.I. 1980. Computer-analyzed two-dimensional electrophoresis of proteins. *Trends Biochem. Sci. 5:* 281.

NEUROBIOLOGY

The Neurobiology summer program underwent two innovative changes during the past year. In addition to our traditional lecture and laboratory courses, we are now offering a new type of workshop designed to facilitate the development and the dissemination of selected new techniques important for neurobiological research. The topic in 1980 was the internal perfusion or dialysis of isolated nerve cell bodies. The extension and development of such current techniques are achieved by bringing together several pioneers in the field who have independently developed versions of a particular method along with investigators who are interested in learning these techniques.

The second current development was the introduction of small conferences, held at Banbury Center, whose proceedings are to be published as Cold Spring Harbor Reports in the Neurosciences. Two such meetings were held this past year: "Molluscan Nerve Cells" and "Monoclonal Antibodies against Neural Antigens" (see Summer Meetings section).

The year-round Neurobiology group has been greatly enhanced by the addition of Susan Hockfield to our staff. Susan's expertise in vertebrate neuroanatomy as well as in light and electron microscopy techniques promises to add an exciting new dimension to the work already in progress in Jones Lab.

A central issue in Neurobiology concerns the principles underlying neural network formation. Another major question involves the mechanisms by which network activity subserving organized behaviors is modulated by chemical moieties such as monoamine neurotransmitters and peptides. Here at Cold Spring Harbor, we use the leech central nervous system, with its relatively small number of large, identifiable neurons, as a model system to address these issues.

B. Zipser, S. Hockfield, R. McKay

Monoclonal Antibodies Specific for Identifiable Leech Neurons

B. Zipser, R. McKay

The ability of a nervous system to generate coherent behavior is dependent on a vast number of precise connections between neurons. Virtually nothing is known about the molecular mechanisms that generate these connections, but all tenable hypotheses ultimately postulate the existence of molecules that differ in kind or quantity from cell to cell to mediate the necessary recognition. The problem of neural specificity is apparent, since neurons typically receive inputs from and send outputs to whole sets of other cells, each of which, in turn, has its own extremely complex characteristic connection pattern. Connections are established during development, but the degree to which marker molecules may be present in adults is unknown, although regeneration studies in adults imply long-term persistence of at least some molecular specificity. It has long been known that there are many chemical differences between neurons which involve such functions as transmitter synthesis. Recent studies on the identification and localization of neuronal peptides have now greatly widened the scope of detectable molecular variation among nerve cells. The relationship of this chemical variation to the mechanism of connection is still unknown. To make progress in this area it will be necessary to develop ways of relating chemical specificity to unique connectivity. Since, by hypothesis, the markers of interest will differ in each neuron with different connections, these molecules must somehow be identified in structured material where the same neuron can be easily identified in repeated experiments. Such a simple system is required not only for the identification of specific neuronal markers, but also for analyzing the rules that govern the establishment of neuronal connections.

As a step toward this goal, we asked whether individual cells and subnetworks of the relatively simple leech nervous system could be distinguished by specific antibodies. Since the putative antigens could, in principle, not be purified a priori, we used the technique of hybridoma formation, which allows the isolation of lymphocyte clones secreting antibodies specific for individual molecules, even though a complex mixture of antigens was used for immunization. Monoclonal antibodies were obtained from lymphocytes of mice immunized with the entire isolated nervous system of the leech and screened on intact ganglia. The results of this study give a clear answer to the question of the existence of chemical markers in individual cells and subnetworks. These specific markers are surprisingly abundant, and extrapolation from our initial sample indicates that it is possible that every cell has one or more chemical markers shared by only small subsets of neurons. Although we still know little about the exact cellular locations of these markers, many are present in all parts of the cell, including in the long axonal projections and neural terminals. Indeed, the situation seems quite analogous to a color-coded electric cable containing many wires, where each wire has its own unique molecule (dye) to facilitate proper recognition and connection at terminals. The existence of specific markers is far from sufficient to explain the mechanism of neural connection specificity, but it does give us a handle on how the problem can be attacked at a previously inaccessible level. In addition to any light these monoclonal antibodies may throw on the mechanism of connectivity, they will be of value in broadening our understanding of leech neurobiology, since they clearly elucidate whole systems of neurons together with their axonal patterns.

Of the 475 individual hybridomas screened, 3 came from a small preliminary test, 43 from one major fusion, and 429 from another. Of these, 64% secreted antibodies that bound to the leech nervous system. Most showed no obvious preference for subsets of neurons but reacted with general nerve-cell components. A few bound to interesting subcellular or extracellular structures, e.g., to antigens specific for the axon hillock of leech neurons (Lan3-15). Two antibodies recognized muscle antigens and stained the large muscle fiber present in each ganglion. Of central concern to us is group 41, which reacted with restricted subsets of neurons or fibers.

An example is illustrated in Figure 1, which shows Lan3-1, a monoclonal antibody that binds to a pair of small bilateral neurons in a midbody ganglion (Fig. 1B). The right and left cell bodies are located at homologous positions on the dorsal surface in each of the 21 midbody ganglia, as shown diagrammatically in Figure 2, which is a schematic representation of the leech nerve cord. In addition to labeling these cell bodies, the antibody also binds to clouds of varicosities in the central ganglionic neuropiles as well as to specific axons in the "connective," the fiber tract that links all ganglia in the 34-ganglion nerve cord.

Several antibodies that bind to homologous neurons in successive midbody ganglia bind to extra neurons in specialized ganglia, in the fused head ganglion or in the supraesophageal ganglion. Ganglia 5 and 6 (G5, G6), which contain almost

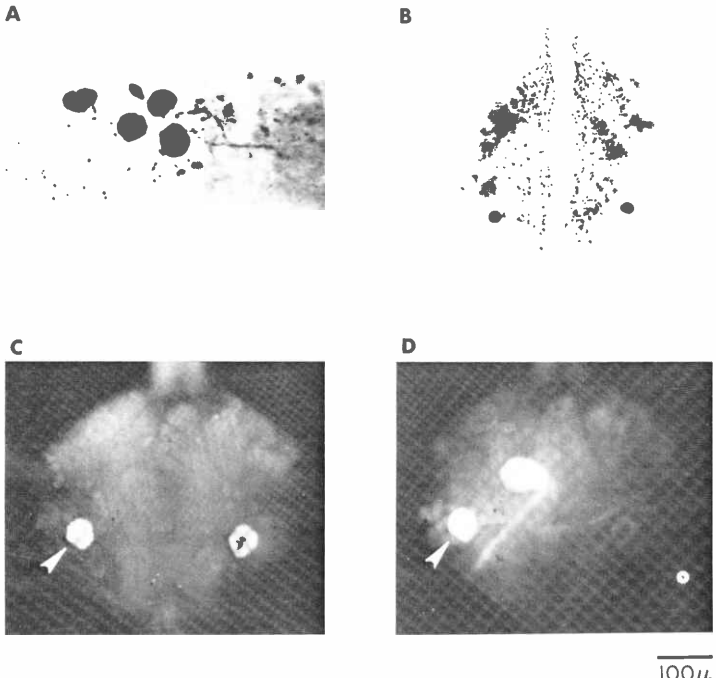

Figure 1
Micrographs of neuronal cell bodies labeled by Lan3-1 in (A) the supraesophageal ganglion, (B) a standard midbody ganglion, and (C) ganglion 6. The identical cell body that is stained immunocytochemically in C (arrow) is identified in D *through a double-labeling experiment.*

twice as many neurons as the standard midbody ganglion, are specialized to subserve reproductive function, and many of their supernumerary neurons innervate the genitalia. Lan3-1 recognizes pairs of large cells in G5 and G6 (Fig. 1C) in addition to the characteristic pair of small cells (Fig. 1B). From previous studies it is known that there is a pair of penile evertor motor neurons (PE) in G6 with the same position and axonal pattern as those stained by Lan3-1 in G6 and a homologous pair in G5 of unknown function. To demonstrate unequivocally that the large cells stained by Lan3-1 in G6 are the previously identified PE neurons, a double-labeling experiment using an injected dye was carried out. A cell body believed to be the left lateral PE cell was impaled with a microelectrode, and its identity was confirmed by its characteristic action potential (20 mV, nonovershooting) and its synaptic relationship with another major PE cell, the rostral PE motor neuron. Following electrophysiological identification, the fluorescent dye Lucifer Yellow was injected through the recording electrode (Fig. 1D). To control for the effect of Lucifer Yellow on antibody staining, the left Retzius cell in the center of the ganglion was also injected. After the ganglion was fixed, it was first incubated with hybridoma supernatant and then treated with rhodamine-conjugated rabbit anti-mouse IgG. The results illustrated in Figure 1, C and D, confirmed that the lateral PE cell contained the antigen to which the monoclonal antibody Lan3-1 binds. The same cell fluoresces yellow with

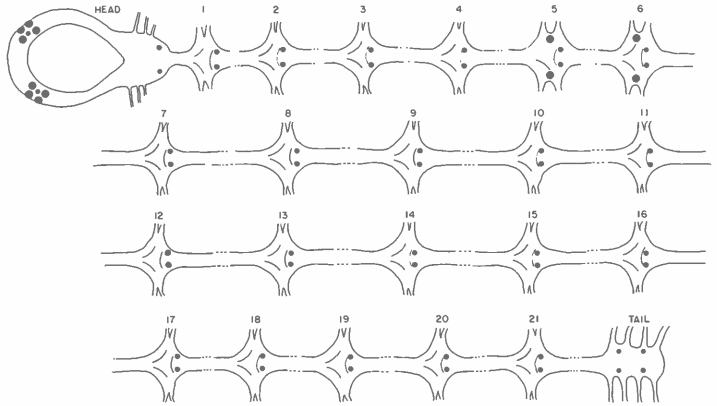

Figure 2
Diagrammatic representation of the Lan3-1 staining pattern along the entire leech nerve cord.

the intrasomatically applied marker Lucifer Yellow (Fig. 1D) and red with the rhodamine-labeled mouse antibody (Fig. 1C).

The subset of neurons labeled by Lan3-1 extends into the head and tail ganglia as well as into the supraesophageal ganglion. The head and tail ganglia contain small pairs of neurons probably analogous to the small pair of cells that regularly repeat in the midbody ganglia. In addition, several large cells occur in the supraesophageal ganglion (Figs. 1A and 2).

Other antibodies were found that labeled cell bodies of neurons in the head, supraesophageal ganglia, or the anterior ganglia but not in any of the regularly repeating midbody ganglia. An example is Lan3-9, which binds to neurons in the supraesophageal ganglion (Fig. 3A). In addition, antigenically related processes occur throughout the entire nerve cord, since fibers in the connective and nerve terminals in midbody ganglia are stained (Fig. 3B,D).

Several of the monoclonal antibodies we iso-

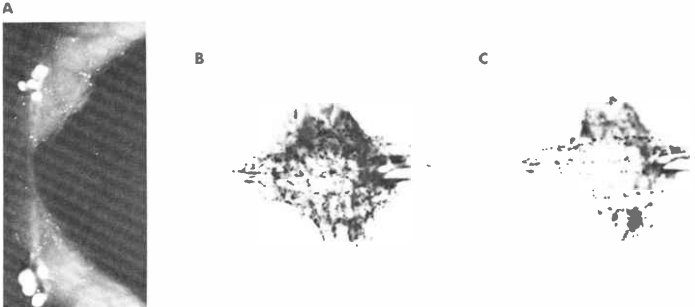

Figure 3
Staining pattern of Lan3-9.

lated bind to mechanosensory neurons present in the typical 400-neuron midbody ganglion, which selectively respond to touch, pressure, and pain in clearly defined receptive fields on the skin of the leech. The typical mechanosensory cell body sends its axons (1) into the ipsilateral roots to innervate its own ganglion's receptive field and (2) via the ipsilateral connective into neighboring ganglia to innervate partial receptive fields in adjacent body segments. These neurons have been extensively characterized electrophysiologically and through intracellular marker injections.

The four neurons responding to noxious mechanical stimuli (the nociceptive cells) all share a common antigen recognized by the monoclonal antibody Lan3-2 (Fig. 4D). This antigen is present in the cell body and all along the axonic and neuritic processes. It labels the ipsilateral projections and highlights a dense railroad-track-like pattern arising from the close packing of right and left axons running through the nerve cord. The monoclonal antibody Lan3-5 (Fig. 4C) labels the cell bodies and processes of three pairs of unidentified neurons and the two pairs of pressure cells. The pressure cells confine their axons to the ipsilateral ganglion and connective, whereas the unidentified neurons cross to the contralateral side, which allows them the possible role of integrative interneurons. The antibody Lan3-6 binds to about 26 neurons, including the pressure cells, possibly the touch cells, and unidentified neurons (Fig. 4B) in the typical posterior midbody ganglion. An additional antibody that binds to pressure cells is Lan3-7 (Fig. 4A). It is likely that these antibodies bind to different pressure-cell antigenic determinants as they stain different sets of neurons.

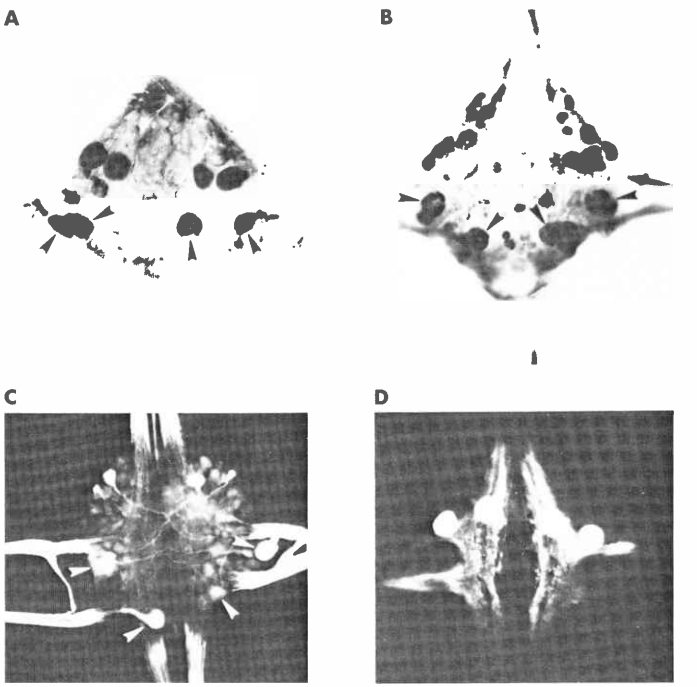

Figure 4
Mechanosensory cells stained by four different antibodies.

Opioid Peptides and Receptors

B. Zipser, G. Stefano, J. Farrar

During this past year research aimed at peptidergic network modulation was carried out largely by collaborative efforts during winter workshops and the Pain Workshop (see below). J. Farrar (Uniformed Services University of Health Sciences, Bethesda, Maryland) has assayed leech tissue for Met-enkephalin in a radioimmunoassay using an antiserum with very low cross-reactivity to Leu-enkephalin. A whole leech nerve cord contains 2–3 pmoles of Met-enkephalin-like immunoreactivity. Currently, we are using the same antiserum to localize in immunocytochemical experiments neurons carrying this chemical moiety.

Collaborative experiments with G. Stefano (Medgar Evers College, Brooklyn, NY), have provided evidence for opiate-receptor-binding sites in the leech CNS (Fig. 5). Experiments aimed at their anatomical localization are in progress in collaboration with C. Pert (NIH). In electrophysiological research, Leu-enkephalin and Met-enkephalin effects or those of their more stable analogs are now being assayed on identified neurons.

The Effect of Serotonin on the Leech Mating Behavior

E. McGlade, W. Higgins, B. Zipser

Experiments directed at the monoaminergic modulation of leech mating behavior were begun in collaboration with William Higgins (University of Maryland) during a winter workshop. They are currently being continued full time by E. McGlade and W. Higgins (University of Maryland, Baltimore). Cholinergic and serotonergic neurons are found in the vicinity of the leech penile eversion muscle (PEM), and acetylcholine introduces contractures of this muscle. Contractures of the PEM induced by either acetylcholine or electric-field stimulation are potentiated by low doses of serotonin (5×10^{-9} M). In addition, serotonin accelerates relaxation. However, serotonin by itself does not alter the resting tension. Other biogenic amines investigated (norepinephrine, epinephrine, and dopamine) do not alter the resting tension or the acetylcholine-induced response. The purpose of this investigation is to determine the basis for the change in acetylcholine responses produced by serotonin. This information would help to characterize the role of serotonin in the regulation of PEM contractility and perhaps clarify a modulatory role that amines play in intercellular communication.

To characterize the effects of serotonin, the site of action of serotonin must be determined. If the cholinergic receptor sites on the muscle are effectively blocked and serotonin still alters the events induced by electric-field stimulation, then serotonin acts on the muscle. The PEM acetylcholine receptor is atypical; it is not blocked by conventional cholinergic blocking agents (D-tubocurarine, hexomethonium, or atropine). However, benzoquinonium is a potent acetylcholine antagonist in this system (5×10^{-6} M benzoquinonium inhibits 10^{-3} M acetylcholine). Knowledge of the potent acetylcholine antagonist should facilitate further study of the site and mode of the action of serotonin.

In experiments utilizing benzoquinonium, serotonin continued to potentiate the response elicited by electric-field stimulation and accelerated relaxation. These data suggest that serotonin acts on the muscle, but they do not exclude the possibility that serotonin also acts at the cholinergic motorneurons.

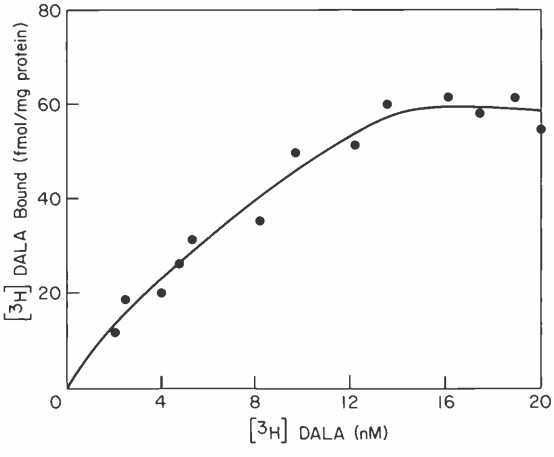

Figure 5
Opiate-receptor-binding sites demonstrated in biochemical experiments using the stable Met-enkaphalin analog (D-Ala²)-Met-Enkephalin.

Publications

McGlade, E., W. Higgins, and B. Zipser. Effect of serotonin on leech mating behavior. (In preparation.)

Zipser, B. 1980. Identification of specific leech neurons immunoreactive to enkephalin. *Nature 283:* 857.

Zipser, B. and R. McKay. 1981. Monoclonal antibodies distinguish identifiable neurons in the leech. *Nature* (in press).

Stefano, G. and B. Zipser. Opiate receptor binding in the leech. (In preparation.)

Invertebrate Neurobiology Workshop, April 3–April 30

ORGANIZER

Augustine, George J., Ph.D., University of Maryland, College Park

In 1980 the Invertebrate Neurobiology Workshop followed closely the spirit and intent of the previous year's workshop. The major emphasis was again on localized neural networks and how they may be influenced by neurohormones. This problem, although of general relevance to the function of all nervous systems, can be advantageously studied in invertebrates because of the relative simplicity of many invertebrate nervous systems and the ease with which these simple nervous networks can be isolated and maintained in vitro. Much attention was focused on the nervous system of the horseshoe crab (*Limulus*), with other work done on the leech. One novel feature of the workshop was the interest of several participants in neuropeptides, molecules that are currently receiving much attention as potential neurotransmitters or neurohormones in many nervous systems.

One avenue of study in *Limulus* involved identification of potential neurohormones and their sites of action. J. Benson, W. Watson, and G. Augustine found that the neuropeptide proctolin excited *Limulus* heart muscle and, in conjunction with R. Sullivan at the University of Hawaii, found that a proctolinlike peptide was found in the cardiac ganglion, in close association with the heart muscle. Another peptide was isolated from the *Limulus* nervous system by R. Dores; this novel peptide was found to act upon neurons of the *Limulus* cardiac ganglion in experiments by W. Watson and G. Augustine. W. Watson and G. Wyse considered the role of biogenic amines as neurohormones in *Limulus* by measuring circulating levels of amines and correlating these measurements with behavioral activity. They found that dopamine, an amine previously found in the *Limulus* nervous system, fulfilled many of the criteria required of a circulating neurohormone. The clotting reaction of *Limulus* blood cells was studied by E. McGlade, to consider how blood clotting was affected by biogenic amines that circulate in the blood.

The second series of *Limulus* projects involved characterization of the cardiac ganglion, a small neural network that is the target of several apparent neurohormones. P. Riordan and G. Augustine examined the ultrastructure of cardiac ganglion neurons to identify individual neurons, their connectivity, and content. The physiological properties of cardiac ganglion motoneurons were analyzed by D. McCulloh and G. Augustine in an attempt to determine the ionic mechanisms that permit these cells to generate rhythmic electrical activity. M. Iadarola also examined the cardiac ganglion network, determining the effects of antiepileptic drugs on cardiac ganglion electrical activity.

The possible physiological roles of opiate peptides in the leech nervous system were considered by J. Farah and B. Zipser. They used immunocytochemical techniques to discover identifiable neurons that may contain the opiate peptide Met-enkephalin.

In summary, through the use of a variety of approaches, the participants in this workshop were able to identify several roles that various neuropeptides and amines may play in regulating the activity of neural networks.

PARTICIPANTS

Benson, Jack A., Ph.D., Friedrich-Miescher Institut, Basel, Switzerland
Dores, Robert M., Ph.D., University of Colorado, Denver
Farah, John M., B.S., Uniformed Services University, Bethesda, Maryland
Iadarola, Michael J., Ph.D., Georgetown University, Washington, DC
McCulloh, David H., M.S., University of Maryland, College Park
McGlade, Ellen K., M.S., University of Maryland, College Park
Riordan, G. Patrick, M.S., Georgetown University, Washington, DC
Watson, Winsor H., Ph.D., University of New Hampshire, Durham
Wyse, Gordon A., Ph.D., University of Massachusetts, Amherst

PRESENTATIONS

Augustine, G. *Alterations in neurotransmitter release produced by the food dye Erythrosin B.*
Benson, J. *Cyclic AMP is the second messenger in the physiological response of a mollusc neuron to 5-hydroxytryptamine.*
Dores, R. *Endorphins in the lizard nervous system.*
Farah, J. *Dopaminergic inhibition of β-endorphin secretion in the rat.*
Iadarola, M. *Compartmentalization and metabolism of GABA in the mammalian central nervous system.*
McCulloh, D. *Membrane events associated with fertilization of the rabbit egg.*
Stewart, W., National Institutes of Health, Bethesda, Maryland. *Use of the fluorescent dye Lucifer Yellow in the study of neuronal structure.*

Workshop on Intracellular Staining with Lucifer Yellow, April 3–April 30

ORGANIZERS

Stewart, Walter W., B.A., NIAMDD, National Institutes of Health, Bethesda, Maryland
Keyser, Kent, Ph.D., NIAMDD, National Institutes of Health, Bethesda, Maryland

To facilitate our work on the morphology of identified neurons in isogenic lines of snails, we needed some fairly rapid ways of characterizing both the physiology and the morphology of a single cell. Two methods were developed from studies carried out jointly at Cold Spring Harbor Laboratory and the National Institutes of Health. These new methods, which we are currently using, will probably be useful to other neurophysiologists as well.

Locating Individual Neurons in the Electron Microscope by means of a Fluorescent Dye

Ordinarily, it is quite difficult to recognize a particular neuron in thin section using an electron microscope. A couple of methods have been used, but none were suitable for our purpose. We developed a novel method based on the intracellular injection of the fluorescent dye Lucifer Yellow CH.

A single live neuron is impaled with a dye-filled electrode, and the dye is ejected iontophoretically. The tissue is then fixed in an aldehyde-containing fixative and processed as usual for electron microscopy, but with one important difference. Osmium tetroxide quenches the fluorescence of Lucifer Yellow (and of almost all fluorescent compounds) and therefore cannot be used. It was possible to generate adequate contrast by treating the tissue with an acetone solution of hafnium tetrachloride in the cold. Typical results are shown in Figure 1. A giant neuron in the right parietal ganglion of the snail *B. glabrata* was injected with dye, treated as described, and thin-sectioned. The silver section, about 700 Å thick, was picked up on a hexagonal grid and viewed in a fluorescence microscope (Fig. 1A). The injected cell is immediately apparent. When the same section was viewed with the electron microscope after lead and uranium staining (Fig. 1B), the injected cell was easy to identify in the electron micrograph by correspondence with the light micrograph. Figure 1 C and D, which are details from Figure 1 A and B, respectively, show that a very precise match can be made between the two images. In the same way, very fine processes of the injected cell can be recognized in complex neuropil. Figure 1E is a further enlargement of the electron micrograph and shows that the ultrastructural preservation is quite good, although the definition of the membranous structures is not as good as when osmium postfixation is employed. It also shows the characteristic granules that occur in the soma and the processes of this particular neuron.

In Vivo Backfilling with Lucifer Yellow CH and Subsequent Physiological Characterization

Backfilling is a morphological technique for identifying the neuronal somata that send processes out a particular nerve. Unfortunately, with all previously available techniques, the cells themselves are killed by the backfilling or by the subsequent procedure for visualizing the backfilling. As a result, they cannot be characterized physiologically. We needed a technique for locating a cell body morphologically that would also be compatible with physiological characterization of the live neuron. A successful procedure was developed using a fluorescent dye. The left salivary nerve of a *B. glabrata* was cut, immersed in a pool of Lucifer Yellow CH, and backfilled using 30 nA of current for 60 minutes. Two large dye-filled cell bodies were easily seen in the buccal ganglion by their fluorescence. The two neurons were impaled with microelectrodes and shown to be electrically coupled. The electrophysiological behavior of these cells appears quite normal even though they are filled with fluorescent dye. The live preparation was then removed and photographed using epifluorescence. After photography, the cells were then impaled again and were shown to be still electrically coupled. Thus, neither the backfilling nor the photography caused detectable damage to the cells.

PARTICIPANTS

Ned Feder, M.D.; Enid Applegate, B.S.; Leslie Knipling, B.S.; Margaret Shea, B.A.; Hope Stanton, B.S.; NIAMDD, National Institutes of Health, Bethesda, Maryland

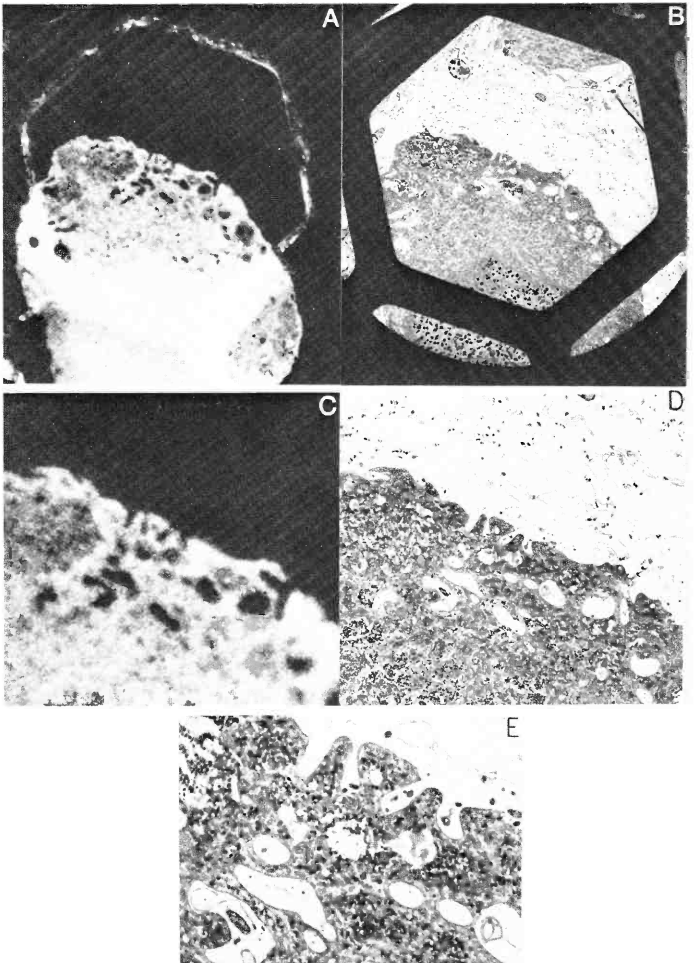

Figure 1
Light and electron micrographs of a dye-injected neuron in the right parietal ganglion of the snail B. glabrata.

Exploratory Synapse Workshop, July 13–July 14

ORGANIZERS

Byrne, John H, Ph.D., University of Pittsburgh, Pennsylvania
Meiri, Halina, Ph.D., Hebrew University Medical School, Jerusalem, Israel
Rahamimoff, Rami, M.D., Hebrew University Medical School, Jerusalem, Israel

Following the Synapse Course, a 2-day workshop was held to demonstrate experimental techniques utilized in electrophysiological analysis of synaptic transmission. Two different experimental preparations were utilized. Using abdominal ganglia of the *Aplysia*, students examined some of the basic properties of synaptic transmission and synaptic plasticity with current-voltage clamp technique. Students also examined the time- and voltage-dependence of ionic currents underlying the synaptic somatic action potential. Using the frog neuromuscular junction, the properties of miniature end-plate potentials were examined. In addition, the students voltage-clamped the muscle and examined the voltage-dependence of the end-plate current in the presence and absence of various cholinergic blocking agents.

PARTICIPANTS

Hoch, Daniel B., M.S., University of North Carolina, Chapel Hill
Kolton, Lihu, M.S., Hebrew University Medical School, Jerusalem, Israel
Lewis, Richard S., B.S., California Institute of Technology, Pasadena
Schehr, Robert S., B.A., Columbia University, New York, New York

Pain Workshop, August 1–August 28

INSTRUCTOR

Jessell, Thomas M., Ph.D., St. George's Hospital Medical School, London, England

GUEST LECTURERS

Black, I., M.D., Cornell University, New York
Kelley, D.B., Ph.D., Princeton University, New Jersey

In a continuation of the theme of the 1979 Pain Workshop, a number of varied and complementary approaches have been used to investigate the cellular mechanisms that underlie pain transmission and its regulation within the spinal cord. Studies on dissociated neurons maintained in cell culture provided the opportunity to examine the membrane properties and cellular interactions between identified sensory and central neurons. A. Mudge and M. Yamamoto used immunocytochemical techniques to identify the potential transmitters substance P and somatostatin in dorsal root ganglion neurons maintained in culture. Techniques to isolate and grow neurons from the raphe nuclei of embryonic rats were developed by M. Yamamoto, S. Hockfield, and E. Glazer, in order to examine the role of monoamine-containing neurons in the regulation of nociceptive input to the spinal cord. Using an antibody directed against synaptosomal plasma membranes from rat cortex, A. Matus was able to demonstrate both intracellular and surface staining of cultured dorsal root ganglion neurons, whereas nonneuronal cells were devoid of staining, suggesting that primary sensory neurons derived from the neural crest share common antigenic determinants with central synaptic plasma membranes. P. Hand and A. Mudge developed [^{3}H]2-deoxyglucose-labeling techniques to provide a method of detecting the activity of individual primary sensory neurons grown in culture. The electrophysiological properties of the same neurons were examined by R. McBurney and J. Kelly. Intracellular recording from individual neurons revealed that the homovanillic acid derivative capsaicin, which activates and then destroys nociceptive sensory afferents, produced a marked prolongation of sensory neuron action potentials, probably due to the inactivation of potassium channels. If these events were reproduced at the central terminals of sensory neurons, a large increase in transmitter release from nociceptive afferents might be expected. C. Pert used autoradiographic techniques to visualize opiate receptors on dorsal root ganglion neurons in culture in an attempt to compare the density of receptors on the soma and processes of individual neurons.

The use of lectins as neuronal tracers was developed by J.D. Coulter and M.A. Ruda at the 1979 Pain Workshop. J.D. Coulter, D. Katz, and T. Jessell, with E. Theriault, used the same techniques this past year to trace cutaneous and visceral sensory projections in vertebrates and, with M. Yamamoto, successfully used peroxidase double

staining to visualize the distributions of vibrissal sensory afferents and substance P within the trigeminal nucleus. In complementary studies, P. Hand and C. Hand used 2-deoxyglucose autoradiographic techniques to trace ascending sensory pathways activated by capsaicin applied to the vibrissal region.

In addition to the experimental projects outlined above, each participant presented an informal description of their own year-round research activities. I. Black visited from Cornell University and provided a detailed account of the developmental neurobiology of monoamine and peptide neurons, and D. Kelly discussed the technical advances in [^{3}H] 2-deoxyglucose labeling of single neurons initiated at Princeton University. The interaction with Birgit Zipser and Ron McKay throughout the workshop was also fundamental in generating both ideas and discussion.

PARTICIPANTS

Coulter, Joe Dan, Ph.D., Marine Biomedical Research Institute, Galveston, Texas
Glazer, Ellyn J., Ph.D., University of California Medical School, San Francisco
Hand, Carol, B.S., University of Pennsylvania, Philadelphia
Hand, Peter J., Ph.D., University of Pennsylvania, Philadelphia
Hockfield, Susan, Ph.D., University of California, San Francisco
Katz, David M., Ph.D., State University of New York, Stony Brook
Kelly, John S., M.D., Ph.D., St. George's Hospital Medical School, London, England
Matus, Andrew I., Ph.D., Friedrich Miescher Institut, Basel, Switzerland
McBurney, Robert N., Ph.D., University of Newcastle-upon-Tyne, England
Mudge, Anne W., Ph.D., Harvard Medical School, Boston, Massachusetts
Pert, Candace B., Ph.D., National Institutes of Health, Bethesda, Maryland
Theriault, Elizabeth, B.S., McMaster University, Ontario, Canada
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Perfusion Workshop, August 12–August 22

ORGANIZER

Horn, Richard, Ph.D., Yale University, New Haven, Connecticut

INSTRUCTORS

Byerly, William L., Ph.D., University of California, Los Angeles
Horn, Richard, Ph.D., Yale University, New Haven, Connecticut
Lee, Kai S., Ph.D., Yale University, New Haven, Connecticut
Reuter, Harald, M.D., Pharmakologisches Institut, Bern, Switzerland
Thompson, Stuart, Ph.D., Hopkins Marine Station, Pacific Grove, California

In recent years, many different scientists have developed methods that permit them to vary, in a controlled manner, the intracellular content of isolated cells. Although such manipulations had been possible in cylindrical structures (such as the squid giant axon) for some time, the ability to perform such experiments on spherical structures is quite a recent development. First accomplished by Kostuk's group (Bogomoletz Institute) in snail neurons, similar techniques were later developed by other scientists, each of whom made his own personal modification.

The main objective of the Perfusion Workshop was to allow a critical comparison of the variations of the internal perfusion method and, at the same time, to train other scientists so that the technique could become more generally available. In the workshop, molluscan neurons were used as the experimental preparation. The students moved from one setup to the next, learning from each of the five "innovators" the technique that he had developed. Following this period of "training," the students did a research project with one of the instructors. These projects centered either on a technical problem or on the use of the internal perfusion technique for better understanding a physiological phenomenon.

The experimental sessions were complemented by extensive discussions in which the different techniques were compared and evaluated, and in which the limitations of the techniques were brought to light. J. Stern (Brandeis University) gave a seminar describing the use of the technique for the study of cells of very small dimensions.

The extent to which the instructors continued to modify their own setups as a function of what they learned from one another made it very clear that the workshop was as useful for them as it was for the "uninitiated," who returned to their own labs with a new and important experimental tool for studying membrane functions.

PARTICIPANTS

Ascher, Phillipe, Ph.D., Ecole Normale Superieure, Paris, France
Ashcroft, Fran, Ph.D., University of Leicester, England
de Peyer, Jacques, Ph.D., Pharmakologisches Institut, Bern, Switzerland
Huang, Mae, Ph.D., National Institutes of Health, Bethesda, Maryland
Junge, Douglas, Ph.D., University of California, Los Angeles
Kehoe, JacSue, Ph.D., Ecole Normale Superieure, Paris, France
Lewis, Richard, B.S., California Institute of Technology, Pasadena
Moody, Bill, Ph.D., University of California, Los Angeles
Stimers, Joseph R., Ph.D., University of Southern California, Los Angeles
Tillotson, Douglas, Ph.D., Boston University Medical School, Massachusetts

45th COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Movable Genetic Elements, May 28—June 4

In the early 1950s, Barbara McClintock's analysis of crosses between genetically marked corn plants led her to postulate that the activity of key genes was under the control of genetic elements that had the capacity to move from one chromosomal site to another. She called them "controlling elements," noting that when one was inserted next to a gene it inhibited that gene's activity. Conversely, when a control element moved away from a gene, the activity of that gene suddenly reappeared. Movement of control elements thus results in abrupt phenotypic changes, and, because of their high mutability, the respective genes they affect were first erroneously thought to be basically different from ordinary genes.

The idea that genetic elements could move with such facility from one chromosomal site to another flew strongly in the face of conventional genetic wisdom. Confirmation in other organisms thus had to occur before the far-reaching consequences of the corn plant message became generally appreciated.

Most important were the independent observations in the late 1960s of Jim Shapiro and Peter Starlinger that certain highly pleiotropic mutations in *E. coli* were the result of the insertion of descrete DNA segments (insertion sequences or IS's) that had the capacity to jump from one chromosomal site to another. Molecular characterization of these elements became possible soon afterward with the arrival of the restriction enzymes and the recombinant DNA cloning procedures. Quickly it became obvious that IS-like elements were to be found not only in bacteria, but perhaps in all organisms. Equally important was the discovery that closely spaced pairs of IS's can move as units ("transposons") carrying along the genes lying between them. Such transposons bore many similarities with the phage Mu, leading Ahmad Bukhari, Jim Shapiro, and Sankar Adhya to convene a meeting on DNA insertion elements at Cold Spring Harbor in May of 1976. With this meeting, IS elements and transposons moved to the center of the genetic world, commencing a frenzy of experimentation that has shown no sign of abatement.

Choosing "Movable Genetic Elements" as our 1980 Symposium topic was a virtually unavoidable decision, one that became even more appropriate after the invitations went out, when rumors began that the structures of integrated retroviruses were remarkably similar to those of transposons. So our most able Symposium organizers, Ahmad Bukhari and Jim Hicks, had great difficulty in keeping the number of presentations within the bounds of sanity. In doing so, they received invaluable advice from Sidney Brenner, Peter Day, Harrison Echols, J. R. S. Fincham, Walter Gehring, Mel Green, Ira Herskowitz, Lee Hood, Amar Klar, Phil Leder, Barbara McClintock, Howard Nash, Heinz Saedler, Jim Shapiro, Peter Starlinger, Jeff Strathern, and Bob Weisberg.

We were obviously most pleased that Barbara McClintock, who typically not wanting to give a

formal presentation, nevertheless gave an informal summary of her latest ideas. And most appropriately on the opening night, Bentley Glass briefly recapitulated many of Barbara's key contributions to genetics.

The program contained 103 scheduled presentations and 14 informal presentations. The total attendance at this Symposium was 302. That we could invite so many participants reflects the substantial financial support again provided by the National Institutes of Health, the National Science Foundation, and the Department of Energy. Because of the rapidly escalating air fares, we needed still additional help, and we wish to acknowledge major support from the Cetus Corporation, Bethesda Research Laboratories, and New England BioLabs.

Welcoming Remarks: J. D. WATSON, Cold Spring Harbor Laboratory

Introduction: H. BENTLEY GLASS, State University of New York, Stony Brook: Movable Genetic Elements and Barbara McClintock

Opening Address: A. CAMPBELL, Stanford University, California

Session 1: Inversion Elements in Bacteria

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

T. IINO and K. KUTSUKAKE, Dept. of Biology, Faculty of Science, University of Tokyo, Japan: The *trans*-acting genes of bacteriophages P1 and Mu mediating inversion of a specific DNA segment involved in flagellar phase variation of *Salmonella*.

M. SILVERMAN, J. ZIEG, and M. SIMON, Biology Dept., University of California, San Diego: The mechanism of phase variation.

R. HARSHEY, Cold Spring Harbor Laboratory, New York: Flip-flop control of gene expression in bacteriophage Mu.

Session 2: Characterization of Transposable Element Families

Chairperson: H. SAEDLER, University of Freiburg, Federal Republic of Germany

J. GAFNER, H. EIBEL, M. BRENNAN, A. STOTZ, and P. PHILIPPSEN, Dept. of Microbiology, Biozentrum, University of Basel, Switzerland: Characterization of a mobile element in yeast.

G. M. RUBIN, W. J. BROREIN, JR., P. DUNSMUIR, R. LEVIS, E. STROBEL, and E. YOUNG, Dept. of Biological Chemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: "*Copia*-like" transposable elements in the *Drosophila* genome.

H. E. SCHWARTZ and M. W. YOUNG, Rockefeller University, New York, New York: Expression of middle repetitive DNA in *Drosophila melanogaster*.

N. A. TCHURIKOV,[1] E. S. ZELENTSOVA,[1] Y. V. ILYIN,[1] E. V. ANANIEV,[2] and G. P. GEORGIEV,[1] [1]Institute of Molecular Biology and [2]Institute of Molecular Genetics, USSR Academy of Sciences, Moscow: Mobile dispersed genes in *Drosophila melanogaster*.

E. O. LONG and I. B. DAWID, Laboratory of Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: Structure and expression of type-2 ribosomal DNA insertions in *Drosophila melanogaster*.

J. K. LIM, Biology Dept., University of Wisconsin, Eau Claire: Restrictive modifications of the *Drosophila* X chromosome.

Session 3: Genetic Effects of Transposition in Drosophila *and Yeast*

Chairperson: P. STARLINGER, University of Köln, Federal Republic of Germany

G. ISING and K. BLOCK, Institute of Genetics, University of Lund, Sweden: Derivation-dependent distribution of insertion sites for a *Drosophila* transposon.

B. RASMUSON, Dept. of Genetics, University of Umea, Sweden: A case of gene regulation in *Drosophila melanogaster* associated with the dispersed gene family Dm plasmid 225.

H. GREER, M. IGO, and F. DEBRUIJN, Biology Dept., Harvard University, Cambridge, Massachusetts: A yeast transposable element which carries the *his4C* gene.

G. R. FINK, D. CHALEFF, T. DONAHUE, P. FARABAUGH, S. SILVERMAN, and S. ROEDER, Dept. of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Unusual genetic events associated with a transposable element in yeast.

S. SCHERER and R. W. DAVIS, Dept. of Biochemistry, Stanford University School of Medicine, California: Recombination and expression of DNA sequences in or near a yeast transposable element.

F. SHERMAN, J. I. STILES, B. ERREDE, T. S. CARDILLO, L. FRIEDMAN, and S. CONSAUL, Dept. of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, New York: Overproduction of isocytochromes c due to transposable rearrangements in mutants of yeast.

Session 4: Mechanisms of DNA Transposition in Bacteria. I.

Chairperson: J. SHAPIRO, University of Chicago, Illinois

W. ARBER, S. IIDA, and H. J. REIF, Dept. of Microbiology, Biozentrum der Universität Basel, Switzerland: Biology o transposable elements in *E. coli*.
R. KLAER, S. KÜHN, P. HABERMANN, D. PFEIFER, E. TILLMANN, I. ST. GIRONS, H.-J. FRITZ, and P. STARLINGER, Institut für Genetik, Universität Köln, Federal Republic of Germany: The transposition of IS*4*.
D. F. GRINDLEY and C. M. CLARKE, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Structure and function of the transposon, Tn*903*.
M. S. GUYER and J. L. ROSNER, Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland: The functional components of Tn*9*.

Session 5: Mechanisms of DNA Transposition in Bacteria. II.

Chairperson: N. ZINDER, Rockefeller University, New York, New York

S. ROTHSTEIN, R. JORGENSEN, J. YIN, Z. YONG-DI, R. JOHNSON, and W. REZNIKOFF, Dept. of Biochemistry, University of Wisconsin, Madison: Genetic organization of Tn*5*—The inverted repeats are different.
E.-A. AUERSWALD and H. SCHALLER, Dept. of Microbiology, University of Heidelberg, Federal Republic of Germany: Structural analysis of transposon Tn*5* and of its imprecise excision.
D. E. BERG, C. EGNER, B. J. HIRSCHEL, and T. D. TLSTY, Dept. of Microbiology, Washington University Medical School, St. Louis, Missouri: A mobile recombinational switch derived from transposon Tn*5* (Km^r).
D. BIEK and J. R. ROTH, Dept. of Biology, University of Utah, Salt Lake City: Regulation of Tn*5* transposition.

Session 6: Mechanisms of DNA Transposition in Bacteria. III.

Chairperson: S. COHEN, Stanford University, California

N. KLECKNER,[1] S. M. HALLING,[1] and T. J. FOSTER,[2] [1]The Biological Laboratories, Harvard University, Cambridge, Massachusetts; [2]Moyne Institute, Trinity College, Dublin, Ireland: The tetracycline-resistance transposon Tn*10*.
J. H. MILLER, M. P. CALOS, M. HOFER, and D. GALAS, Dept. of Molecular Biology, University of Geneva, Switzerland: Genetic and sequence analysis of transpositions in the *lac* region of *E. coli*.
H. SAEDLER, J. CULLUM, P. NEVERS, B. SCHUMACHER, and H. SOMMER, Institute für Biologie III, University of Freiburg, Federal Republic of Germany: IS*1*-induced deletions and inversions.
G. B. SMIRNOV, T. S. ILYINA, Y. M. ROMANOVA, A. P. MARKOV, and E. V. NECHAEVA, Gamaleya Institute for Epidemiology and Microbiology, Moscow, USSR: Mutants of *E. coli* affected in the processes of transposition and genomic rearrangements.
N. DATTA, M. NUGENT, and H. RICHARDS, Bacteriology Dept., Royal Postgraduate Medical School, London, England: Transposons in medically important bacteria.
M. SO, Cold Spring Harbor Laboratory, New York: Studies on the mechanism of dissemination of a pathogenic determinant of enterotoxigenic *E. coli*.
R. NOVICK,[1] E. MURPHY,[1] S. KHAN,[1] and J. KROLEWSKI,[2] [1]Public Health Research Institute; [2]New York University School of Medicine, New York: Hitchhiking transposons and other site-specific recombination systems in *Staphylococcus aureus*.
M. CHANDLER, M. CLERGET, and L. CARO, Dept. of Molecular Biology, University of Geneva, Switzerland: IS*1*-promoted events associated with drug-resistance plasmids.

Session 7: Controlling Elements and Unstable Genes in Plants

Chairperson: G. FINK, Cornell University, Ithaca, New York

P. A. PETERSON, Agronomy Dept., Iowa State University, Ames: Diverse expression of controlling element components in maize—Test of a model.
H. K. DOONER, Dept. of Genetics, University of Wisconsin, Madison: Effects of the controlling element *Ds* on *Bz*-gene function in maize.
B. BURR and F. A. BURR, Biology Dept., Brookhaven National Laboratory, Upton, New York: Detection of changes in maize DNA at the *Shrunken* locus due to the intervention of *Ds* elements.
F. SALAMINI, Istituto sperimentale per la Cerealicoltura, Sezione di Bergamo, Italy: Controlling elements and insertion mutations at the *opaque-2* locus of maize.
G. R. K. SASTRY, K. M. ASLAM, and V. JEFFRIES, Dept. of Genetics, University of Leeds, England: The role of controlling elements in the instability for flower color in *Antirrhinum majus* and *Impatiens balsamina*.
R. FLAVELL, M. O'DELL, J. R. BEDBROOK, and J. HUTCHINSON, Plant Breeding Institute, Trumpington, Cambridge, England: Evidence for and the role of sequence translocation during the evolution of plant chromosomes.
R. J. MANS,[1] B. D. KIM,[1] M. F. CONDE,[2] D. R. PRING,[2] C. S. LEVINGS III,[3] S. J. GABAY-LAUGHNAN,[4] and J. R. LAUGHNAN,[4] [1]Dept. of Biochemistry and Molecular Biology; [2]Department of Plant Pathology, University of Florida, Gainesville; [3]Genetics Department, North Carolina State University, Raleigh; [4]Dept. of Genetics and Development, University of Illinois, Urbana: Transposition events in maize mitochondrial DNA.

B. Decaris, F. Francou, A. Kouassi, C. Lefort, and G. Rizet, Laboratoire de Génétique, Université Paris-Sud, Orsay, France: Genetic instability in *Ascobolus immersus*—Modalities of back-mutations, intragenic mapping of unstable sites, and unstable insertion. Preliminary biochemical data.

Session 8: Mechanisms of DNA Transposition in Bacteria. IV.

Chairperson: S. Brenner, Medical Research Council, Cambridge, England

G. Chaconas, R. Harshey, N. Sarvetnick, and A. I. Bukhari, Cold Spring Harbor Laboratory, New York: Molecular mechanism of transposition.

N. Symonds, A. Coelho, D. Leach, and S. Maynard-Smith, School of Biology, University of Sussex, Brighton, England: Transposition studies with phage Mu.

D. Kamp and R. Kahmann, Max-Planck-Institut für Biochemie, München, Federal Republic of Germany: Two pathways in bacteriophage Mu transposition?

M. M. Howe and J. W. Schumm, Dept. of Bacteriology, University of Wisconsin, Madison: Transposition of bacteriophage Mu—Properties of lambda phages containing both ends of Mu.

P. van de Putte, M. Giphart-Gassler, N. Goosen, T. Goosen, and E. van Leerdam, Laboratory of Molecular Genetics, University of Leiden, The Netherlands: The integration and replication of multicopy plasmids containing different fragments of bacteriophage Mu.

L. Desmet,[1] M. Faelen,[1] N. Lefebvre,[3] A. Resibois,[2] A. Toussaint,[1] and F. Van Gijsegem,[1] [1]Laboratoire de Génétique, [2]Laboratoire de Microscopie Electronique, [3]Laboratoire de Microbiologie, Université Libre de Bruxelles, Belgium: Mu-induced chromosomal rearrangements in enterobacteria.

E. Piruzian, V. Andrianov, M. Mogutov, E. Krivtsova, V. Yuzeeva, and A. Vetoshkin, Institute of Molecular Genetics, Moscow, USSR: Specificity of bacteriophage Mu integration in DNA from different sources.

Session 9: Organization of Immunoglobulin Genes

Chairperson: P. Leder, NICHHD, National Institutes of Health, Bethesda, Maryland

S. Tonegawa, H. Sakano, R. Maki, W. Roder, A. Traunbacher, and Y. Kurosawa, Basel Institute for Immunology, Switzerland: Somatic recombination and differential RNA splicing during lymphocyte differentiation.

P. Leder, E. Max, J. Seidman, and P. Hieter, Laboratory of Molecular Genetics, NICHHD, National Institutes of Health, Bethesda, Maryland: Organization and reorganization of immunoglobulin genes.

T. H. Rabbitts, D. L. Bentley, G. E. A. R. Matthyssens, Medical Research Council Laboratory of Molecular Biology, Cambridge, England: Multiple organizational changes during the differentiation of immunoglobulin genes.

R. Wall,[1] E. Choi,[1] M. Keuhl,[2] and J. Rogers,[1] [1]Molecular Biology Institute, University of California, Los Angeles; [2]Dept. of Microbiology and Immunology, University of Virginia School of Medicine, Charlottesville: DNA rearrangements and RNA processing in immunoglobulin-gene expression.

L. Hood, K. Calame, S. Crews, M. Davis, P. Early, S. Kim, H. Huang, T. Hunkapiller, D. Livant, K. Moore, and B. Taylor, Division of Biology, California Institute of Technology, Pasadena: The organization and rearrangement of immunoglobulin heavy-chain genes.

K. B. Marcu, N. Arnheim, J. Banerji, N. A. Penncavage, R. Lang, P. Seperack, and R. Miesfeld, Biochemistry Dept., State University of New York, Stony Brook: Studies on the nature and instability of DNA sequences flanking the mouse immunoglobulin heavy-chain constant-region genes.

F. R. Blattner,[1] J. L. Slightom,[1] N. Newell,[1] J. Richards,[1] G. Goldberg,[1] C.-P Liu,[1] P. W. Tucker,[2] K. Marcu,[3] and J. F. Mushinski,[4] [1]Dept. of Genetics, University of Wisconsin, Madison; [2]University of Mississippi Medical Center, Jackson; [3]State University of New York, Stony Brook; [4]National Institutes of Health, Bethesda, Maryland: Studies on the structure of the heavy-chain locus of the mouse.

T. Honjo, T. Kataoka, Y. Yamawaki-Kataoka, M. Obata, N. Takahashi, T. Kawakami, Y. Yaoita, and A. Shimizu, Dept. of Genetics, Osaka University Medical School, Japan: Deletion of immunoglobulin heavy-chain genes accompanies the class switch recombination.

R. P. Perry, C. Coleclough, and M. Weigert, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Reorganization and expression of immunoglobulin genes—The status of allelic elements.

Session 10: Movable Elements in Differentiation

Chairperson: I. Herskowitz, University of Oregon, Eugene

P. Borst,[1] A. C. C. Frasch,[1] A. Bernards,[1] J. H. J. Hoeijmakers,[1] and G. A. M. Cross,[2] [1]Laboratory of Biochemistry, University of Amsterdam, The Netherlands; [2]The Wellcome Research Laboratories, Beckenham, Kent, England: Antigenic variation in trypanosomes involves rearrangements in nuclear DNA.

R. O. Williams,[1] K. B. Marcu,[2] J. R. Young,[1] and P. A. O. Majiwa,[1] [1]International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya; [2]Dept. of Biochemistry, State University of New York, Stony Brook: Contextural genomic rearrangements of variant antigen genes in *Trypanosoma brucei*.

S. Longacre,[1] U. Hibner,[1] T. Baltz,[2] and H. Eisen,[1] [1]Unité d'Immunoparasitologie, Institut Pasteur, Paris, France; [2]Université de Bordeaux, France: Antigenic variation in *T. equiperdum*.

P. Nisen and L. Shapiro, Albert Einstein College of Medicine, Bronx, New York: Insertion element nucleotide sequences in precursor and mature *E. coli* ribosomal RNA and cell-cycle-associated rearrangement of *Caulobacter crescentus* inverted-repeat DNA.

J. Rine, D. Hagen, R. Jensen, G. Sprague, Jr., and I. Herskowitz, Institute of Molecular Biology and Dept. of Biology, University of Oregon, Eugene: The switching pattern of homothallic yeast and its control.

K. A. Nasmyth,[1] K. Tatchell,[1] B. D. Hall,[1] C. Astell,[2] and M. Smith,[2] [1]Dept. of Genetics, University of Washington, Seattle; [2]Dept. of Biochemistry, University of British Columbia, Vancouver: The transcripts made at the yeast mating-type locus.

A. Klar,[1] J. Hicks,[1] H. Strathern,[1] and J. Broach,[2] [1]Cold Spring Harbor Laboratory, New York; [2]State University of New York, Stony Brook: Mechanism of transposition and transcriptional regulation of the mating-type genes in yeast.

J. Haber, B. Weiffenbach, D. Rogers, and J. McCusker, Dept. of Biology, Brandeis University, Waltham, Massachusetts: Chromosomal rearrangements accompanying yeast mating-type conversions.

Session 11: Biochemistry of Recombination and λ Integration

Chairperson: F. Stahl, University of Oregon, Eugene

H. Potter,[1] D. Dressler,[1] C. DasGupta,[2] T. Shibata,[2] R. Cunningham,[2] and C. Radding,[2] [1]Harvard University, Cambridge, Massachusetts; [2]Yale University, New Haven, Connecticut: Homologous pairing in genetic recombination.

N. R. Cozzarelli, P. O. Brown, A. Morrison, K. N. Kreuzer, and R. Otter, University of Chicago, Illinois: The shaping of DNA by *E. coli* topoisomerases.

M. Gellert, L. M. Fisher, K. Mizuuchi, and M. H. O'Dea, Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland: Studies on the mechanism of DNA gyrase.

H. Ikeda, K. Moriya, and T. Matsumoto, Institute of Medical Science, University of Tokyo, Japan: Studies of illegitimate recombination—In vitro insertion of plasmid DNA into phage λ genome.

H. A. Nash, NIMH, National Institutes of Health, Bethesda, Maryland: λ integration—Genes and proteins.

R. Weisberg,[1] C. Foeller,[2] L. Enquist,[1] and A. Landy,[2] [1]National Institutes of Health, Bethesda, Maryland; [2]Division of Biology and Medicine, Brown University, Providence, Rhode Island: λ integration—Exchange of genetic information between virus and host.

K. Mizuuchi,[1] M. Mizuuchi,[1] P.-L. Hsu,[2] W. Ross,[2] and A. Landy,[2] [1]Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland; [2]Division of Biology and Medicine, Brown University, Providence, Rhode Island: The λ phage *att* sites—Interaction with Int protein, functional limits, and the crossover event.

H. I. Miller,[1] J. Abraham,[1] B. Benedik,[2] A. Campbell,[2] D. Court,[3] H. Echols,[1] R. Fischer,[1] G. Guarneros,[4] D. Mascarenhas,[2] and D. Schindler,[1] [1]University of California, Berkeley; [2]Stanford University, California; [3]NCI, National Institutes of Health, Bethesda, Maryland; [4]Centro de Investigacion, Mexico City, Mexico: Regulation of the integration-excision reaction by bacteriophage λ.

Session 12: Mechanisms of DNA Transposition in Bacteria. V.

Chairperson: W. Szybalski, University of Wisconsin, Madison

F. Heffron,[1] C. Morita,[1] R. E. Gill,[2] and S. Falkow,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Microbiology and Immunology, University of Washington, Seattle: Studies on the internal resolution site (IRS) of Tn*3*.

L. A. MacHattie, C. J. Muster, J. A. Shapiro, and D. M. Shah, Dept. of Microbiology, University of Chicago, Illinois: Origin and breakdown of replicon fusion cointegrates mediated by transposable elements.

M. J. Casadaban, J. Chou, P. G. Lemaux, C. A. Miller, C.-P. D. Tu, and S. N. Cohen, Stanford University School of Medicine, California: Tn*3*—Mechanism and control of transposition.

D. Sherratt and A. Arthur, Dept. of Genetics, University of Glasgow, Scotland: Transposon-specified site-specific recombination systems.

E. Ohtsubo, M. Zenilman, H. Ohtsubo, M. McCormick, C. Machida, and Y. Machida, Dept. of Microbiology, State University of New York, Stony Brook: Mechanisms of plasmid cointegration mediated by translocatable DNA elements.

N. Sternberg, D. Hamilton, M. Yarmolinsky, S. Austin, and R. Hoess, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: Site-specific recombination and the P1 plasmid way of life.

L. J. Wallace, J. M. Ward, P. M. Bennett, M. K. Robinson, and M. H. Richmond, Dept. of Bacteriology, University of Bristol, England: Transposition immunity.

R. Schmitt,[1] J. Altenbuchner,[1] K. Wiebauer,[1] W. Arnold,[2] A. Puhler,[2] and F. Schöffl,[2] [1]Lehrstuhl für Genetick, Universität Regensburg; [2]Institut für Mikrobiologie, Universität Erlangen-Nürnberg, Federal Republic of Germany: Basis of transposition and gene amplification by Tn*1721* and related Tc transposons.

Session 13: Virus as a Movable Element

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

K. R. Yamamoto, V. L. Chandler, J. C. Ring, and D. S. Ucker, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Mammary tumor virus DNA—A movable genetic element that specifies hormone-regulated transcription.

G. F. Vande Woude,[1] D. G. Blair,[2] R. Dhar,[1] L. Enquist,[1] W. L. McClements,[1] and M. Oskarsson,[1] Laboratory of [1]Molecular Virology and [2]Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: Properties of the terminal-repeat sequence of integrated Moloney sarcoma provirus.

J. G. Sutcliffe, T. M. Shinnick, and R. A. Lerner, Scripps Clinic and Research Foundation, La Jolla, California: Moloney leukemia virus is a transposon—Nucleotide sequence analysis identifies genes and replication details.

D. Baltimore, C. Shoemaker, S. Goff, M. Paskind, E. Gilboa, and S. Mitra, Massachusetts Institute of Technology, Cambridge: Structure of cloned retrovirus circular DNA—Implications for viral integration.

H. M. Temin and K. Shimotohno, McArdle Laboratory, University of Wisconsin, Madison: Similarity of nucleotide sequences of cell-virus junctions of an avian retrovirus and of transposable elements.

J. Majors, R. Swanstrom, S. Hughes, W. DeLorbe, A. P. Czernilofsky, J. M. Bishop, and H. E. Varmus, Dept. of Microbiology, University of California, San Francisco: Structural and functional homologies between retroviral proviruses and transposable elements.

M. Botchan,[1] S. Conrad,[1] P. Bullock,[1] W. Gish,[1] D. Hanahan,[2] and Y. Gluzman,[2] [1]University of California, Berkeley; [2]Cold Spring Harbor Laboratory, New York: Excision of SV40 sequences from cellular chromosomes.

R. Sager, A. Anisowicz, and N. Howell, Sidney Farber Cancer Institute, Cambridge, Massachusetts: DNA rearrangements in SV40-transformed mouse cell lines, hybrids, and cybrids.

Session 14: Arrangements of Clustered and Dispersed Gene Families

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

J. R. Bedbrook,[1] J. Jones,[2] and R. Flavell,[2] [1]Division of Plant Industry, CSIRO, Canberra, Australia; [2]Plant Breeding Institute, Cambridge, England: Evidence for nonhomology-dependent recombination in the evolution of repeated sequences in *Secale* species.

E. F. Fritsch, C . K. J. Shen, R. M. Lawn, and T. Maniatis, Division of Biology, California Institute of Technology, Pasadena: The organization of repetitive sequences in mammalian globin-gene clusters.

B. de Crombrugghe, G. Vogeli, H. Ohkubo, V. E. Avvedimento, M. Mudryj, and I. Pastan, National Institutes of Health, Bethesda, Maryland: Repetitive structure in the chick α2-collagen gene.

R. T. Schimke, P. C. Brown, C. Simonsen, M. McGrogan, D. P. Setzer, D. Slate, and G. Crouse, Dept. of Biological Sciences, Stanford University, California: Organization and localization of dihydrofolate-reductase-gene sequences in methotrexate-resistant cultured cells and in the mouse genome.

M. Carlson, B. Osmond, and D. Botstein, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The *SUC* genes of yeast—A dispersed multigene family of known function.

L. Singh, I. F. Purdom, and K. W. Jones, Institute of Animal Genetics, University of Edinburgh, Scotland: Conserved sex-chromosome-associated transposable nucleotide sequences in eukaryotes.

K. Saigo,[1] L. Millstein,[2] and C. A. Thomas,[2] [1]Scripps Clinic and Research Foundation, La Jolla, California; [2]Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan: The reshuffling of the histone-gene cluster in *Drosophila melanogaster* and the insertion of a mobile element.

Session 15: Recombination of Heterologous Genomes

Chairman: H. Lewis, National Science Foundation, Washington, DC

J. Collins, Gesellschaft für Biotechnologische Forschung, Braunschweig-Stöckheim, Federal Republic of Germany: On the instability of palindromic DNA in *E. coli*—Use as a method of super-high-frequency site-specific mutagenesis.

M. Wigler, M. Perucho, D. Levy, and D. Hanahan, Cold Spring Harbor Laboratory, New York: DNA-mediated gene transfer in animal cells—Theory and applications.

G. B. Ruvkun and F. M. Ausubel, Dept. of Biology, Harvard University, Cambridge, Massachusetts: Replacement of nitrogen fixation *(nif)* genes from *Rhizobium meliloti* with Tn*5*-mutagenized, cloned homologous *nif* genes.

M. Van Montagu,[1] M. De Beuckeleer,[1] M. Lemmers,[1] M. Holsters,[1] P. O'Farrell,[2] A. Depicker,[1] P. Dhaese,[1] G. Engler,[1] J. P. Hernalsteens,[1] and J. Schell,[1] [1]Depts. of Genetics and Histology, State University Gent, Belgium; [2]Dept. of Biophysics and Biochemistry, University of California, San Francisco: T-DNA—A transferrable element of the Ti plasmids of *Agrobacterium tumefaciens*.

Summary: M. Yarmolinsky, Frederick Cancer Research Center, Frederick, Maryland

SUMMER MEETINGS

Biology of the Vascular Endothelial Cell, May 6—May 11

Arranged by
Stephen Blose, *Cold Spring Harbor Laboratory, New York*
Eric Jaffe, *Cornell University Medical College, New York, New York*
Denis Gospodarowicz, *University of California, San Francisco*

105 participants

Opening Remarks: S. H. Blose, Cold Spring Harbor Laboratory, New York

Session 1: Cell Structure and Motility

Chairperson: S. Blose, Cold Spring Harbor Laboratory, New York

S. H. Blose, Cold Spring Harbor Laboratory, New York: Functions of 10-nm filaments in vascular endothelial cells.

A. I. Gotlieb,[1] W. Spector,[1] L. Subrahmanyan,[2] and V. I. Kalnins,[2] [1]Dept. of Pathology; [2]Dept. of Anatomy, University of Toronto, Ontario, Canada: Cytoskeleton and cell movement in cultured endothelial and smooth-muscle cells.

R. R. Bürk,[1] P. Clopath,[2] and K. Müller,[2] [1]Friedrich Miescher-Institut, Basel, Switzerland; [2]Research Dept., Pharmaceuticals Division, Ciba-Geigy Ltd., Basel, Switzerland: An inhibitor of endothelial-cell migration.

B. R. Zetter, C. J. Scheiner, and M. Klagsbrun, Harvard Medical School and Children's Hospital Medical Center, Boston, Massachusetts: Quantitative analysis of capillary endothelial-cell migration.

E. Bogenmann and B. Sordat, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Characterization of primary and cloned bovine endothelial cells grown on collagen gels.

I. Hüttner,[1] P. MoCostabella,[2] C. de Chastonay,[2] and G. Gabbiani,[2] [1]Dept. of Pathology, McGill University, Montreal, Quebec, Canada; [2]Dept. of Pathology, University of Geneva, Switzerland: Organization of cell junctions in rat aortic endothelium during normal and hypertensive states.

U. S. Ryan and J. W. Ryan, Dept. of Medicine, University of Miami School of Medicine, Florida: Morphological interactions between pulmonary endothelial cells.

Session 2: Growth Factors. I.

Chairperson: D. Gospodarowicz, University of California Medical Center, San Francisco

R. A. Brown,[1] J. B. Weiss,[1] S. Kumar,[2] and P. Phillips,[2] [1]Dept. of Rheumatology, Manchester University, England; [2]Dept. of Paediatric Oncology, Christie Hospital, Manchester, England: A low-molecular-weight endothelial-cell-stimulating angiogenesis factor (ESAF) from synovial fluids of diseased articular joints.

S. Watt, K. Wallis, and A. Fenselau, Dept. of Physiological Chemistry, Johns Hopkins University, Baltimore, Maryland: Partial purification of an endothelial-cell-growth stimulatory factor from Walker 256 rat tumor.

A. M. Schor,[1] S. L. Schor,[2] J. B. Weiss,[3] R. A. Brown,[3] S. Kumar,[1] and P. Phillips,[1] [1]Clinical Research Laboratory, [2]CRC Dept. of Medical Oncology, Christie Hospital, Manchester, England; [3]Dept. of Rheumatology and Medical Biochemistry, University of Manchester, England: Collagen substratum required for the stimulation of capillary endothelial cells in vitro by a low-molecular-weight angiogenic factor.

R. S. Langer,[1,2] H. Conn,[2] J. Vacanti,[2] and J. Folkman,[2,3] [1]Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Surgery, Children's Hospital Medical Center, Boston, Massachusetts; [3]Dept. of Surgery, Harvard Medical School, Boston, Massachusetts, Inhibition of tumor angiogenesis.

J. J. Castellot Jr.,[1] M. J. Karnovsky,[1] and B. M. Spiegelman,[2] [1]Dept. of Pathology, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A potent stimulation of vascular endothelial-cell growth by differentiated 3T3-adipocytes.

P. D'Amore, B. Glaser, S. Brunson, and A. Fenselau, Dept. of Physiological Chemistry and Wilmer Ophthalmological Institute, Johns Hopkins School of Medicine and Hospital, Baltimore, Maryland: Demonstration and partial purification of a vasoproliferative substance from mammalian retina.

Session 3: Growth Factors. II.

Chairperson: B. Zetter, Children's Hospital Medical Center, Boston, Massachusetts

J. J. Castellot, Jr., M. L. Addonizio, and M. J. Karnovsky, Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Vascular endothelial cells produce a heparinlike inhibitor of smooth-muscle-cell growth.

P. Bowman, A. L. Betz, and G. W. Goldstein, Depts. of Pediatrics and Neurology, University of Michigan, Ann Arbor: Brain capillary endothelial cells in culture.

J. Folkman,[1] C. Haudenschild,[2] and B. Zetter,[1] [1]Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts; [2]Mallory Institute, Boston City Hospital, Massachusetts: Long-term culture of capillary endothelial cells.

E. McCall, J. Povey, and D. C. Dumonde, Dept. of Immunology, St. Thomas' Hospital, London, England: Culture of porcine endothelial cells on expanded microporous polytetrafluoroethylene (PTFE) membranes.

B. J. Weimann, H. Kuhn, and H. R. Baumgartner, Pharma Research 3, F. Hoffman-La Roche & Company Ltd., Basel, Switzerland: Human (HEC) and bovine (BEC) endothelial-cell injury by homocysteine (HO).

C. K. Dorey and S. B. Rybicki, School of Dental Medicine, Southern Illinois University, Edwardsville: Ornithine decarboxylase levels depressed in endothelial cells exposed to EGI in vitro.

Session 4: Cell Metabolism. I.

Chairperson: L. L. Slakey, University of Massachusetts, Amherst

S. F. Ching, L. W. Hayes, and L. L. Slakey, Dept. of Biochemistry, University of Massachusetts, Amherst: Synthesis of angiotensin-converting enzyme by swine arterial endothelial cells.

D. B. Rubin, Cardio-Vascular Research Institute, University of California, San Francisco: Hydrolysis of an angiotensin analog by endothelial cells and fibroblasts in vitro.

W. E. Laug, Children's Hospital, University of Southern California School of Medicine, Los Angeles: Secretion of different plasminogen activators and anchorage-independent growth of bovine endothelial cells.

D. J. Loskutoff, Dept. of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Effect of thrombin on endothelial-cell-mediated fibrinolysis.

J.-P. Tauber and D. Gospodarowicz, Cancer Research Institute and Dept. of Medicine, University of California Medical Center, San Francisco: Interaction of HDL with cultured vascular endothelial cells and up-regulation of HDL receptor sites by 25-hydroxycholesterol.

C. Busch,[1] J. Dawes,[2] D. S. Pepper,[3] and Å. Wasteson,[4] [1]Dept. of Pathology and [4]Medical Chemistry, University of Uppsala, Sweden; [2]MRC Radioimmunoassay Team, [3]South-East Scotland Regional Blood Transfusion Centre, Edinburgh: Binding of platelet factor 4 to cultured human umbilical-vein endothelial cells.

J. W. Ryan, L. R. Hendricks, G. H. Fisher, and U. S. Ryan, Dept. of Medicine, University of Miami School of Medicine, Florida: Metabolism of bradykinin by bovine pulmonary endothelial cells in culture.

Session 5: Cell Metabolism. II.

Chairperson: E. Jaffe, Cornell University Medical College, New York, New York

R. I. Levin,[1] B. B. Weksler,[2] and E. A. Jaffe,[2] Depts. of Medicine, [1]New York University School of Medicine, New York; [2]Cornell University Medical College, New York, New York: Nitroglycerin induces production of prostacyclin by human endothelial cells.

W. HOPE,[1] T. J. MARTIN,[1] R. D. NOLAN,[1] C. N. CHESTERMAN,[2] and F. J. MORGAN,[3] [1]Dept. of Medicine, University of Melbourne, Repatriation General Hospital, Australia; [2]St. Vincent's Hospital, [3]St. Vincent's School of Medical Research, Victoria, Australia: Human β-thromboglobulin inhibits 6-oxo-prostaglandin F, α production by bovine aortic endothelial cells.

C. SEILLAN,[1], C. ODY,[1] F. RUSSO-MARIE,[1] W. SIESS,[2] and F. DRAY,[2] [1]INSERM Unité 7, Necker Hospital, Paris; [2]Pasteur Institute, Paris, France: Determination of cyclooxygenase products using high-pressure liquid chromatography and radioimmunoassay in pig endothelial and smooth-muscle cells in culture.

J. D. PEARSON, J. S. CARLETON, and J. L. GORDON, ARC Institute of Animal Physiology, Babraham, Cambridge, United Kingdom: Metabolism of exogenous nucleotides by endothelial cells.

D. SHEPRO[1] and H. B. HECHTMAN,[2] [1]Biological Science Center, Boston University, Massachusetts; [2]Harvard Medical School, Boston, Massachusetts: Saturable-carrier-mediated and nonfacilitative diffusion of serotonin by intimal and microvessel endothelium in vitro.

P. A. CANCILLA and L. E. DEBAULT, Dept. of Pathology and Arteriosclerosis, Specialized Center of Research, College of Medicine, University of Iowa, Iowa City: Demonstration of an A and L system for neutral amino acid transport in a cerebral endothelial cell line.

Session 6: Endothelial-cell–White-blood-cell Interaction

Chairperson: J. L. GORDON, ARC Institute of Animal Physiology, Cambridge, England

D. BURGER,[1] D. FORD,[1] A. HAMBLIN,[2] and D. C. DUMONDE,[2] [1]Surgical Research Laboratory, VA Medical Center, Portland, Oregon; [2]Department of Immunology, St. Thomas' Hospital, London: Activation of human T-lymphocytes by lymphocyte–endothelial-cell interaction.

H. HIRSCHBERG[1] and O. J. BERGH,[2] [1]Dept. of Neurosurgery, [2]Tissue Typing Laboratory, The National Hospital, Oslo, Norway: Immunological function of human vascular endothelial cells.

D. C. DUMONDE,[1] E. KASP-GROCHOWSKA,[1] P. JOSE,[2] and T. J. WILLIAMS,[2] [1]Dept. of Immunology, St. Thomas' Hospital, London; [2]Dept. of Pharmacology, Royal College of Surgeons, London: Modulation of lymphocyte transformation by soluble products of cultured endothelial cells.

W. L. FORD, P. ANDREWS, S. FOSSUM, M. E. SMITH, and R. W. STODDART, Dept. of Experimental Pathology, University of Manchester, England: The interaction of lymphocytes with vascular endothelium.

J. L. GORDON, J. D. PEARSON, and J. E. BEESLEY, ARC Institute of Animal Physiology, Babraham, Cambridge, United Kingdom: Granulocyte adhesion to and migration through vascular endothelium in culture.

R. AUERBACH, D. M. FORM, L. KUBAI, V. MUTHUKKARUPPAN, D. J. RHUDE, and Y. A. SIDKY, Dept. of Zoology, University of Wisconsin, Madison: A comparison of lymphocyte-induced and tumor-induced angiogenesis in the mouse.

Session 7: Cell Attachment and Substrate

Chairperson: H. KLEINMAN, NIDR, National Institutes of Health, Bethesda, Maryland

V. P. TERRANOVA,[1] D. H. ROHRBACH,[1] J. C. MURRAY,[1] G. R. MARTIN,[1] and S. H. YUSPA,[2] [1]NIDR, [2]NCI, National Institutes of Health, Bethesda, Maryland: The role of laminin in epidermal-cell attachment to basement-membrane collagen.

G. R. GROTENDORST, H. K. KLEINMAN and G. R. MARTIN, Laboratory of Developmental Biology and Anomalies, NIDR, National Institutes of Health, Bethesda, Maryland: Attachment of arterial smooth-muscle cells to collagens.

E. PEARLSTEIN, Dept. of Pathology, New York University Medical Center, New York: Comparison of fibronectin-mediated cellular adhesion to extracellular microexudates and vascular subendothelial matrices.

D. H. AUSPRUNK, Dept. of Surgery, Children's Hospital Medical Center and Dept. of Anatomy, Harvard Medical School, Boston, Massachusetts: Glycosaminoglycans in the extracellular matrix of regenerating and nonregenerating endothelial cells.

R. F. DYCK,[1] M. LOCKWOOD,[2] V. DUANCE,[3] M. KERSHAW,[4] and M. B. PEPYS,[1] [1]Immunological Medicine Unit and [2]Renal Unit, Dept. of Medicine, Royal Postgraduate Medical School, London, England; [3]University of Bristol, England; [4]St. Mary's Hospital Medical School, London, England: Amyloid P-component—A constituent of normal human basement membrane.

Session 8: Tumor-cell Interaction with Endothelial Cells

Chairperson: G. NICOLSON, College of Medicine, University of California, Irvine

R. GONZALEZ,[1] R. H. KRAMER,[1] E. RUOSLAHTI,[3] and G. L. NICOLSON,[1,2] [1]Dept. of Developmental and Cell Biology, [2]Dept. of Physiology, College of Medicine, University of California, Irvine; [3]La Jolla Cancer Research Foundation, California: Adhesion of metastatic human and mouse melanoma cells to vascular endothelial cells and their extracellular matrix—role of fibronectin.

R. H. KRAMER,[1] K. G. VOGEL,[2] R. GONZALEZ,[1] and G. L. NICOLSON,[1,3] [1]Dept. of Developmental and Cell Biology, [2]Dept. of Biology, University of New Mexico, Albuquerque; [3]Dept. of Physiology, College of Medicine, University of California, Irvine: Metastatic tumor-cell invasion of vascular endothelial-cell monolayers and interaction with associated extracellular matrix.

G. Poste,[1] J. Doll,[1] C. Akers,[2] and R. E. Baier,[2] [1]Dept. of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York; [2]Calspan Corporation, Buffalo, New York: The interaction of tumor cells with endothelial cells and subendothelial components in perfused blood vessels.

P. A. Jones[1] and H. B. Neustein,[2] [1]Division of Hematology-Oncology and [2]Dept. of Pathology, Children's Hospital, University of Southern California School of Medicine, Los Angeles: Construction of an artificial blood-vessel wall and its destruction by human tumor cells.

L. Ossowski and E. Reich, Rockefeller University, New York, New York: A quantitative model for experimental study of metastasis.

W. Düchting and G. Dehl, Dept. of Electrical Engineering, University of Siegen, Federal Republic of Germany: Spatial structure and temporal propagation of normal and malignant cell growth—Modeling and computer simulation.

Session 9: Endothelial-cell Matrix and Expression of Phenotype

Chairperson: D. Rifkin, New York University Medical Center, New York

L. E. DeBault, Dept. of Pathology and the Atherosclerosis Specialized Center of Research, University of Iowa, College of Medicine, Iowa City: Endothelial/glial cocultures and gamma-glutamyl transpeptidase induction mediated by cell-to-cell contact.

D. Gospodarowicz, Cancer Research Institute, University of California Medical Center, San Francisco: Cell proliferation and the extracellular matrix.

I. Vlodavsky, G. Greenburg, L. K. Johnson, N. Savion, and D. Gospodarowicz, Cancer Research Institute, University of California Medical Center, San Francisco: Structural and functional alterations in vascular endothelial cells associated with the formation of a confluent cell monolayer and with the withdrawal of fibroblast growth factor.

D. Moscatelli,[1] E. Jaffe,[2] and D. B. Rifkin,[1] [1]Dept. of Cell Biology, New York University Medical Center, New York; [2]Division of Hematology-Oncology, Cornell University Medical College, New York, New York: Tetradecanoyl phorbol acetate stimulates latent collagenase production by cultured human endothelial cells.

J. B. Weiss and R. A. Brown, K. A. Sedowofia, Dept. of Rheumatology, University of Manchester, England: Endothelial-cell-stimulating angiogenesis factor (ESAF) stimulates a latent collagenolytic enzyme to partially degrade basement-membrane collagen.

Forms of Microtubule Organization in Cells, May 14—May 17

Arranged by
Guenter Albrecht-Buehler, *Cold Spring Harbor Laboratory, New York*
Richard G. W. Anderson, *University of Texas Health Science Center, Dallas*

59 participants

Opening Remarks: K. R. Porter, University of Colorado, Boulder

Session 1: Structure and Chemistry of Microtubules

Chairperson: L. Amos, Medical Research Council Laboratory, Cambridge, England

L. A. Amos, MRC Laboratory of Molecular Biology, Cambridge, England: Microtubule structure and polarity.
L. Wilson, R. Margolis, and K. Farrell, Dept. of Biological Sciences, University of California, Santa Barbara: Pharmacological probes in the elucidation of microtubule assembly and function.
D. B. Murphy, Dept. of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland: The identity and purification of a microtubule-associated ATPase.
P. B. Schiff, J. Parness, and S. B. Horwitz, Depts. of Cell Biology and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York: Taxol stabilizes labile microtubules.

Session 2: Microtubules in the Cytoplasmic Matrix

Chairperson: F. Solomon, Massachusetts Institute of Technology, Cambridge

J. Wolosewick, University of Illinois, Chicago: Observations on microtubules in cells embedded in an extractable matrix (polyethylene glycol).
A. Duerr, D. Pallas, G. Zieve, and F. Solomon, Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Proteins associated with microtubules in cultured cells.
K. Luby, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Microtubules and intracellular transport—What can we learn from the fish chromatophore?
E. Frixione[1] and L. Tilney,[2] [1]Centro de Investigacion del IPN, Mexico City, Mexico; [2]Dept. of Biology, University of Pennsylvania, Philadelphia: Association of microtubules and pigment granules, as observed in isolated cytoplasm of crayfish retinula cells.
M. Schliwa, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Structural association of microtubules with cytoplasmic filaments.
R. J. Lasek, S. Brady, and M. Tytell, Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio: Axonal transport of microtubules and associated proteins.

Session 3: Poster Session

B. Cherksey, J. A. Zadunaisky, and R. B. Murphy, Dept. of Chemistry and Dept. of Physiology and Biophysics, New York University, New York: Cytoskeletal involvement in regulation of antagonist-beta-adrenergic receptor interaction in frog erythrocyte membrane.
M. De Brabander, G. Geuens, R. Nuydens, R. Willebrords, and J. De Mey, Laboratory of Oncology, Janssen Pharmaceutica Research Laboratories, Beerse, Belgium: The effects of metabolic inhibitors on nucleated and random microtubule assembly in intact cells.
J. De Mey, M. Moeremans, G. Geuens, R. Nuydens, and M. De Brabander, Laboratory of Oncology, Janssen Pharmaceutica Research Laboratories, Beerse, Belgium: A colloidal gold labeled antibody method for LM and EM visualization of microtubules and microtubule-associated calmodulin in cultured cells.

G. Erler, Institute of Zoology, Wilhelms University, Münster, Federal Republic of Germany: Are microtubules involved in mechanosensory transduction?

I. Gozes[1] and C. J. Barnstable,[2] [1]Laboratory of Neural and Endocrine Regulation, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Neurobiology, Harvard Medical School, Boston, Massachusetts: Identification of multiple tubulin forms by monoclonal antibodies.

I. Gozes[1] and K. J. Sweadner,[2] [1]Laboratory of Neural and Endocrine Regulation, Massachusetts Institute of Technology, Cambridge; and [2]Dept. of Neurobiology, Harvard Medical School, Boston, Massachusetts: Multiple tubulin forms are expressed by a single neuron.

D. Scandella, E. White, M. Cole, and E. R. Katz, Dept. of Microbiology, State University of New York, Stony Brook: Mutants resistant to mitotic inhibitors in the cellular slime mold, *Dictyostelium discoideum*.

Session 4: Microtubules in Various Cell Types

Chairperson: J. B. Tucker, St. Andrews University, Fife, Scotland

J. S. Hyams[1] and H. Stebbings,[2] [1]Dept. of Botany and Microbiology, University College, London; [2]Dept. of Biological Sciences, University of Exeter, England: Microtubule-associated transport in the insect ovary.

J. B. Tucker, Dept. of Zoology, St. Andrews University, Fife, Scotland: Intercellular coordination of microtubule organization in certain insect epithelia.

R. E. Sinden, Dept. of Zoology and Applied Entomology, Imperial College, University of London, United Kingdom: Microtubule organization during microgametogenesis of malarial parasites.

W. E. Foor, Dept. of Biological Sciences, Wayne State University, Detroit, Michigan: Microtubules and microfilaments within nematode spermatocytes and spermatozoa.

D. Phillips, Center for Biomedical Research, Rockefeller University, New York, New York: Insect sperm flagella.

D. T. Woodrum, and R. W. Linck, Dept. of Anatomy, Harvard Medical School, Boston, Massachusetts: Arrangement of microtubules and crossbridges in the axostyle of *Saccinobaculus*.

Session 5: Centrioles and Basal Bodies

Chairperson: R. G. W. Anderson, University of Texas, Dallas

R. G. W. Anderson and A. K. Floyd, Dept. of Cell Biology, University of Texas Health Science Center, Dallas: Biochemical properties of isolated oviduct basal bodies.

B. R. Brinkley and D. A. Pepper, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Analysis of centrioles and kinetochores as tubulin assembly sites in mammalian cells.

M. Borneno, Dépt. de Biologie Moléculaire, Institute Pasteur, Paris, France: Centrioles and the nuclear envelope.

M. W. Berns,[1] S. Brenner,[2] and S. P. Peterson,[3] [1]Dept. of Developmental and Cell Biology, University of California, Irvine; [2]Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas, [3]Ph.D.-M.D. Program, University of Miami School of Medicine, Florida: The centriolar region in mitosis.

G. Albrecht-Buehler and A. Bushnell, Cold Spring Harbor Laboratory, New York: Centrioles in migrating tissue-culture cells.

J. P. Chang and P. C. Moller, Division of Cell Biology, University of Texas Medical Branch, Galveston: Tubulin and basal body formation during ciliogenesis.

S. Tamm, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts: Large-scale formation of free kinetosomes in Australian termite flagellates.

Session 6: Microtubules and Sensory Processes

Chairperson: R. A. Bloodgood, Albert Einstein College of Medicine, New York, New York

R. A. Bloodgood, Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Flagellar membrane dynamics.

D. T. Moran, Dept. of Anatomy, University of Colorado Health Sciences Center, Denver: Microtubule organization in sensory cilia.

U. Thurm, J. Gödde, H. Kastrup, T. Keil, W. Völker, and B. Vohwinkel, Institute of Zoology, University Münster, Federal Republic of Germany: A microtubule-cytomembrane complex serving mechano-electric transduction.

H. Machemer,[1] A. Ogura,[2] and J. de Peyer,[3] [1]Abteilung für Biologie, Ruhr-Universität Bochum, Federal Republic of Germany; [2]Zoological Institute, University of Tokyo, Japan; [3]Pharmakologisches Institut, Universität Bern, Switzerland: Ciliate protozoan mechanosensitivity—No microtubules involved?

A. J. Hudspeth, D. P. Corey, and R. Jacobs, Division of Biology, California Institute of Technology, Pasadena: The transduction apparatus of vertebrate hair cells.

Session 7: Cilia and Flagella

Chairperson: S. L. Tamm, Marine Biology Laboratory, Woods Hole, Massachusetts

D. N. Wheatley, Dept. of Pathology, University of Aberdeen, Scotland: Special features of primary cilia in cultured cells.

S. L. Tamm, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts: Ciliary beat direction—Ionic, nervous, and structural control.

P. Satir, J. Wais-Steder, and J. Avolio, Albert Einstein College of Medicine, Bronx, New York: Mechanomorphology of microtubule sliding.

C. K. Omoto and G. B. Witman, Dept. of Biology, Princeton University, New Jersey: Rotation of the central pair of microtubules in cilia and flagella.

D. R. Mitchell and F. D. Warner, Dept. of Biology, Syracuse University, New York: Dynein-microtubule interactions in tetrahymena cilia.

C. Silflow,[1] P. Lefebvre,[2] T. McKeithan,[3] and J. Rosenbaum,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [3]Dept. of Pathology, University of Chicago School of Medicine, Illinois: Control of flagellar protein synthesis during flagellar regeneration in *Chlamydomonas*.

RNA Tumor Viruses, May 21—May 25

Arranged by
William S. Hayward, *Rockefeller University, New York, New York*
John Taylor, *Institute for Cancer Research, Fox Chase, Pennsylvania*

376 participants

Session 1: Synthesis and Structure of Viral DNA

Chairperson: H. Varmus, University of California, San Francisco

J. Majors and H. E. Varmus, Dept. of Microbiology and Immunology, University of California, San Francisco: The structure of integrated mouse mammary tumor virus DNA.

K. Shimotohno, S. Mizutani, and H. M. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Nucleotide sequence of the terminal repeat of integrated spleen necrosis virus and the junctions between cellular and viral DNA.

C.-M. Wei,[1] G. L. Hager,[1] and D. R. Lowy,[2] [1]Tumor Virus Genetics Branch; [2]Dermatology Branch, NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning of infectious circular DNA intermediate of Moloney murine leukemia virus.

D. R. Lowy and E. H. Chang, Dermatology Branch, NCI, National Institutes of Health, Bethesda, Maryland: Functional organization of the Harvey murine sarcoma virus genome.

W. L. McClements,[1] D. G. Blair,[2] M. Oskarsson,[1] T. G. Wood,[1] and G. F. Vande Woude,[1] Laboratories of [1]Molecular Virology and [2]Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: Leukemia virus sequences required for transformation by *src*/*sarc*.

J. G. Sutcliffe, T. M. Shinnick, and R. A. Lerner, Scripps Clinic and Research Foundation, La Jolla, California: Nucleotide sequence of Moloney leukemia virus—The 3' end reveals details of replication, analogy to bacterial transposons, and an unexpected gene.

P. Luciw, H. Opperman, H. Varmus, and J. M. Bishop, Dept. of Microbiology, University of California, San Francisco: Transfection with cloned avian sarcoma virus (ASV) DNA—Integration and gene expression in nonpermissive cells.

L. Boone and A. M. Skalka, Roche Institute of Molecular Biology, Nutley, New Jersey: Kinetics of synthesis and structure of proviral DNA made in vitro by the melittin permeabilized RAV-2 virion.

R. P. Junghans, L. Boone, and A. M. Skalka, Roche Institute of Molecular Biology, Nutley, New Jersey: Models for reverse transcription and recombination from electron microscope analysis of in vitro synthesized products.

R. Swanstrom, W. De Lorbe, P. Hackett, J. M. Bishop, and H. E. Varmus, Dept. of Microbiology and Immunology, University of California, San Francisco: Functional aspects of nucleotide sequences in the genome of avian sarcoma virus.

G. Ju and A. M. Skalka, Roche Institute of Molecular Biology, Nutley, New Jersey: Size heterogeneity and nucleotide sequence analysis of the direct repeats of avian retroviruses.

A. P. Czernilofsky,[1] E. Tischer,[2] B. DeLorbe,[1] H. Varmus,[1] R. Swanstrom,[1] H. Goodman,[2] and J. M. Bishop,[1] [1]Dept. of Microbiology and Immunology; [2]Dept. of Biochemistry and Biophysics, University of California, San Francisco: The *src* gene of ASV—Nucleotide sequence of the gene and its flanking regions in the viral genome.

C. Shoemaker, S. Goff, E. Gilboa, S. Mitra, and D. Baltimore, Massachusetts Institute of Technology, Cambridge: Structure of a molecularly cloned Moloney virus circular DNA with an inverted segment—Implications for integration.

Session 2: Viral RNA Structure and Expression

Chairperson: W. Hayward, Rockefeller University, New York, New York

T. YAMAMOTO, J. S. TYAGI, J. FAGAN, G. JAY, B. DE CROMBRUGGHE, and I. PASTAN, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Structural and functional features of the common region of avian sarcoma virus.

G. P. GASIC and W. S. HAYWARD, Rockefeller University, New York, New York: Sequence analysis of the 5' leader of ALV.

D. DINA, E. W. BENZ, JR., R. WYDRO, and B. NADAL-GINARD, Albert Einstein College of Medicine, Bronx, New York: Moloney murine sarcoma virus DNA is a transcriptional unit.

D. SCHWARTZ, R. TIZARD, and W. GILBERT, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: The nucleotide sequence of Rous sarcoma virus determined by analysis of single-stranded cDNA.

C. M. STOLTZFUS, Dept. of Microbiology, University of Iowa, Iowa City: Evidence for a possible role of RNA methylations in the formation of subgenomic avian sarcoma virus RNAs.

D. L. ROBERTSON and H. E. VARMUS, Dept. of Microbiology, University of California, San Francisco: Gene regulation of mouse mammary tumor virus.

D. S. UCKER and K. R. YAMAMOTO, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Analysis of transcription at the integration site of a hormone-responsive MTV provirus.

N. A. JENKINS and G. M. COOPER, Sidney Farber Cancer Institute, Boston, Massachusetts: Integration, expression and infectivity of exogenously-acquired RAV-O DNAs.

J. J. O'REAR, S. MIZUTANI, and H. M. TEMIN, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Infectious and noninfectious proviruses of spleen necrosis virus cloned in Charon 4A.

J. I. MULLINS,[1] M. NICHOLSON,[2] J. CASEY,[1] K. BURCK,[1] and N. DAVIDSON,[1] [1]California Institute of Technology, Pasadena; [2]The Childrens Hospital of Los Angeles, California: Sequence arrangement and biological activity of cloned integrated forms of FeLV DNA.

D. JÄHNER, H. STUHLMANN, and R. JAENISCH, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Hamburg, Federal Republic of Germany: Germ line integration of Moloney leukemia virus—Characterization of independently derived mouse sublines.

R. MICHALIDES,[1] R. VAN NIE,[1] R. NUSSE,[1] N. HYNES,[2] and B. GRONER,[2] [1]Netherlands Cancer Institute, Amsterdam; [2]Swiss Cancer Institute, Lausanne, Switzerland: Regulation of mouse mammary tumor virus (MMTV) expression in mouse strains GR and GR/Mtv-2^{-}.

Session 3: Poster Session—Retrovirus Biochemistry

Synthesis and Structure of Viral DNA

C. A. OMER and A. J. FARAS, Dept. of Microbiology, Minneapolis, Minnesota: DNA sequence analysis of $tRNA^{trp}$ initiated RSV cDNA longer than 101 nucleotides.

E. C. WOODLAND and P. R. SHANK, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Detection of supercoiled avian sarcoma virus DNA molecules of 1/3 to 1/2 genome length.

T. GILMER, L. RAFIELD, P. HIGHFIELD, T. PUGATSCH, G. GILMARTIN, and J. PARSONS, Dept. of Microbiology, University of Virginia, Charlottesville: Structure and biological activity of cloned avian sarcoma virus DNA.

J. C. OLSEN and K. F. WATSON, Dept. of Chemistry, University of Montana, Missoula: Reverse transcription of avian myeloblastosis virus 35S RNA—Identification and characterization of (+) DNA of discrete sizes synthesized in the reconstructed reaction.

R. FRIEDRICH, Institute of Tumor Immunology, University of Freiburg, Federal Republic of Germany: Are both retroviral RNA subunits involved in the synthesis of one DNA molecule?

L. DESGROSEILLERS, E. RASSART, and P. JOLICOEUR, Institut de Recherches Cliniques, Université de Montréal, Quebec, Canada: Study of in vitro synthesis of Balb/c endogenous MuLV DNA.

C. VAN BEVEREN,[1] J. G. GODDARD,[2] A. BERNS,[1] and I. M. VERMA,[2] [1]Laboratory of Biochemistry, University of Nijmegen, The Netherlands; [2]Tumor Virology Laboratory, Salk Institute, San Diego, California: Structure of Moloney murine leukemia viral DNA—Nucleotide sequence of the 5'-long terminal repeat and adjacent cellular sequences.

W. L. MCCLEMENTS, R. DHAR, and G. F. VANDE WOUDE, Laboratory of Molecular Virology, NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence of provirus-host junctions of integrated Moloney sarcoma virus.

L. BACHELER and H. FAN, Tumor Virology Laboratory, Salk Institute, San Diego, California: Cloning of integrated Moloney murine leukemia virus DNA sequences from infected mouse cells.

N. TSUCHIDA and S.-I. UESUGI, Wistar Institute, Philadelphia, Pennsylvania: Molecular cloning of Ki-MSV genome size DNA.

E. BUETTI, B. GRONER, N. HYNES, and H. DIGGELMANN, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Molecular cloning of cellular unintegrated DNA forms of mouse mammary tumor virus (MMTV).

N. HYNES,[1,2] B. GRONER,[1,2] R. MICHALIDES,[3] and N. KENNEDY,[2] [1]Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland; [2]Kernforschungszentrum, Karlsruhe, Germany; [3]Dutch Cancer Institute, Amsterdam, The Netherlands: Isolation of the endogenous MMTV proviruses of the GR mouse and their molecular comparison.

M. NODA, T. TAMURA, and T. TAKANO, Dept. of Microbiology, Keio University School of Medicine, Tokyo, Japan: Restriction mapping and integration mechanism of baboon endogenous virus (M7) DNA.

E. GELMANN, S. JOSEPHS, A. CETTA, R. C. GALLO, and F. WONG-STAAL, NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning and restriction enzyme maps of two strains of baboon endogenous virus.

N. Battula and G. J. Todaro, NCI, National Institutes of Health, Bethesda, Maryland: Physical map of infectious baboon type C viral DNA and sites of integration.

S. J. O'Brien, C. A. Winkler, W. G. Nash, R. S. Lemons, and J. S. Martenson, NCI, National Institutes of Health, Bethesda, Maryland: Control of integration of baboon endogenous virus in human chromosomes by the *BEVI* locus—Genetic studies.

Viral RNA—Structure and Expression

G. G. Lovinger and G. Schochetman, Frederick Cancer Research Center, Frederick, Maryland: 5' terminal nucleotide sequences—Features common to terminal noncoding sequences of known eukaryotic messenger RNAS.

M. Hatanaka, Y. Tomita, and R. Kominami, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: The "C" region of retrovirus genome in normal human DNA.

R. Klemenz, M. Reinhardt, and H. Diggelmann, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Sequence determination of the 3' end of mouse mammary tumor virus RNA.

C. Dickson and G. G. Peters, Imperial Cancer Research Fund, London, England: Coding potential of the mouse mammary tumor virus genome RNA as determined by in vitro translation analysis C.

K. G. Murti, M. Bondurant, and A. Tereba, St. Jude Children's Research Hospital, Memphis, Tennessee: Secondary structure of the genomic RNAs of Moloney MuLV and Rous sarcoma virus (RSV) as determined by electron microscopy.

B. K. Pal,[1] C. S. Shimizu,[1] and M. M. C. Lai,[2] Departments of [1]Pathology and [2]Microbiology, University of Southern California School of Medicine, Los Angeles: Genomic analysis of wild mouse retroviruses.

P. Vigier,[1] F. Catala,[1] G. Goubin,[1] M. Roussel,[2] and V. Krsmanovic,[3] [1]Institut Curie, Orsay, France; [2]INSERM, Pasteur Institute, Lille, France; [3]Unité de Virologie, INSERM, Lyon, France: Blockage of expression of the avian sarcoma virus genome in transformed tumorigenic mammalian cells.

A. Panet,[1,2] C. Czarniecki,[1] and R. M. Friedman,[1] [1]National Institutes of Health, Bethesda, Maryland; [2]The Hebrew University, Jerusalem, Israel: Interferon treatment and arrest of cell cycle appear to inhibit murine leukemia virus production by similar mechanisms.

I. Balazs, Memorial Sloan-Kettering Cancer Center, New York, New York: Rate of *gag* and *env* protein synthesis and virus production in synchronized mouse cells infected with Rauscher leukemia virus.

J. Doehmer,[1] I. Rademacher,[2] and K. Willecke,[2] [1]Tumor Virology Laboratory, Salk Institute, San Diego, California; [2]Institut für Zellbiologie, Universität Essen, Federal Republic of Germany: Expression of p30 antigen in fibroblasts of Balb/MÖ mice after fusion with mouse thymocytes.

Viral Proteins

E. Hunter and J. M. Hardwick, Dept. of Microbiology, University of Alabama, Birmingham: A mutant of Rous sarcoma virus defective in glycoprotein synthesis.

C. W. Rettenmier and H. Hanafusa, Rockefeller University, New York, New York: Comparative peptide analysis of avian oncoviral structural protein markers.

S. Oroszlan, T. D. Copeland, and L. E. Henderson, Frederick Cancer Research Center, Frederick, Maryland: Amino acid sequence analysis of retrovirus structural proteins—A progress report.

J. Kopchick,[1] J. Harless,[2] R. Hewitt,[2] and R. Arlinghaus,[3] [1]Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Dept. of Environmental Biology, [3]Dept. of Tumor Virology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Evidence for a viral coded endonuclease of Rauscher murine leukemia virus.

G. F. Gerard, Institute for Molecular Virology, St. Louis, Missouri: Nature of the nuclease activities found in lysates of Moloney murine leukemia virus.

D. Gerlier,[1] S. Gisselbrecht,[2] B. Guillemain,[3] and J. F. Doré,[1] [1]INSERM, Lyon, France; [2]INSERM, Paris, France; [3]INSERM, Bordeaux, France: Quantitative determination of gross cell surface antigen (GCSAa) and p30 level in murine retrovirus infected cell lines.

J. K. Collins and B. Chesebro, Rocky Mountain Laboratories, NIAID, National Institutes of Health, Hamilton, Montana: Incorporation of $pr65^{gag}$ into a replication-defective virus produced by a Friend virus-induced erythroleukemia cell line.

N. Burnette, Fred Hutchinson Cancer Research Center, Seattle, Washington: Analysis of intracellular MuLV polyproteins by Western blotting.

R. Goodenow, E. Olcott, A. Declevé, M. Lieberman, and H. S. Kaplan, Dept. of Radiology, Stanford University School of Medicine, California: Evidence for type-specific antigenic sites on the p30 of radiation leukemia virus.

S. Anderson and R. Naso, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: GR-mouse mammary tumor virus polyproteins—A possible fused glycosylated polyprotein.

G. C. Sen, J. Racevskis, and N. H. Sarkar, Sloan-Kettering Cancer Center, New York, New York: In vitro synthesis of murine mammary tumor viral proteins.

S. Whiteley and R. Naso, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Intracellular percursor polyproteins of primate and human-isolate retroviruses.

R. Hehlmann,[1] H. Schetters,[1] and V. Erfle,[2] [1]Medizinische Polikilnik der Universität, München, Federal Republic of Germany; [2]Gesellschaft für Strahlenforschung, Neuherberg, Federal Republic of Germany: Enzyme immunoassy for the detection of C-type viral proteins.

D. Portetelle,[1,2] C. Bruck,[1] Y. Cleuter,[1] M. Mammerickx,[3] and A. Burny,[1,2] [1]Dept. of Molecular Biology, University of Brussels, Belgium; [2]Faculty of Agronomy, Gembloux, Belgium; [3]National Institute for Veterinary Research Uccle, Belgium: In animals infected by bovine leukemia virus (BLV) antibodies to envelope glycoprotein gp51 are directed against the carbohydrate moiety.

Session 4: Transforming Proteins

Chairperson: T. Hunter, Salk Institute, San Diego, California

J. Brugge,[1] E. Erikson,[2] R. Erikson,[2] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Dept. of Pathology, University of Colorado Medical Center, Denver: Interaction of the transforming protein of avian sarcoma virus with cellular proteins.

S. A. Courtneidge, A. D. Levinson, and J. M. Bishop, Dept. of Microbiology and Immunology, University of California Medical Center, San Francisco: The nature of the association of pp60 *src and pp60 proto-src* with the plasma membrane of SR-D transformed and uninfected rat cells.

A. R. Goldberg, J. G. Krueger, E. Wang, and E. A. Garber, Rockefeller University, New York, New York: The intracellular location of pp60 *src* in RSV-transformed avian and mammalian cells.

L. Rohrschneider and K. Shriver, Fred Hutchinson Cancer Research Center, Seattle, Washington: Adhesion plaques of Rous sarcoma virus transformed cells contain the src gene product.

B. Sefton, T. Hunter, and K. Beemon, Tumor Virology Laboratory, Salk Institute, San Diego, California: Rous sarcoma virus transforming protein phosphorylates tyrosine in vivo.

K. Radke, T. Gilmore, and G. S. Martin, Zoology Dept., University of California, Berkeley: A 36,000 molecular weight cellular polypeptide containing phosphotyrosine in Rous sarcoma virus-transformed fibroblasts.

F. Poirier, G. Calothy, R. E. Karess, and H. Hanafusa, Rockefeller University, New York, New York: Induction of neuro-retinal cell proliferation by Rous sarcoma virus and pp60 *src* kinase activity.

T. Y. Shih, H. Langbeheim, A. Papageorge, P. Stokes, M. Weeks, and E. Scolnick, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: The p21 src of Harvey murine sarcoma virus—Characterization and purification.

D. Kabat, M. Ruta, and T. Fitting, School of Medicine, University of Oregon Health Sciences Center, Portland: Genetic analyses of plasma membrane glycoproteins encoded by cloned Rauscher and Friend SFFV's and by MuLV's.

F. H. Reynolds, Jr., W. J. M. Van de Ven, J. Blomberg, and J. R. Stephenson, NCI, Frederick Cancer Research Center, Maryland: Abelson murine leukemia transformation-defective mutants with impaired polyprotein associated protein kinase activity.

W. J. M. Van de Ven, F. H. Reynolds, Jr., and J. R. Stephenson, NCI, Frederick Cancer Research Center, Maryland: Polyproteins encoded by independent isolates of feline sarcoma virus possess common sequences within their nonstructural components.

M. Barbacid,[1] K. Beemon,[2] and S. Devare,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Tumor Virology Laboratory, Salk Institute, San Diego, California: The major gene product of ST-FeSV is a polyprotein of viral and cellular origin with an associated protein kinase activity that phosphorylates tyrosine residues.

Session 5: Virus Proteins

Chairperson: R. Eisenman, Fred Hutchinson Research Center, Seattle, Washington

D. P. Grandgenett,[1] W. Mason,[2] T. Cloppin,[3] S. Oraszlan,[3] M. Golomb,[1] and T. Misra,[1] [1]Institute for Molecular Virology, St. Louis, Missouri; [2]Institute for Cancer Research, Philadelphia, Pennsylvania; [3]Frederick Cancer Research Center, Bethesda, Maryland: Characterization of DNA endonuclease of avian retrovirus p32 *pol* and $\alpha\beta$ DNA polymerase.

R. Eisenman,[1] P. Heater,[2] P. Tsichlis,[2] C. S. Barker,[2] and J. Coffin,[2] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Tufts University School of Medicine, Boston, Massachusetts: Analysis of an avian retrovirus deletion mutant defective in the processing of its *gag* polyprotein.

P. Traktman and D. Baltimore, Massachusetts Institute of Technology, Cambridge: Relationship of murine pr180 *gag·pol* to virion maturation.

H. P. Ghosh and J. Ro, Dept. of Biochemistry, McMaster University, Hamilton, Ontario, Canada: RNA tumor virus maturation and assembly—Defective virus maturation of a temperature sensitive mutant of Rous sarcoma virus with impaired processing of reverse transcriptase precursor.

E. C. Murphy, Jr., S.-M. Mong, and R. B. Arlinghaus, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston: Suppression of murine retrovirus polypeptide termination—The effect of amber suppressor tRNA on the cell-free translation of R-MuLV, Mo-MuLV, and Mo-MuSV 124 RNA.

R. B. Pepinsky and V. M. Vogt, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Analysis of *gag* protein-membrane interaction by cross-linking studies.

S. Edwards and H. Fan, Tumor Virology Laboratory, Salk Institute, San Diego, California: Studies of glycosylated M-MuLV *gag* polyprotein.

A. M. Schultz, T. D. Copeland, and S. Oroszlan, Frederick Cancer Research Center, Frederick, Maryland: Structural Characterization of Rauscher leukemia virus *gag* and *env* polyproteins.

C. J. M. Saris, H. C. M. van Eenbergen, R. Liskamp, and H. P. J. Bloemers, Dept. of Biochemistry, University of Nijmegen, The Netherlands: Leader sequence and glycosylation of Mo-MuLV *gag*-precursor proteins.

H. Niman and J. Elder, Dept. of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Localization of recombinant-specific domains of murine retrovirus GP70's using monospecific hybridoma antibodies and peptide fingerprinting.

Session 6: Poster Session — Transformation

Transforming Proteins

S. D. Tsen, Y. S. E. Cheng, M. L. Walsh, R. Lee, E. Wolinsky, and L. B. Chen, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Cellular aspects of transformation induced by *src* gene product.

M. Owada, P. Donner, A. Scott, T. Bunte, and K. Moelling, Max-Planck-Institute for Molecular Genetics, Berlin, Federal Republic of Germany: The transformation-specific protein pp60 ^{src} from avian sarcoma viruses.

Y. S. E. Cheng, C. Y. Cheng, R. Lee, S. D. Tsen, and L. B. Chen, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Alteration in the phosphorylation of polypeptides in Rous sarcoma virus transformed cells.

J. G. Burr, G. Dreyfuss, S. Penman, and J. M. Buchanan, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Association of the *src* gene product of Rous sarcoma virus with cytoskeletal structures of chick embryo fibroblasts.

A. Tanaka, N. Kobayashi, and A. Kaji, University of Pennsylvania, Philadelphia: Phosphorylation and protein kinase activity in differentiated myotubes and chondrocytes infected with Rous sarcoma virus.

A. F. Lau, R. A. Krzyzek, and A. J. Faras, Dept. of Microbiology, University of Minnesota, Minneapolis: *src* gene product in ASV-infected field vole cells—Correlation of pp60 ^{src} protein kinase activity with tumorigenicity and subcellular localization of pp60 ^{src}.

C. Krycève-Martinerie,[1] J. M. Biquard,[1] D. Lawrence,[1] P. Vigier,[1] S. Barlati,[2] and P. Mignatti,[2] [1]Institut Curie, Faculté des Sciences, Orsay, France: [2]Laboratorio di Genetica Biochimica, CNR, Pavia, Italy: Transformed cells secrete a glycoprotein which favors the expression of transformation parameters controlled by the *src* gene of ASV.

C. B. Boschek,[1] B. M. Jockusch,[2] R. R. Friss,[1] R. Back,[1] and H. Bauer,[1] [1]Institut für Virologie, FB Humanmedizin der Justus-Liebig-Universität, Giessen, Federal Republic of Germany: The distribution and organization of cytoskeletal proteins in neoplastic transformation.

J. L. Toy and R. A. Weiss, Imperial Cancer Research Fund, London, England: Biological and biochemical studies on Abelson murine leukemia virus (A-MuLV).

E. Canaani, A. Eva, K. Robbins, S. Tronick, and S. A. Aaronson, NCI, National Institutes of Health, Bethesda, Maryland: Deletion mutants of Moloney sarcoma virus.

R. Arlinghaus,[1] E. C. Murphy,[1] Jr., J. P. Horn,[1] S.-M. Mong,[1] and T. G. Wood,[2] [1]Dept. of Tumor Virology, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston; [2]Laboratory of DNA Tumor Viruses, NCI, National Institutes of Health Bethesda, Maryland: Translation products of the 124 strain of Moloney murine sarcoma virus (Mo-MuSV)—Characterization of a 23,000 dalton candidate *src* gene product.

S. Rasheed[1] and H. Young,[2] [1]Dept. of Pathology, University of Southern California School of Medicine, Los Angeles; [2]Frederick Cancer Research Center, Frederick, Maryland: Independent rat sarcoma virus isolates code for the same transforming protein in rat or heterologous transformed cells.

D. A. Scheinberg and M. Strand, Johns Hopkins University School of Medicine, Baltimore, Maryland: Transformation sensitive antigens associated with Kirsten sarcoma virus pseudotypes and transformed cells.

G. R. Anderson, Dept. of Microbiology, University of Pittsburgh School of Medicine, Pennsylvania: A thermolabile LDH in cells infected with TS Kirsten sarcoma virus.

H.-J. Thiel, E. Broughton, A. Butchko, D. Bolognesi, and T. Matthews, Duke University Medical Center, Durham, North Carolina: Characterization of antigens in SSV transformed nonproducer cells.

Mechanisms of Transformation

Y.-K. T. Fung,[1] H.-J. Kung,[1] L. B. Crittenden,[2] and A. M. Fadly,[2] [1]Dept. of Biochemistry, Michigan State University, E. Lansing: [2]Regional Poultry Research Laboratory, United States Department of Agriculture, E. Lansing, Michigan: The structure of the exogenous proviral DNA in lymphoid leukosis tumors induced by an avian leukosis virus (RAV-1).

D. J. Fujita,[1] C. B. Boschek,[2] A. Ziemiecki,[2] and R. R. Friis,[2] [1]Cancer Research Laboratory and Dept. of Biochemistry, University of Western Ontario, London, Canada; [2]Institut für Virolgie, Justus Leebig Universität, Giessen, Federal Republic of Germany: An avian sarcoma virus temperature-sensitive mutant that produces an aberrant transformation.

J. L. Sabran, K. Hsia, and A. Kaji, Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Infection of multinucleated myotubes with Rous sarcoma virus.

H. E. Varmus,[1] N. Quintrell,[1] H. Opperman,[1] A. Levinson,[1] and J. Wyke,[2] [1]Dept. of Microbiology, University of California, San Francisco; [2]Imperial Cancer Research Fund, London, England: Multiple mechanisms of reversion of an ASV-transformed rat cell.

U. G. ROVIGATTI, R. A. WEISS, and J. A. WYKE, Imperial Cancer Research Fund, London, England: Reexpression of the transformed phenotype in revertants from avian sarcoma virus (ASV) transformed rat cells.

L. M. SOUZA, J. N. STROMMER, D. G. BERGMANN, and M. A. BALUDA, University of California School of Medicine and Molecular Biology Institute, Los Angeles: The presumptive avian myeloblastosis virus genome contains a cellular substitution in the envelope region.

J. H. CHEN, Life Sciences Biomedical Research Institute, St. Petersburg, Florida: Expression of endogenous AMV information in different chicken cells.

S. WRIGHT[1,3] and S. HARMON,[2,3] [1]Depts. of Medicine and Cellular, Viral, and Molecular Biology, University of Utah School of Medicine; [2]Dept. of Biology, University of Utah, Salt Lake City; [3]Viral Oncology Laboratory, Veterans Administration Medical Center, Salt Lake City, Utah: In vitro translation of avian myeloblastosis virus (AMV) genomic RNA yields nonstructural proteins.

S. M. ANDERSON and H. HANAFUSA, Rockefeller University, New York, New York: Transcription and translation products of avian erythro-blastosis virus.

L. GAZZOLO,[1] J. SAMARUT,[2] M. BOUABDELLI,[2] and J. P. BLANCHET,[2] [1]Unité de Virologie, INSERM, Lyon, France; [2]Dépt. of Biologie, Générale et Appliquée, Villeurbanne, France: Identification of the target cells transformed by avian erythroblastosis virus.

T. C. WONG and M. M. C. LAI, Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: Characterization of avian erythroblastosis virus-specific sequences in normal cells.

C. MOSCOVICI, S. PESSANO, and M. G. MOSCOVICI, Veterans Administration Medical Center and College of Medicine, University of Florida, Gainesville: Induction of avian acute leukemia in Japanese quail.

T. PAWSON, T. -H. KUNG, and G. S. MARTIN, Dept. of Zoology, University of California, Berkeley: Gene product of Fujinami avian sarcoma virus.

W. D. HANKINS[1] and S. B. KRANTZ,[1,2] [1]Dept. of Medicine, Vanderbilt University Medical School, Nashville, Tennessee; [2]Veterans Administration Hospital, Nashville, Tennessee: Helper MuLV is not required for in vitro erythroid transformation of hemopoietic cells by Friend virus.

Y. IKAWA,[1] Y. KOBAYASHI,[1] and S. HINO,[2] [1]Dept. of Viral Oncology, Cancer Institute, Tokyo; [2]Dept. of Virus Infection, Institute of Medical Science, University of Tokyo, Japan: SFFV-specific glycoprotein (gp55)—Oligosaccharide chains and appearance in the perinuclear area of SFFV-infected cells.

J. A. BILELLO,[1] I. B. PRAGNELL,[2] G. WARNECKE,[1] C. ARBUTHNOT,[2] P. NOBIS,[1] C. JASMIN,[2] and W. OSTERTAG,[2] [1]Dept. of Molecular Biology, University of Hamburg, Federal Republic of Germany; [2]Beatson Institute for Cancer Research, Glasgow, Scotland: Characterization of myeloproliferative virus—A defective fibroblast transforming virus, which induced spleen foci in adult mice.

G. C. BALDWIN and R. C. NOWINSKI, Fred Hutchinson Cancer Research Center, Seattle, Washington: Viruses produced by AKR leukemias.

D. L. BUCHHAGEN,[1] F. S. PEDERSEN,[2] C. Y. CHEN,[3] E. F. HAYS,[4] and W. A. HASELTINE,[4] [1]State University of New York, Downstate Medical Center, Brooklyn; [2]University of Aarhus, Denmark; [3]Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles; [4]Sidney Farber Cancer Institute, Boston Massachusetts: AKR MCF-247 virus infects and replicates in cells of the AKR mouse lymphoma it accelerates.

K. VAN DEN BERG, V. KRUMP, and P. BENTVELZEN, Radiobiological Institute TNO, Rijswijk, The Netherlands: Direct evidence for the presence of transforming sequences in the genome of normal Balb/c mice.

V. ERFLE,[1] R. HEHLMANN,[2] H. SCHETTERS,[2] J. SCHMIDT,[1] and A. LUZ,[1] [1]Abt. für Pathologie, GSF, Neuherberg, Federal Republic of Germany; [2]Med. Poliklinik, Universität München, Federal Republic of Germany: Time course of endogenous C-type virus expression in radiation-induced murine osteosarcomas and leukemias.

M. HAAS and J. H. ELDER, Dept. of Cell Biology, Weizmann Institute of Science, Rehovot, Israel, and Dept. of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California: T-cell and B-cell malignant lymphomas in C57BL/6 mice induced by different recombinant retroviruses.

L. J. L. VAN GRIENSVEN,[1] T. J. STOOF,[1] J. N. M. MOL,[1] and M. VOGT,[2] [1]Dept. of Pathology, Faculty of Medicine, Erasmus University, Rotterdam, The Netherlands; [2]Tumor Virology Laboratory, Salk Institute, San Diego, California: An analysis of R-MuLV complex—Properties of the different components.

N. ROSENBERG[1] and O. N. WITTE,[2] [1]Cancer Research Center, Tufts University Medical School, Boston, Massachusetts; [2]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Abelson murine leukemia virus mutants deficient in lymphoid cell transformation.

P. C. KIMBALL,[1,2] M. C. SIMON,[2] and N. K. MISHRA,[3] [1]Comprehensive Cancer Center and [2]Department of Microbiology, Ohio State University, Columbus: [3]United States Food and Drug Administration, Silver Springs, Maryland: On the role of progenitors of sarcoma virus genes in chemical carcinogenesis in the rat.

G. VECCHIO,[1] A. FUSCO,[1] D. TRAMONTANO,[1] A. PINTO,[1] and N. TSUCHIDA,[2] [1]Centro di Endocrinologia ed Oncologia Sperimentale del CNR, University of Naples, Italy; [2]Wistar Institute, Philadelphia, Pennsylvania: Transformation of rat thyroid epithelial cells by the Kirsten murine sarcoma virus.

M. JONES,[1] R. A. BOSSELMAN,[1] F. v.d. HOORN,[2] A. BERNS,[2] and I. M. VERMA,[1] [1]Tumor Virology Laboratory, Salk Institute, San Diego, California; [2]Laboratory of Biochemistry, University of Nijmegen, The Netherlands: Identification and molecular cloning of Moloney mouse sarcoma virus specific sequences from uninfected mouse cells.

Z. F. ROSENBERG,[1] B. G. SAHAGAN,[1] H. W. SNYDER, JR.,[2] and W. A. HASELTINE,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]Laboratory of Viral Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York: Biochemical characterization of cells transformed by feline sarcoma virus (FeSV) proviral DNA.

L. Donner,[1] L. Turek,[1] S. Ruscetti,[2] L. A. Fedele,[1] and C. J. Sherr,[1] [1]Laboratories of Viral Carcinogenesis, [2]Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: Transformation-defective mutants of Snyder-Theilen feline sarcoma virus.

G. A. Dekaban and J. K. Ball, Dept. of Biochemistry, University of Western Ontario, London, Canada: A highly leukemogenic virus extensively related to the mouse mammary tumor virus.

P. Roe, A. S. Berkower, and F. Lilly, Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Friend virus produced by superinfected erythroleukemia cells has the same tropism as the superinfecting virus.

J. N. M. Mol,[1] W. Vonk,[1] I.B. Pragnell,[2] and T.J. Stoof,[1] [1]Dept. of Pathology, Faculty of Medicine, Erasmus University, Rotterdam, The Netherlands; [2]Beatson Institute for Cancer Research, Glasgow, Scotland: On the relationship between Friend spleen focus-forming virus (SFFV) and its helper virus.

M. E. MacDonald,[1] T. W. Mak,[2] and A. Bernstein,[2] [1]Ontario Cancer Institute, Ontario, Canada; [2]University of Toronto, Canada: Biological and molecular analysis of different clonal isolates of replication-defective and replication-competent Friend leukemia virus.

W. G. Hesselink, A. C. M. van der Kemp, and H. P. J. Bloemers, Dept. of Biochemistry, University of Nijmegen, The Netherlands: Moloney cell surface antigen (MCSA) is related to an *env*-gene produce of a type C virus that is serologically distinct from Moloney murine leukemia virus.

R. S. Schwartz, R. Khiroya, C. Y. Thomas, P. N. Tsichlis, and J. M. Coffin, Tufts University School of Medicine, Boston, Massachusetts: Biologic and genetic analysis of HRS/J murine leukemia viruses.

Session 7: Poster Session

Endogenous Viruses

S. M. Astrin[1] and L. B. Crittenden,[2] [1]Institute for Cancer Research, Philadelphia, Pennsylvania; [2]Regional Poultry Research Laboratory, East Lansing, Michigan: Development of a line of chickens lacking endogenous viral genes.

E. J. Smith and L. B. Crittenden, Regional Poultry Research Laboratory, United States Dept. of Agriculture, East Lansing, Michigan: Segregation of chicken endogenous viral (*ev*) loci *ev*7 and *ev*12 with the expression of infectious subgroup E viruses.

B. Baker, H. E. Varmus, and J. M. Bishop, Dept. of Microbiology and Immunology, University of California, San Francisco: Characteristics of retrovirus RNA in uninfected chicken cells.

H. Robinson[1] and S. Astrin,[2] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]Institute for Cancer Research, Philadelphia, Pennsylvania: Replication defective endogenous viruses that express subgroup E envelope antigens interfere with subgroup E virus infections.

M. Boccara, N. Pluquet, C. Rommens, and D. Stehelin, INSERM, Pasteur Institute, Lille, France: Detection and characterization of chicken specific sequences (unrelated to ASLV or RAV-O) in the nd-B77 avian sarcoma virus.

T. I. Bonner, E. Birkenmeier, N. Battula, and G. J. Todaro, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health Bethesda, Maryland: Endogenous type C virus-related sequences in chimpanzee.

E. Rassart and P. Jolicoeur, Institut de Recherches Cliniques, University of Montreal, Quebec, Canada: Studies of murine endogenous proviral DNA.

D. Dolberg, L. Bacheler, and H. Fan, Tumor Virology Laboratory, Salk Institute, San Diego, California: Study of endogenous MuLV-related sequences in mouse cells.

F. S. Pedersen, D. L. Buchhagen, R. L. Crowther, and W. A. Haseltine, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Comparative finger-print and sequence analysis of the genomes of the Akv virus and an in vitro passaged, leukemogenic gross A virus.

Genetics and Biology

R. H. Bassin,[1] B. I. Gerwin,[1] J. G. Levin,[2] G. Duran-Troise,[1] B. M. Benjers,[1] and A. Rein,[1] [1]Laboratory of Tumor Viruse Genetics, NCI, [2]Laboratory of Molecular Genetics, NICHD, National Institutes of Health, Bethesda, Maryland: Abrogation of Fv-1 restriction by murine leukemia virus—Requirement for genomic RNA but not for DNA synthesis.

L. -C. Tsui[1] and W. K. Yang,[2] [1]Oak Ridge Graduate School of Biomedical Science, University of Tennessee; [2]Oak Ridge National Laboratory, Tennessee: Effects of cellular extracts on the in vitro DNA synthesis of murine ecotropic viruses.

S. Dattagupta, J. Chinsky, and R. Soeiro, Department of Medicine and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Protein structural studies of Fv-1 host range variants.

A. Mayer, Dept. of Pathology, New York University Medical Center, New York: Radiation induction of thymic lymphoma in mice is not influenced by alleles at the murine *Fv-1* locus restrictive for cellular infection by MuLV.

J. Silver and N. Teich, Imperial Cancer Research Fund, London, England: Susceptibility of *Fv-2*^{rr} bone marrow to Friend virus induced erythropoiesis in *Fv-2*^{rr}/*Fv-2*^{ss} bone marrow chimeras.

C. Moroni,[1] R. Papoian,[1] F. Jay,[1] and G. Schumann,[2] [1]Friedrich Miescher-Institut, Basel, Switzerland; [2]Pharmaceuticals Division, Ciba-Geigy Ltd., Basel, Switzerland: Effect on B- and T-cell proliferation by anti-Friend leukemia virus serum.

J. Merregaert, M. Barbacid, and S. A. Aaronson, NCI, National Institutes of Health, Bethesda, Maryland: The genetic map of murine leukemia viruses.

J. Zazra,[1] A. Barkas,[1] M. Reitz,[2] R. C. Gallo,[3] and W. Prensky,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Litton Bionetics, Kensington, Maryland; [3]NCI, National Institutes of Health, Bethesda, Maryland: Recombination between SSV and murine helper virus.

G. Schochetman, L. O. Arthur, B. Altrock, and R. J. Massey, Frederick Cancer Research Center, Frederick, Maryland: Mouse mammary tumor viruses (MMTVs), biological recognition and oncogenicity.

A. B. Vaidya,[1] C. A. Long,[1] and J. B. Sheffield,[2] [1]Hahnemann Medical College, Philadelphia; [2]Temple University, Philadelphia, Pennsylvania: Biochemical and biological studies on Mu-MTV produced by a lymphoma cell line—A possible morphogenesis mutant?

A. Haberman and L. F. Velicer, Dept. of Microbiology and Public Health, Michigan State University, East Lansing: A feline oncornavirus with envelope properties of both feline leukemia virus and RD-114.

S. S. Ved Brat and W. Prensky, Memorial Sloan-Kettering Cancer Center, New York, New York: Monoclonal antibody to FOCMA, the feline oncornavirus-associated cell membrane antigen.

F. K. Yoshimura and J. Yamamura, Fred Hutchinson Cancer Research Center, Seattle, Washington: Characterization of replication-defective mutants of Moloney murine leukemia virus.

J. Links and O. Tol, Netherlands Cancer Institute, Amsterdam: Chemical and spontaneous induction of B-type and C-type retroviruses in mouse kidney cells.

A. Pinter,[1] N. M. Teich,[1] and T. M. Dexter,[2] [1]Imperial Cancer Research Fund, London; [2]Paterson Laboratories, Manchester, England: Characterization of a bone marrow cell line transformed by Friend murine leukemia virus.

M. Pettigrew and M. Linial, Fred Hutchinson Cancer Research Center, Seattle, Washington: Nonrescuable clones of ASV transformed rat cells.

G. S. Martin,[1] W. -H. Lee,[2] Depts of [1]Zoology and [2]Molecular Biology, University of California, Berkeley: Recombination between deletion mutants of Rous Sarcoma virus.

M. F. D. Notter,[1] J. F. Leary,[2] and P. B. Balduzzi,[1] Depts. of [1]Microbiology and [2]Pathology, University of Rochester School of Medicine and Dentistry, New York: Specific and nonspecific receptors for avian tumor viruses.

D. Niwa,[1] and K. Toyoshima,[2] M. Yutsudo,[2] H. Sugiyama,[2] S. Tahara,[2] and T. Sugahara,[1] [1]Faculty of Medicine, Kyoto University; [2]Research Institute for Microbial Diseases, Osaka University, Suita, Japan: Sensitivity to γ-ray of avian sarcoma and murine leukemia viruses.

T. Kawakami and L. Sun, and Comparative Oncology Laboratory, University of California, Davis: Replication-defective gibbon ape leukemia virus infection in human lymphoctyes.

E. Hefti,[1] C. Raineri,[1] S. Panem,[1] E. Clark,[2] and W. E. Giddens, Jr.,[2] [1]Dept. of Pathology, University of Chicago, Illinois; [2]Regional Primate Research Center, University of Washington, Seattle: Comparison of a new macaque viral isolate, macacca nemestrina virus (MNV-1), to type C and type D retroviruses.

J. Löwer, R. Löwer, and R. Kurth, Friedrich-Miescher-Laboratory, Max-Planck-Institut, Tübingen, Federal Republic of Germany: Human teratocarcinoma cells can be induced to produce retrovirus particles.

J. M. Prevost,[1] C. Birkenwald,[1] R. Kettmann,[2] D. Dekegel,[2,3] and A. Burny,[2] and [1]Institut J. Bordet; [2]Universit(e Libre de Bruxelles; [3]Institut Pasteur du Brabant, Bruxelles, Belgium: Molecular characterization of a retrovirus isolated from a human osteosarcoma cell line.

R. C. Mellors, J. W. Mellors, S. Maeda, L. Jerabek, H. Ueno, and I. Zervoudakis, Hospital for Special Surgery and New York Hospital-Cornell University Medical College, New York: Normal human placental antigen is immunologically related to major structural (p26) protein of simian sarcoma associated virus as shown by solid phase enzymoimmunoassay.

D. Hughes,[1] D. Frisby,[1] I. B. Pragnell,[1] F. Smadja-Joffe,[2] B. Klein,[3] K. Vehmeyer,[4] B. Fagg,[5] and W. Ostertag,[1] [1]Beatson Institute, Glasgow, Scotland; [2]ICIG, Villejuif, France; [3]Hopital St. Elo, Montpellier, France; [4]Max-Planck-Institut, Goettingen, Federal Republic of Germany; [5]University of California, Irvine: A murine myeloproliferative virus—molecular and biological analysis.

Session 8: Endogenous Viruses

Chairperson: S. Astrin, Institute for Cancer Research, Philadelphia, Pennsylvania

A. Tereba,[1] S. Astrin,[2] and M. M. C. Lai,[3] [1]Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee; [2]Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania; [3]Dept. of Microbiology, University of Southern California Medical Center, Los Angeles: Nonrandom chromosomal distribution of endogenous retrovirus loci in white leghorn chickens.

F. Hishinuma,[1] P. J. DeBona,[1] S. Astrin,[2] and A. M. Skalka,[1] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Studies on integrated endogenous avian proviruses and their integration sites.

S. Hughes,[1] P. Shank,[2] J. M. Bishop,[3] and H. Varmus,[3] [1]Cold Spring Harbor Laboratory, New York; [2]Division of Biology and Medicine, Brown University, Providence, Rhode Island; [3]Dept. of Microbiology and Immunology, University of California, San Francisco: Organization of the endogenous proviruses of chickens—Implications for origin and expression.

W. Kane, T. Hsu, G. Marden, and J. Taylor, Institute for Cancer Research, Philadelphia, Pennsylvania: Application of a novel strategy for the detection of retrovirus-related RNA sequences in uninfected cells.

S. K. Chattopadhyay,[1] M. R. Lander,[1] E. Rands,[2] and D. R. Lowy,[2] [1]Pediatric Oncology and [2]Dermatology Branches, NCI, National Institutes of Health, Bethesda, Maryland: The structure of endogenous murine leukemia virus DNA genomes.

D. L. Steffen,[1] B. A. Taylor,[2] and R. A. Weinberg,[1] [1]Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Jackson Laboratory, Bar Harbor, Maine: Genetic instability of endogenous murine leukemia proviruses.

A. Berns,[1] W. Quint,[1] H. v.d. Putten,[1] W. Quax,[1] M. Lai,[2] R. Bosselman,[2] and I. Verma,[2] [1]Dept. of Biochemistry, University of Nijmegen, The Netherlands; [2]Salk Institute, La Jolla, California: The characterization of endogenous Moloney and AKR viral sequences.

E. Keshet, Y. Shaul, J. Kaminchick, and H. Aviv, Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Heterogeneity of "virus-like" genes encoding retrovirus-associated 30S RNA and their organization within the mouse genome.

H. A. Young,[1] M. A. Gonda,[1] D. Jones,[2] K. Nagashima,[1] R. Ellis,[2] and E. M. Scolnick,[2] [1]Biological Carcinogenesis Program, Frederick Cancer Research Center, Maryland; [2]Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: Comparison of endogenous rat replication defective (30s) retrovirus and Harvey sarcoma virus DNA — Analysis by restriction enzyme mapping, southern blotting and hetero-duplex conditions.

R. Callahan,[1] E. L. Kuff,[2] K. K. Lueders,[2] and E. Birkenmeier,[1] Laboratories of [1]Viral Carcinogenesis and [2]Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: A structural comparison of the genomes of intracisternal A-particles of *Mus musculus* and the endogenous retrovirus (M432) of *M. Cervicolor.*

Session 9: Mechanisms of Transformation. I.

Chairperson: E. Scolnick, NCI, National Institutes of Health, Bethesda, Maryland

S. Goff, O. N. Witte, and D. Baltimore, Massachusetts Institute of Technology, Cambridge: Analysis of Abelson viral mutants and the homologous cellular gene using cloned Abelson viral DNA.

V. Rotter, O. N. Witte, and D. Baltimore, Massachusetts Institute of Technology, Cambridge: Abelson virus-induced tumors elicit antibodies against a host cell protein, P50.

D. J. Grunwald, K. Miller, P. Jelen, C. Sinaiko, J. Timmins, and R. Risser, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Characterization of Abelson virus lymphomas and antisera made to them.

A. Oliff, D. Linemeyer, S. Ruscetti, and E. Scolnick, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: A subgenomic fragment of molecularly cloned Friend murine leukemia virus (F-MuLV) DNA contains the gene(s) responsible for F-MuLV induced proliferation of hematopoietic precursors.

R. Anand,[1] S. Ruscetti,[2] R.A. Steeves,[1] and F. Lilly,[1] [1]Albert Einstein College of Medicine, Bronx, New York; [2]NCI, National Institutes of Health, Bethesda, Maryland: The spleen focus-forming virus is specifically neutralized by antisera to certain *gag* gene-encoded proteins.

R. A. Bosselman,[1] L. J. L. D. van Griensven,[2] M. Vogt,[1] and I. M. Verma,[1] [1]Tumor Virology Laboratory, Salk Institute, San Diego, California; [2]Dept. of Experimental Pathology, Erasmus University, Rotterdam, The Netherlands: F-SFFV, Mo-MCF virus and xenotropic viruses Balb-virus 2 and NZB virus share *env* gene sequence homology between 1.7 and 2.5 KB for the genomic 3′ end.

B. G. Neel,[1] S. M. Astrin,[2] and W. S. Hayward,[1] [1]Rockefeller University, New York, New York; [2]Institute for Cancer Research, Philadelphia, Pennsylvania: Avian lymphoid leukosis is correlated with the appearance of discrete new RNAs containing viral and cellular genetic information.

G. Payne,[1] L. B. Crittenden,[2] J. M. Bishop,[3] and H. E. Varmus,[3] [1]Dept. of Biochemistry and Biophysics, [3]Dept. of Microbiology, University of California, San Francisco; [2]Regional Poultry Research Laboratory, United States Department of Agriculture, East Lansing, Michigan: The structure of RAV-2 proviruses present in tumors from chickens with RAV-2 induced leukosis.

G. M. Cooper,[1] and P. E. Neiman,[2] [1]Sidney Farber Cancer Institute, Boston, Massachusetts; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Transforming genes of avian leukosis virus-induced neoplasms.

M. Roussel, D. Leprince, S. Saule, M. B. Raes, C. Lagrou, and D. Stehelin, INSERM, Pasteur Institute, Lille, France: Genomic structure of avian myeloblastosis viruses.

B. Vennstrom, D. Sheiness, T. Gonda, and J. Michael Bishop, Dept. of Microbiology, University of California, San Francisco: The cellular progenitor of the oncogene of AEV—Characterization of the DNA locus and its RNA transcripts.

Session 10: Mechanisms of Transformation. II.

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

E. M. Durban and D. Boettiger, Dept. of Microbiology, University of Pennsylvania, Philadelphia: Differential effects of transforming avian RNA tumor viruses on avian macrophages.

T. J. Gonda, D. Sheiness, B. Vennstrom, H. Oppermann, J. M. Bishop, M. G. Moscovici, and C. Moscovici, University of California, San Francisco; and Veterans Administration Hospital, Gainesville, Florida: Avian defective leukemia viruses — Strategies of oncogene expression

P. N. TSICHLIS,[1] J. M. COFFIN,[2] and H. L. ROBINSON,[2] [1]Tufts University School of Medicine, Boston, Massachusetts; [2]Worcester Foundation for Experimental Biology, Massachusetts: Differences between endogenous and exogenous avian oncovirus genomes — Role of the C region.

P. N. TSICHLIS, C. BARKER, and J. M. COFFIN, Tufts University School of Medicine, Boston, Massachusetts: A genetic region that maps between *env* and *src* affects the growth of the transforming avian retroviruses.

H. HANAFUSA, L.-H. WANG, S. M. ANDERSON, R. E. KARESS, R. FELDMAN, M. SHIBUYA, and T. HANAFUSA, Rockefeller University, New York, New York: A new transforming gene of Fujinami sarcoma virus.

W.-H. LEE,[1] K. BISTER,[1] A. PAWSON,[2] T. ROBINS,[1] C. MOSCOVICI,[3] and P. DUESBERG,[1] Departments of [1]Molecular Biology and [2]Zoology, University of California, Berkeley; [3]Virus Research Laboratory, Veterans Administration Hospital, Gainesville, Florida: Fujinami sarcoma virus — An avian RNA tumor virus with a unique transforming gene.

M. YOSHIDA,[1] S. KAWAI,[2] H. SUGIYAMA,[3] and K. TOYOSHIMA,[3] [1]Cancer Institute, Tokyo; [2]Institute of Medical Science, University of Tokyo; [3]Research Institute for Microbial Diseases, Osaka University, Japan: Newly isolated avian sarcoma virus, Y73, contains transforming gene unrelated to *src* sequences of RSV.

J. C. NEIL, M. L. BREITMAN, and P. K. VOGT, Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: A new transforming gene in avian sarcoma.

M. L. LUNG,[1] N. HOPKINS,[1] J. W. HARTLEY,[2] and W. P. ROWE,[2] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: "Identifying and localizing nucleotide sequences that distinguish the genomes of two biologically defined classes of MCF viruses."

W. A. HASELTINE, F. S. PEDERSEN, and R. A. CROWTHER, Harvard Medical School and Sidney Farber Cancer Institute, Boston, Massachusetts: Origin of leukemic AKR viruses.

E. P. REDDY,[1] S. ZAIN,[2] S. TRONICK,[1] K. ROBBINS,[3] E. CANAANI,[1] and S. A. AARONSON,[1] [1]National Cancer Institute, Bethesda, Maryland; [2]University of Rochester Medical School, New York, [3]Hazleton Laboratories, Inc., Vienna, Virginia: Nucleotide sequence analysis of the transforming gene of Moloney sarcoma virus.

Session 11: Genetics and Biology

Chairperson: H. HANAFUSA, Rockefeller University, New York, New York

B. POIESZ, F. RUSCETTI, H. RHO, A. GAZDAR, P. BUNN, J. MINNA, and R. GALLO, NCI, National Institutes of Health, Bethesda, Maryland: Isolation of novel type-C retrovirus particles from cultured and fresh lymphocytes from two patients with cutaneous T-cell lymphomas.

F. RUSCETTI, B. POIESZ, M. REITZ, V. KALYANARAMAN, and R. GALLO, NCI, National Institutes of Health, Bethesda, Maryland: Analysis of novel type-C retroviral particles isolated from cultured and fresh lymphocytes from two patients with cutaneous T-cell lymphomas.

P. JOLICOEUR and E. RASSART, Institut de Recherches Cliniques, Université de Montreal, Quebec, Canada: Studies of linear viral DNA from Fv-1 permissive and resistant mouse cells infected with MuLV.

W. K. YANG,[1] J. O. KIGGANS, JR.,[1] C. Y. OU,[1] D. M. YANG,[1] R. H. BASSIN,[2] R. W. TENNANT,[1] and A. BROWN,[3] [1]Biology Division, Oak Ridge National Laboratory, Tennessee; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]University of Tennessee, Knoxville: Covalently closed circular DNAs of murine ecotropic type C retroviruses — Effects of Fv-1 restriction and inhibition of early protein synthesis.

S. KAWAI,[1] T. KOYAMA,[1] and F. HARADA,[2] [1]Institute of Medical Science, University of Tokyo; [2]National Cancer Center, Tokyo, Japan: A mutant of Rous sarcoma virus which contains 30-40S RNA instead of 70S RNA in virions.

D. W. STACEY,[1] B. R. CULLEN,[1] and L. -H. WANG,[2] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Rockefeller University, New York, New York: Participation of *env* mRNA in active provirus formation and recombination.

L.-H. WANG, M. BECKSON, and H. HANAFUSA, Rockefeller University, New York, New York: Generation of replication-defective sarcoma virus via recombination between viral and cellular sequences.

S. CHEN,[1] F. DURAN-STRUUCK,[2] F. LILLY,[3] and M. DURAN-REYNALS,[2] [1]Dept. of Biological Sciences, Columbia University, New York; Depts. of [2]Pathology and [3]Genetics, Albert Einstein College of Medicine, Bronx, New York: Genetic and nongenetic factors in MuLV expression in the DBA/2 X RF cross.

E. STOCKERT, P. V. O'DONNELL, Y. OBATA, and L. J. OLD, Memorial Sloan-Kettering Cancer Center, New York, New York: Inhibition of AKR leukemogenesis by SMX-1, a dualtropic murine leukemia virus.

R. RISSER, D. J. GRUNWALD, P. JELEN, and J. TIMMINS, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The unusual "anti-self H-2" response of hybrid mice to immunization with a semisyngeneic Abelson virus lymphoma.

J. AZOCAR,[1] M. G. BALDINI,[1] and M. ESSEX,[2] [1]Hematology Research, Memorial Hospital, Pawtucket, Rhode Island; [2]Harvard University School of Public Health, Boston, Massachusetts: Mechanism of incorporation of histocompatibility antigens in the envelope of RNA tumor viruses during virus maturation.

C. A. LONG, A. B. VAIDYA, and U. J. DUMASWALA, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania: Influence of the major histocompatibility complex on susceptibility to MuMTV's.

SV40, Polyoma, and Adenoviruses, August 13 — August 17

Arranged by
Terri Grodzicker, *Cold Spring Harbor Laboratory, New York*
Michael Botchan, *University of California, Berkeley*

353 participants

Session 1: SV40/Polyoma—Transcription

Chairperson: R. KAMEN, Imperial Cancer Research Fund Laboratories, London, England

L. DANDOLO,[1,2] D. BLANGY,[1] and R. KAMEN,[2] [1]Institut de Rescherches Scientifiques sur le Cancer, Villejuif, France; [2]Imperial Cancer Research Fund, London, England: Regulation of polyoma virus transcription in murine teratocarcinoma cells.

C. KAHANA, D. GIDONI, D. CANAANI, and Y. GRONER, Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Transcriptional initiation and subsequent capping of SV40 early and late mRNAs occur in vivo and in vitro at multiple nucleotide sequences including pyrimidines.

D. MATHIS, C. BENOIST, and P. CHAMBON, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité de Biologie Moléculaire et de Génie Génétique de l'INSERM, Strasbourg, France: Comparison of the in vivo and in vitro expression of the SV40 early region.

P. K. GHOSH,[1] Y. GLUZMAN,[2] R. FRISQUE,[2] and P. LEBOWITZ,[1] [1]Dept. of Medicine, Yale University, New Haven, Connecticut; [2]Cold Spring Harbor Laboratory, New York: Studies on the 5' termini of early viral mRNAs in cells infected and transformed by wild-type SV40 and transformed by "origin-defective" mutants.

U. HANSEN, H. HANDA, C. CEPKO, D. TENEN, D. LIVINGSTON, J. MANLEY, M. GEFTER, and P. A. SHARP, Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Transcription in vitro of SV40 DNA.

R. TJIAN, D. RIO, A. ROBBINS, and R. MYERS, Dept. of Biochemistry, University of California, Berkeley: Modulation of SV40 early transcription in vitro by a purified tumor antigen.

E. B. JAKOBOVITS,[1] S. BRATOSIN,[1] O. LAUB,[1] S. SAROGOSTI,[2] M. YANIV,[2] and Y. ALONI,[1] [1]Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel; [2]Dept. of Molecular Biology, Institut Pasteur, Paris, France: Correlations between the structure of the SV40 minichromosome and the regulation of its gene expression.

C. DEVERE-TYNDALL and R. KAMEN, Imperial Cancer Research Fund, London, England: Deletion mutants of polyoma virus DNA in the noncoding sequences between the replication origin and the beginning of the late region.

P. GRUSS and G. KHOURY, NCI, National Institutes of Health, Bethesda, Maryland: Use of recombinant DNA molecules to investigate regulatory signals for mRNA biosynthesis.

M. FITZGERALD and T. SHENK, Dept. of Microbiology, University of Connecticut Health Center, Farmington: The hexanucleotide, AAUAAA, forms part of the signal for polyadenylation of SV40 late mRNAs.

Session 2: Adenoviruses—Transcription

Chairperson: J. FLINT, Princeton University, New Jersey

S.-L. HU[1] and J. L. MANLEY,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Identification of major late promoter of Ad2.

G.-J. WU, Dept. of Microbiology, Emory University School of Medicine, Atlanta, Georgia: Transcription of Ad2 DNA by calf thymus RNA polymerase II and KB cellular RNA polymerase II in a soluble nuclear extract.

V. W. YANG,[1] S. J. FLINT,[1] M. R. LERNER,[2] and J. A. STEITZ,[2] [1]Dept. of Biochemical Sciences, Princeton University, New Jersey; [2]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: The role of small nuclear ribonucleoproteins (snRNPs) in the splicing of Ad2 early RNA precursors.

A. J. Berk,[1] T. F. Osborne,[1] R. E. Schell,[1] and S. J. Berget,[2] [1]Molecular Biology Institute, University of California, Los Angeles; [2]Dept. of Biochemistry, Rice University, Houston, Texas: Upstream DNA sequence required for expression of the Ad2 pre-early genes in HeLa cells.

M. C. Wilson, F. Cross, and J. E. Darnell, Jr., Rockefeller University, New York, New York: Metabolism of E1A and E1B mRNA species during adenovirus lytic cycle.

A. Babich and J. R. Nevins, Rockefeller University, New York, New York: The stability of the early adenovirus mRNAs is controlled by the 72K DNA binding protein.

A. R. Shaw and E. B. Ziff, Rockefeller University, New York, New York: Transcripts from the Ad2 major late promoter are initiated at early times and processed to yield mRNA.

G. P. Thomas and M. B. Mathews, Cold Spring Harbor Laboratory, New York: DNA replication obligatorily precedes late gene expression of adenovirus and SV40.

H. Persson, H.-J. Monstein, and L. Philipson, Biomedical Center, University of Uppsala, Sweden: A translational control of adenovirus early gene expression.

R. Guilfoyle and R. Weinmann, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Deletion mutants around the control regions of VA_1 RNA.

D. M. Fowlkes and T. Shenk, Dept. of Microbiology, University of Connecticut Health Center, Farmington: In vitro analysis of the adenovirus VA RNA polymerase III initiation site.

Session 3: Poster Session—SV40 and Polyoma

J. C. Alwine[1] and G. Khoury,[2] [1]Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia; [2]NCI, National Institutes of Health, Bethesda, Maryland: The SV40-associated small RNA (SAS-RNA)— Mapping on the SV40 genome and characterization of its synthesis.

E. Amtmann and G. Sauer, Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Transformation by episomal papilloma virus DNA.

M. Bastin and C. Parent, Dépt. de Microbiologie, Centre Hospitalier Universitaire de Sherbrooke, Quebec, Canada: Tumorigenicity of recombinant plasmids containing defective polyoma virus DNA molecules.

E. A. Baumann, D. Stedman, A. Fuks, and R. Hand, McGill Cancer Centre, Montreal, Quebec, Canada: Comparison of the immunoreactivity of SV40 large T antigen and D2 hybrid protein.

P. Beard, M. Kaneko, and K. Nyfeler-Schriefer, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: The carcinogen acetylaminofluorene binds preferentially to a specific region on the SV40 chromosome.

M. A. R Bender and W. W. Brockman, Dept. of Microbiology, University of Michigan, Ann Arbor: SV40 sequences in untransformed mouse cells.

A. Ben-Zeev, M. Horowitz, H. Skolnik, R. Abulafia, O. Laub, and Y. Aloni, Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: SV40-specific RNA is associated with the cytoskeletal framework.

J. Buitenwerf, W. de Jong, and J. van der Noordaa, Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, Mauritskade, The Netherlands: Characterization of SV40-transformed human liver cells.

K. Chandrasekaran and P. T. Mora, NCI, National Institutes of Health, Bethesda, Maryland: Synthesis of 55,000-m.w. cellular protein as a correlate of the SV40 early gene expression and not of tumorigenicity in the mouse.

D. Cosman and M. J. Tevethia, Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Characterization of an atypical *tsA* mutant of SV40.

L. Crawford and D. Pim, Imperial Cancer Research Fund, London, England: The presence of 53K protein in human tumor cell lines.

L. Dailey, V. Colantuoni, R. Fenton, G. Della Valle, and C. Basilico, Dept. of Pathology, New York University School of Medicine, New York: Excision and the evolution of polyoma-transformed cell lines.

L. Daya-Grosjean, A. Sarasin, and R. Monier, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: The effect of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on cell transformation by SV40 mutants.

R. J. Deans and W. R. Folk, Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Cloning of polyoma virus tumor antigen coding sequences in *E. coli*.

L. Delbecchi and P. Bourgaux, Dépt. de Microbiologie, Centre Hospitalier Universitaire de Sherbrooke, Quebec, Canada: Absence of complementation between resident and superinfecting polyoma virus genomes in permissive transformed cells.

W. Deppert,[1] E. Gurney,[2] and R. Harrison,[2] [1]Dept. of Biochemistry, University of Ulm, Federal Republic of Germany; [2]Dept. of Biology, University of Utah, Salt Lake City: Monoclonal antibodies against SV40 tumor antigens—Characterization of antigenic binding sites using Ad2/SV40 hybrid viruses.

R. G. Fenton and C. Basilico, Dept. of Pathology, New York University School of Medicine, New York: Transcription in polyoma-transformed rat cells and their cured derivatives.

M. M. Fluck,[1] R. Shaikh,[2] and T. L. Benjamin,[2] [1]Microbiology Department, Michigan State University, East Lansing; [2]Pathology Department, Harvard Medical School, Boston, Massachusetts: The role of the hr-t and ts-a functions of polyoma virus.

E. Georges, M. Vasseur, J. Aghion, and D. Blangy, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Control of polyoma virus expression in murine embryonal carcinoma cells.

R. D. Gerard and W. A. Scott, Dept. of Biochemistry, University of Miami, Florida: Deletion mutant mapping of the nuclease-sensitive region in SV40 chromatin.

P. K. GHOSH,[1] J. MERTZ,[2] A. BARKAN,[2] S. WEISSMAN,[3] M. PIATAK,[3] T. SHENK,[4] P. ROY,[3] and P. LEBOWITZ,[1] Depts. of [1]Medicine and [3]Human Genetics, Yale University, New Haven, Connecticut; [2]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; [4]Dept. of Microbiology, University of Connecticut Medical Center, Farmington: Structure of late mRNAs produced by SV40 late leader deletion mutants.

M. GRIGORYAN, D. KRAMEROV, and E. LUKANIDIN, Institute of Molecular Biology, Academy of Sciences of USSR, Moscow: Initiation points for DNA replication in mammalian cells.

J. D. HARE,[1] B. J. POMERANTZ,[2] and E. K. THOMAS,[3] [1]Dept. of Microbiology, University of Rochester Medical Center, New York; [2]McGill University, Montreal, Quebec, Canada; [3]Dept. of Pathology, Harvard University, Cambridge, Massachusetts: A mutation in polyoma virus affecting expression of late functions at a post transcriptional level.

C. A. HEILMAN, M.-F. LAW, and P. M. HOWLEY, NCI, National Institutes of Health, Bethesda, Maryland: Cloning of human papillomavirus genomic DNAs and analysis of homologous polynucleotide sequences.

W. C. HEISER and W. ECKHART, Tumor Virology Laboratory, Salk Institute, San Diego, California: Transcription in polyoma-virus-infected 3T6 cells.

K. HERCULES, E. BURCH-JAFFE, and B.J. RYDER, Dept. of Biological Chemistry and the Molecular Biology Institute, University of California, Los Angeles: Metastasis of mutant SV40-induced tumors.

A. HORWICH,[1] A. H. KOOP,[2] and W. ECKHART,[1] [1]Tumor Virology Laboratory, [2]Regulatory Biology Laboratory, Salk Institute, San Diego, California: Expression of the polyoma small-T-antigen gene in *E. coli*.

H. C. ISOM,[1] M. J. TEVETHIA,[1] J. M. TAYLOR,[1] and J. W. KREIDER,[1,2] Depts. of [1]Microbiology and [2]Pathology, Pennsylvania State University College of Medicine, Hershey: Tumorigenicity of SV40-transformed rat hepatocytes.

P. KAHN and S. SHIN, Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Tumor formation by SV40-transformed human cells in immunodeficient mice.

R. KAMEN,[1] J. FAVALORO,[1] P. JAT,[1] R. TREISMAN,[1] and W. FOLK,[2] [1]Imperial Cancer Research Fund, London, England; [2]University of Michigan, Ann Arbor: The 5′ ends of polyoma virus early-region transcripts.

S. LAVI and S. ETKIN, Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Induction of SV40 DNA synthesis in transformed cells treated with chemical carcinogens.

M.-F. LAW, D. R. LOWY, I. DVORETSKY, and P. M. HOWLEY, NCI, National Institutes of Health, Bethesda, Maryland: Transformation of mouse cells by cloned BPV-1 DNA segments and analysis of the state of the viral genome.

S. G. LAZAROWITZ, Dept. of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Properties of an SV40 mutant with transposed T antigen and VP1 genes.

A. M. LEWIS, JR. and J. L. COOK, NIAID, National Institutes of Health, Bethesda, Maryland: The implications of the different tumor-inducing capacities of Ad2 and SV40-transformed hamster cells.

A. LO, C. PRIVES, M. VERDERAME, and R. POLLACK, Dept. of Biological Sciences, Columbia University, New York, New York: Detection of 54K-NVT in SV40-free tumor cells by in vitro formation of complex with SV40 large T.

M. A. MARCHIONNI and D. J. ROUFA, Kansas State University, Manhattan: Replication of viral DNA sequences integrated within the chromatin of SV40-transformed CHL cells.

E. MAY,[1] J. M. JELTSCH,[2] and F. GANNON,[2] [1]Institut de Recherches Scientifiques sur le Cancer, Villejuif; [2]Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, INSERM, Institut de Chimie Biologique, Strasbourg, France: Organization of an integrated SV40 genome coding for a 115K super T antigen.

E. MENDELSOHN, N. BARAN, and H. MANOR, Dept. of Biology, Technion, Haifa, Israel: The integration sites of polyoma virus DNA in an inducible line of polyoma-virus-transformed cells.

J. E. MERTZ, J. J. MILLER, L. A. TRIMBLE, G. Z. HERTZ, and A. BARKAN, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: A small regulatory region of SV40 DNA is both necessary and sufficient for viral RNA synthesis.

B. MILAVETZ, L. DIBERARDINO, and J. A. HUBERMAN, Dept. of Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York: A physical identification of proteins associated with SV40 chromosomes.

T. J. MILLER, D. L. STEPHENS, G. Z. HERTZ, and J. E. MERTZ, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Template structural requirements for SV40 RNA synthesis by RNA polymerase II in *Xenopus* oocytes.

T. MIYAMURA, K. YOSHIIKE, H. W. CHAN, and K. K. TAKEMOTO, NIAID, National Institutes of Health, Bethesda, Maryland: Characterization of JC papovavirus adapted to growth in human embryonic kidney cells.

P. E. MONTANDON[1] and N. H. ACHESON,[1,2] [1]Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland; [2]Dept. of Microbiology, McGill University, Montreal, Quebec, Canada: Mapping DRB-resistant 3S-7S polyoma viral RNAs.

M. MONTENARH, K. PALME, and R. HENNING, Dept. of Biochemistry, University of Ulm, Federal Republic of Germany: Phosphorylation of SV40 T antigen and its affinity to DNA and other polyanions.

K. NESS and W. A. SCOTT, Dept. of Biochemistry, University of Miami School of Medicine, Florida: Mitomycin-induced excision of a specific viral sequence from hamster cells transformed by a variant of SV40.

L. NORKIN, Dept. of Microbiology, University of Massachusetts, Amherst: Host- cell factors in the generation of defective SV40.

M. M. PATER, A. PATER, and G. DI MAYORCA, College of Medicine and Dentistry, New Jersey Medical School, Newark: The arrangement of the genome of the human papovavirus, RFV.

C. A. PETIT,[1] F. DAUTRY,[1] G. CARMICHAEL,[2] and J. FEUNTEUN,[1] [1]Institut de Recherches Scientifiques sur le Cancer, Villejuif, France; [2]Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Multiple mutations in the SV40 early region.

M. Piatak,[1] S. M. Weissman,[1] P. Roy,[1] K. N. Subramanian,[2] and L. Norkin,[3] [1]Dept. of Human Genetics, Yale University, New Haven, Connecticut; [2]Dept. of Microbiology, University of Illinois, Chicago; [3]Dept. of Microbiology, University of Massachusetts, Amherst: Studies on the biogenesis of SV40 late mRNA.

R. S. Powers and R. Pollack, Dept. of Biological Sciences, Columbia University, New York, New York: SV40 transformation and establishment—Absolute and conditional loss of anchorage and hormone requirements for growth.

M. Rassoulzadegan,[1] F. Birg,[2] L. Trejo-Avila,[1] P. Gaudray,[1] and F. Cuzin,[1] [1]Unité de l'INSERM, Marseille; [2]Centre de Biochimie du CNRS, Nice, France: Is the polyoma virus 56K middle T antigen sufficient for the maintenance of transformation?

D. R. Rawlins and N. Muzyczka, Dept. of Immunology and Medical Microbiology, University of Florida Medical College, Gainesville: Construction of a specific amber codon in the SV40 T antigen gene by site-directed mutagenesis.

F. Rentier-Delrue,[1] M. A. Israel,[2] K. K. Takemoto,[2] and P.M. Howley,[1] [1]NCI, [2]NIAID, National Institutes of Health, Bethesda, Maryland: Molecular cloning of the human polyoma virus JC genome.

T. M. Roberts, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: General methods for expressing eukaryotic genes in *E. coli*.

R. Seif, NIAMDD, National Institutes of Health, Bethesda, Maryland: Transformation by polyoma virus or SV40 and transformation by chemical carcinogens.

G. Spangler, J. Griffin, H. Rubin, and D. Livingston, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Identification and characterization of a new, low-molecular-weight SV40 T antigen in a line of SV40-transformed cells.

B. Steinberg and K. Smith, Dept. of Biological Sciences, Columbia University, New York, New York: SV40 transformation diminishes fibroblast-collagen gel contractility.

R. T. Su, G. B. Ogden, and C. Jones, Dept. of Microbiology, University of Kansas, Lawrence: Biological activity of isolated SV40 nucleoprotein complex.

B. Sylla, P. Bourgaux, M. Bastin, and D. Bourgaux-Ramoisy, Dépt. de Microbiologie, Centre Hospitalier Universitaire de Sherbrooke, Quebec, Canada: A unique recombinant between polyoma and mouse DNAs.

L. C. Tack and M. L. DePamphilis, Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: SV40 DNA sequence and nucleosome phasing.

C. S. Thummel, T. L. Burgess, and R. Tjian, Dept. of Biochemistry, University of California, Berkeley: Characterization of SV40 small T antigen overproduced in bacteria.

K. Trevor and J. M. Lehman, Dept. of Pathology, University of Colorado Health Sciences Center, Denver: Fate of the polyoma viral genome in F9 embryonal carcinoma and its differentiated progeny.

C. Wake and J. Wilson, Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas: Defined oligomeric SV40 DNA—A sensitive probe of general recombination in somatic cells.

W. Waldeck and G. Sauer, Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Papovavirus chromatin-associated cellular endonuclease which introduces one double-strand cut in superhelical DNA.

G. Walter,[1] K.-H. Scheidtmann,[1] A. Carbone,[1] A. P. Laudano,[2] and R. F. Doolittle,[2] [1]Institut für Immunbiologie der Albert-Ludwigs-Universität, Freiburg, Federal Republic of Germany; [2]Dept. of Chemistry, University of California, San Diego: Antibodies specific for the carboxy- and amino-terminal regions of SV40 large T antigen.

F. O. Wettstein and J. G. Stevens, Dept. of Microbiology and Immunology, University of California School of Medicine, Los Angeles: Persistence of Shope papilloma virus DNA in an episomal state in nonvirus-producing tumors.

Session 4: Workshop on DNA Sequences

Organizer: T. Gingeras, Cold Spring Harbor Laboratory, New York

R. Chanda,[1] E. Lifson,[1] E. Lee,[1] M. Yablonski,[1] N. Stow,[2] and S. Zain,[1] [1]Dept. of Microbiology and Cancer Center, University of Rochester, New York; [2]Cold Spring Harbor Laboratory, New York: Studies on early gene block III region in Ad2.

J. A. Engler, B. A. Kilpatrick, L. T. Chow, and T. R. Broker, Cold Spring Harbor Laboratory, New York: The sequence of Ad3 and Ad7 DNAs encoding the late promoters.

D. Sciaky, I. Battle, P. Bullock, R. E. Gelinas, T. R. Gingeras, M. Kelly, R. J. Roberts, and C. Yen, Cold Spring Harbor Laboratory, New York: Ad2 sequences.

Session 5: SV40 and Polyoma—Transformation

Chairperson: C. Basilico, New York University School of Medicine, New York

D. Dorsky and T. Benjamin, Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Polyoma mutants with altered T antigens.

K. Chowdhury, D. F. Vanderryn, and M. A. Israel, NIAID, National Institutes of Health, Bethesda, Maryland: A possible role for altered polyoma large T antigens in tumorigenesis.

A.-M. MES and J. A. HASSELL, Dept. of Microbiology, McGill University, Montreal, Quebec, Canada: Mutations in cloned polyoma viral DNA which alter transformation.

U. NOVAK and B. E. GRIFFIN, Imperial Cancer Research Fund, London, England: Transformation of rat-1 cells with subgenomic fragments of polyoma virus DNA.

G. DELLA-VALLE, R. G. FENTON, and C. BASILICO, Dept. of Pathology, New York University School of Medicine, New York: Role of polyoma large T antigen in the initiation of transformation.

Y. GLUZMAN, Cold Spring Harbor Laboratory, New York: Transformed permissive cells which support the replication of early SV40 mutants.

M. BOTCHAN and W. GISH, Dept. of Molecular Biology, University of California, Berkeley: Defective T antigens in human transformed cell lines.

M. POLVINO, M. KRICKER, P. CAMPBELL, and C. N. COLE, Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Mutants with deletions near the 3' end of SV40 have altered growth and transformation properties.

M. VERDERAME, D. ALCORTA, and R. POLLACK, Dept. of Biological Sciences, Columbia University, New York, New York: Disorganization of F-actin in intermediate SV40-transformed cells requires expression of viral antigens.

P. L. KAPLAN,[1] W. C. TOPP,[2] and B. OZANNE,[1] [1]University of Texas Health Science Center, Dallas; [2]Cold Spring Harbor Laboratory, New York: Anchorage-independent SV40 or polyoma-virus-transformed rat cells produce transforming factors.

Session 6: SV40 and Polyoma—Integration

Chairperson: M. BOTCHAN, University of California, Berkeley

P. MOUNTS and T. J. KELLY, JR., Dept. of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: SV40 integration—Evidence for rearrangement of viral and cellular sequences.

J. STRINGER, Cold Spring Harbor Laboratory, New York: Analysis of integrated SV40 DNA cloned from the transformed rat cell lines SVRE9 and SVRE17.

C. E. CLAYTON, M. LOVETT, and P. W. J. RIGBY, Dept. of Biochemistry, Imperial College of Science and Technology, London, England: The structure and expression of the integrated viral DNA in SV40-transformed mouse cells.

V. COLANTUONI, L. DAILEY, and C. BASILICO, Dept. of Pathology, New York University School of Medicine, New York: Amplification of integrated viral DNA sequences in polyoma-transformed cells.

L. LANIA, A HAYDAY, and M. FRIED, Imperial Cancer Research Fund, London, England: Analysis of polyoma virus transformation.

B. R. DHRUVA,[1] B. J. BYRNE,[1] T. SHENK,[2] and K. N. SUBRAMANIAN,[1] [1]University of Illinois Medical Center, Chicago; [2]University of Connecticut Health Center, Farmington: Integration in vitro into SV40 DNA of a sequence that resembles a certain family of interspersed repeated sequences.

H. MANOR,[1] A. NEER,[1] M. ZABEAU,[2] and N. BARAN,[1] [1]Dept. of Biology, Technion, Haifa, Israel; [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Cloning of chromosomally associated polyoma virus DNA from an inducible line of polyoma-virus-transformed cells in a λ bacteriophage vector.

V. B. REDDY and S. M. WEISSMAN, Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Reconstruction of small T antigen gene of SV40 to study transformation of cells using TK marker.

D. MURPHY, J. HISCOTT, and V. DEFENDI, Dept. of Pathology, New York University Medical Center, New York: Instability of integrated viral DNA in SV40-transformed mouse cells.

G. BLANCK, A. LO, and R. POLLACK, Dept. of Biological Sciences, Columbia University, New York, New York: Instability of SV40 integration in 3T3 cell lines which express incomplete transformed phenotypes.

Session 7: Poster Session—Adenoviruses

E. S. ALLEBACH,[1] R. J. MANNINO,[1] W. A. STROHL,[1] and K. RASKA, JR.,[1,2] Depts. of [1]Microbiology and [2]Pathology, Rutgers Medical School, Piscataway, New Jersey: Stimulation of DNA synthesis by an Ad12 T-antigen fraction containing protein kinase activity and encapsulated in liposomes.

C. C. BAKER and E. B. ZIFF, Rockefeller University, New York, New York: Heterogeneous 5' termini of Ad2 mRNAs.

M.-H. BINGER,[1] R. GALOS,[2] J. WILLIAMS,[2] and S. J. FLINT,[1] [1]Dept. of Biochemical Sciences, Princeton University, New Jersey; [2]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburg, Pennsylvania: Expression of the Ad5 ts36 region (early region 5).

M. BROWN and J. WEBER, Dépt. de Microbiologie, Centre Hospitalier Universitaire de Sherbrooke, Quebec, Canada: Corelike organization of intranuclear Ad2 chromatin late in infection.

J. BRUSCA, Q. S. KAPOOR, and G. CHINNADURAI, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Insertion and deletion mutants of Ad2.

L. CARLOCK and N. C. JONES, Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: The isolation and characterization of a mutant of Ad5 that alters a single transcript in region 1A.

T. H. CARTER,[1] C. S. H. YOUNG,[2] and P. B. FISHER,[2] [1]Dept. of Biological Sciences, St. John's University, Jamaica, New York; [2]College of Physicians and Surgeons, Columbia University, New York, New York: TPA alters the transcription program of human Ad5 in HeLa cells.

C. Cepko and P. A. Sharp, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Monoclonal antibodies to Ad2 proteins—Studies on the interaction of hexon and 100K protein using α hexon and α 100K monoclonal antibodies.

L. T. Chow and J. B. Lewis, Cold Spring Harbor Laboratory, New York: Identification of early region 2B RNA and protein.

H. Colandre, L. Aiello, and R. Weinmann, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Effects of adenovirus infection on the distribution of 5' ends cellular hn RNA.

E. Daniell, M. Fedor, and L. Burg, Dept. of Molecular Biology, University of California, Berkeley: Replication and virus production of adenovirus and SV40 in cells inhibited by *n*-butyrate.

R. Deuring and W. Doerfler, Institute of Genetics, University of Cologne, Federal Republic of Germany: An unusual recombinant between human Ad12 DNA and human cell DNA.

F. Eggerding, Dept. of Pathology, University of California, Los Angeles: Processing pathway of Ad2 early mRNA precursors.

H. Esche and M. Brötz, Institute of Genetics, University of Cologne, Federal Republic of Germany: Expression of viral sequences in adenovirus-transformed cells.

A. Fire, J. Manley, K. Berkner, M. Gefter, and P. A. Sharp, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Transcription of adenovirus early regions in soluble whole-cell extracts.

N. W. Fraser,[1] C. C. Baker,[2] M. A. Moore,[2] and E. B. Ziff,[2] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Rockefeller University, New York, New York: The poly-A-sites of Ad2 transcription units.

C. Gambke and W. Deppert, Dept. of Biochemistry, University of Ulm, Federal Republic of Germany: Ad2 100K-protein-mediated transport of hexon into the nuclei of infected cells.

C. Goding, C. H. Shaw, G. E. Blair, and W. C. Russell, National Institute for Medical Research, London, England: ADP ribosylation associated with in vitro systems replicating adenovirus DNA.

D. E. Groff and E. Daniell, Dept. of Molecular Biology, University of California, Berkeley: Ad3 early RNA synthesis in human and hamster cells.

S. Hashimoto, M. Pursley, W. Wold, and M. Green, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Transcription initiation sites of Ad2 early RNA.

H. Jochemsen, P. J. van den Elsen, P. I. Schrier, and A. J. van der Eb, Laboratory for Physiological Chemistry, University of Leiden, The Netherlands: Studies on the role of T antigens of Ad12 and Ad5 in transformation and oncogenicity.

G. Khittoo and J. Weber, Dépt. de Microbiologie, Centre Hospitalier Universitaire de Sherbrooke, Quebec, Canada: The nature of the DNA associated with incomplete particles of Ad2.

C. Laughlin, C. Marcus, F. Jay, and B. Carter, NIAMDD, National Institutes of Health, Bethesda, Maryland: The adenovirus helper function for AAV.

C. Lawrence, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Regulation of fiber mRNA levels in HeLa cells infected with Ad2 and Ad2-SV40 hybrid viruses.

S. Mak and I. Mak, Dept. of Biology, McMaster University, Hamilton, Ontario, Canada: Mutants of adenoviruses defective in initiation of transformation.

T. Matsuo, S. Hashimoto, K. Brackmann, J. Symington, W. Wold, and M. Green, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Ad2 early polypeptides in Ad2-infected and -transformed cells—Cell free translation, immunoprecipitation, 2D-gel electrophoresis, and peptide map analysis.

A. Mirza and J. Weber, Dépt. de Microbiologie, Centre Hospitalier Universitaire de Sherbrooke, Quebec, Canada: Structure of adenovirus chromatin.

J. R. Nevins and M. C. Wilson, Rockefeller University, New York, New York: A portion of the major late adenovirus transcription unit is expressed during early infection.

S. Pincus, J. McDonough, and D. Rekosh, Dept. of Biochemistry, State University of New York, Buffalo: Studies on the protein covalently bound to the ends of adenovirus DNA.

R. Rosenthal, C. Goldenberg, S. Bhaduri, and H. J. Raskas, Dept. of Pathology, Washington University School of Medicine, St. Louis, Missouri: Synthesis of mRNAs from Ad2 early region 2.

M. Rossini, G. J. Jonak, and R. Baserga, Fels Research Institute, Temple University, Philadelphia, Pennsylvania: The role of Ad2 early genes in the induction of cellular DNA synthesis in resting cells.

D. T. Rowe,[1] P. E. Branton,[2] S. Bacchetti,[2] and F. L. Graham,[1,2] Depts. of [1]Biology and [2]Pathology, McMaster University, Hamilton, Ontario, Canada: Studies on the proteins involved in oncogenic transformation by human Ad5.

W. C. Russell,[1] G. Patel,[1] and I. Sharp,[2] [1] National Institute for Medical Research; [2]Public Health Laboratory Service, London, England: Characterization of adenovirus monoclonal antibodies.

K. Segawa, I. Saito, K. Shiroki, and H. Shimojo, Institute of Medical Science, University of Tokyo, Japan: In vitro translation of Ad12-specific mRNA complementary to the transforming gene.

K. Segawa, K. Shiriki, and H. Shimojo, Institute of Medical Science, University of Tokyo, Japan: Phosphorylation of Ad12 T antigen.

J. E. Smart,[1] J. B. Lewis,[1] M. B. Mathews,[1] M. L. Harter,[2] and C. W. Anderson,[3] [1]Cold Spring Harbor Laboratory, New York; [2]College of Medicine and Dentistry, New Jersey Medical School, Newark; [3]Brookhaven National Laboratory, Upton, New York: Adenovirus early proteins—Correlation of amino acid sequence and viral DNA sequence.

D. Spector, D. Halbert, and H. Raskas, Dept. of Pathology, Washington University School of Medicine, St. Louis, Missouri: Expression of integrated viral sequences in adenovirus transformed 293 cells infected with deletion mutants.

S. Stabel, D. Eick, I. Kuhlmann, S. Schirm, and W. Doerfler, Institute of Genetics, University of Cologne, Federal Republic of Germany: Integration and expression of Ad12 DNA in transformed cells, in tumor cells, and revertants.

J. S. Sussenbach, M. G. Kuijk, W. Kruijer, and A. W. M. Rijnders, Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands: Characterization of viral proteins involved in adenovirus DNA replication.

C. Tibbetts, C. Green, S. Sharnick, and L. Kosturko, Dept. of Microbiology, University of Connecticut School of Medicine, Farmington: Cloning, manipulation, and analysis of left-end DNA from Ad3.

M. Tigges and H. J. Raskas, Dept. of Pathology, Washington University School of Medicine, St. Louis, Missouri: Analysis of Ad2 early region 4 RNAs and their polypeptide products.

G. A. Zorn and C. W. Anderson, Biology Dept., Brookhaven National Laboratory, Upton, New York: Expression of fiber in Ad2-infected monkey-human cell hybrids and reconstructed cells.

Session 8: SV40 and Adenoviruses—Replication

Chairperson: M. Horwitz, Albert Einstein College of Medicine, New York, New York

R. M. Myers,[1] R. C. Williams,[2] and R. Tjian,[1] Depts. of [1]Biochemistry and [2]Molecular Biology, University of California, Berkeley: Construction and analysis of SV40 origin mutants defective in T-antigen binding and DNA replication.

R. McKay[1] and D. DiMaio,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: SV40 T-antigen binding to the DNA of regulatory mutants of SV40.

C. Sumida-Yasumoto and G. Khoury, NCI, National Institutes of Health, Bethesda, Maryland: In vitro replication of SV40 DNA—SV40 T-antigen and form-I DNA replication with initiation.

R. T. Hay and M. L. DePamphilis, Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Preferred sequences that initiate Okazaki fragments on SV40 DNA.

N. D. Stow, Cold Spring Harbor Laboratory, New York: The effect of additional DNA sequences linked to the left-hand end of the adenovirus genome on DNA replication.

S. V. Desiderio, M. D. Challberg, and T. J. Kelly, Jr., Dept. of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Adenovirus terminal protein—Structure of the linkage to DNA and characterization of a novel form bound to nascent DNA strands.

M. Longiaru,[1] H. Ariga,[1] B. Friefeld,[1] M. Horwitz,[1] J.-E. Ikeda,[2] J. Lichy,[2] T. Enomoto,[2] and J. Hurwitz,[2] Depts. of [1]Microbiology-Immunology and [2]Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Adenovirus DNA synthesis—Evidence for multiple rounds of initiation in vitro.

M. T. Hsu and D. J. Wolgemuth, Dept. of Molecular Cell Biology, Rockefeller University, New York, New York: Electron microscope analyses of Ad2 transcription, replication, and recombination intermediates isolated from Ad2-infected HeLa cells.

R. L. Fatt, H. Ezoe, and S. Mak, Dept. of Biology, McMaster University, Hamilton, Ontario, Canada: DNA degradation by mutants of adenoviruses.

L. Burg and E. Daniell, Dept. of Molecular Biology, University of California, Berkeley: Core proteins from Ad5 induce superhelical turns in closed circular DNA.

Session 9: Adenoviruses—Expression of Integrated Genomes

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

D. F. Klessig and T. Grodzicker, Cold Spring Harbor Laboratory, New York: Expression of adenovirus genes in human cells cotransformed with the HSV-1 tk gene and Ad2 DNA.

L. Vardimon and W. Doerfler, Institute of Genetics, University of Cologne, Federal Republic of Germany: Integration and methylation patterns of Ad2 DNA in transformed hamster cells.

W. Wold, M. Green, K. Brackmann, and M. Cartas, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Integration and expression of Ad2 early genes.

M. W. van Maarschalkerweerd, L. Visser, A. M. C. B. Reemst, A. D. C. Wassenaar, J. S. Sussenbach, and T. H. Rozijn, Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands: Arrangements and expression of integrated sequences of Ad5 DNA in transformed cells.

K. Fujinaga, Y. Sawada, and T. Kimura, Cancer Research Institute, Sapporo Medical College, Japan: Detailed mappings of Ad12 mRNAs transcribed from the transforming region in infected cells and in transformed cells.

C. Paraskeva,[1] P. H. Gallimore,[1] and A. R. Dunn,[2] [1]Dept. of Cancer Studies, University of Birmingham Medical School, England; [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Properties of a cloned rat liver epithelial cell strain and its adenovirus-transformed derivatives.

B. Lenk, T. Storch, and J. Maizel, NICHHD, National Institutes of Health, Bethesda, Maryland: Association of early adenovirus proteins with the cytoskeleton of HeLa cells.

J. B. Lewis,[1] H. Esche,[2] and M. B. Mathews,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Genetics Dept., University of Cologne, Federal Republic of Germany: Regulation of adenovirus early gene expression.

R. Ricciardi,[1] R. Jones,[1] C. Cepko,[2] P. Sharp,[2] and B. Roberts,[1] [1]Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts; [2]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: An acidic protein encoded within the E1A region of Ad5 DNA is required for the expression of adjacent genes.

Session 10: SV40 and Polyoma—T-Antigen-Associated Cellular Proteins

Chairperson: A. Levine, State University of New York Medical School, Stony Brook

D. P. Lane, Dept. of Zoology and Cancer Research Campaign Unit of Eukaryotic Molecular Genetics, Imperial College, London, England: A cellular protein that shares an antigenic determinant with SV40 large T detected by a monoclonal antibody.

P. Tegtmeyer, State University of New York, Stony Brook: Identification of a cellular protein with antigenic and structural similarities to SV40 T antigen.

E. Harlow and L. Crawford, Imperial Cancer Research Fund, London, England: The complex of SV40 large T antigen with a host 53K protein in monkey cells.

W. Maltzman, D. I. H. Linzer, M. Oren, N. Reich, and A. J. Levine, Dept. of Microbiology, State University of New York, Stony Brook: Characterization of 54K murine nonviral tumor antigens seen in SV40 transformants and other murine cells.

F. McCormick,[1] R. Clarke,[2] and R. Tjian,[2] [1]Translation Laboratory, Imperial Cancer Research Fund, London, England; [2]Dept. of Biochemistry, University of California, Berkeley: Binding of purified SV40 large T antigen to a cellular 53K protein in vitro.

D. S. Greenspan and R. B. Carroll, Dept. of Pathology, New York University School of Medicine, New York: Effects of differential phosphorylation of SV40 T antigen on 48K host tumor antigen and DNA binding.

W. A. Scott and J. P. Hartmann, Dept. of Biochemistry, University of Miami School of Medicine, Florida: Endonuclease-sensitive sites in various SV40 nucleoprotein structures.

G. W. Robinson[1] and L. M. Hallick,[2] Depts. of [1]Biochemistry and [2]Microbiology, University of Oregon Health Sciences Center, Portland: Detection of a nucleosome-free replication origin in SV40 minichromatin by the technique of radioactive-psoralen labeling.

S. Saragosti, G. Moyne, and M. Yaniv, Dépt. de Biologie Moléculaire, Institut Pasteur, Paris, France: Absence of nucleosomes in a fraction of SV40 chromatin between the origin of replication and the region coding for the late leader RNA.

O. Sundin and A. Varshavsky, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers attached to the nuclear matrix.

Session 11: SV40 and Polyoma—T Antigens

Chairperson: P. Tegtmeyer, State University of New York Medical School, Stony Brook

W. Eckhart, M. A. Hutchinson, and T. Hunter, Tumor Virology Laboratory, Salk Institute, San Diego, California: Tyrosine phosphorylation in polyoma T antigens and transformed cells.

B. Schaffhausen and T. Benjamin, Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Studies on middle T antigens of polyoma virus.

P. Gaudray, M. Center, P. Clertant, and F. Cuzin, Centre de Biochimie du CNRS, Nice, France: ATPase activity and binding to chromatin of a polyoma virus early protein.

S. E. Light,[1] F. Hirata,[2] and Y. Ito,[1] [1]NIAID, [2]NIMH, National Institutes of Health, Bethesda, Maryland: Characterization of middle T antigen in the plasma membrane of polyoma-virus-transformed mouse cells.

R. Henning, J. Lange-Mutschler, and W. Deppert, Dept. of Biochemistry, University of Ulm, Federal Republic of Germany: Serological demonstration of SV40 T-antigen-related cell-surface antigens on SV40-transformed cells.

H. R. Soule and J. S. Butel, Dept. of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: Detection and characterization of SV40 surface-associated tumor antigen by enzyme-catalyzed cell-surface iodination.

M. Bradley, J. Griffin, and D. Livingston, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Structural and functional heterogeneity of the large SV40 T antigen.

D. Gidoni,[1] B. Barnet,[2] and C. Prives,[2] [1]Dept. of Virology, Weizmann Institute of Science, Israel; [2]Dept. of Biological Sciences, Columbia University, New York, New York: Properties of different sedimenting forms of SV40 T antigen.

E. Fanning, C. Burger, and B. Nowak, Fakultät für Biologie, Universität Konstanz, Federal Republic of Germany: Detection and characterization of multiple forms of SV40 large T antigen.

E. Gurney and R. Harrison, Dept. of Biology, University of Utah, Salt Lake City: A collection of monoclonal antibodies against SV40 T antigens.

Bacteriophage Meeting, August 19—August 24

Arranged by
Thomas R. Broker, *Cold Spring Harbor Laboratory, New York*
Ahmad Bukhari, *Cold Spring Harbor Laboratory, New York*

143 participants

Session 1: Reactions at Replication Origins and DNA Termini

K. Horiuchi, Rockefeller University, New York, New York: Origin of DNA replication of bacteriophage f1 as the signal for termination.

M. Krevolin and R. Calendar, Dept. of Molecular Biology, University of California, Berkeley: Initiation of P4 DNA replication in vitro.

L. Korsnes and B. H. Lindqvist, Institute of Medical Biology, University of Tromsö, Norway: A comparison of the DNA sequences in the *COS* regions of phage P2 and its satellite P4.

D. W. Bowden and P. Modrich, Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: In vitro characterization of the P2 terminase reaction.

J. M. Hermoso and M. Salas, Centro de Biologiá Molecular, Universidad Autómoma, Madrid, Spain: Protein p3 is linked to the DNA of phage ϕ29 through a phosphoester bond between serine and 5′ dAMP.

C. Escarmís and M. Salas, Centro de Biologiá Molecular, Universidad Autónoma, Madrid, Spain: Nucleotide sequence at the termini of the DNA of *B. subtilis* phage ϕ29.

M. Sumner-Smith, A. Becker, and S. Benchimol, Dept. of Medical Genetics, University of Toronto, Ontario, Canada: DNA packaging in the lambdoid phages—Identification of the products of ϕ80 genes *1* and *2*.

C. E. Snyder, Jr.,[1] R. H. Benzinger,[1] and H. J. Burkardt,[2] [1]Dept. of Biology, University of Virginia, Charlottesville; [2]Institute of Microbiology, University of Erlangen, Federal Republic of Germany: Second-step transfer of bacteriophage T5 DNA—Interaction of the T5 gene-*A2* protein with DNA.

Session 2: Repressors and Integration

D. Cully and A. Garro, Dept. of Microbiology, Mount Sinai School of Medicine, New York, New York: Expression of superinfection immunity to phage ϕ105 by *B. subtilis* carrying a plasmid chimera of pUB110 and *EcoR*I fragment F of ϕ105 DNA.

F. H. Stephenson,[1] W. P. Diehl,[2] and H. Echols,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Biology, San Diego State University, California: Chromosomal rearrangements between the operators in the bacteriophage λ.

A. Johnson, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Action of the λ repressor and *cro* protein at 0_L.

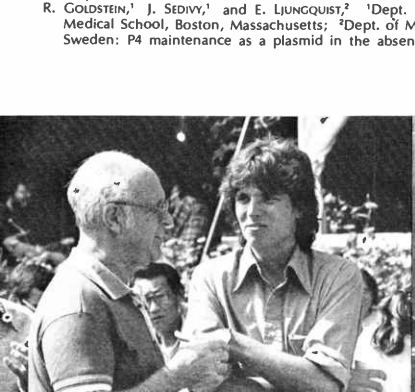
D. Hawley and W. McClure, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Activation of transcription initiation from the λ P_{RM} promoter.

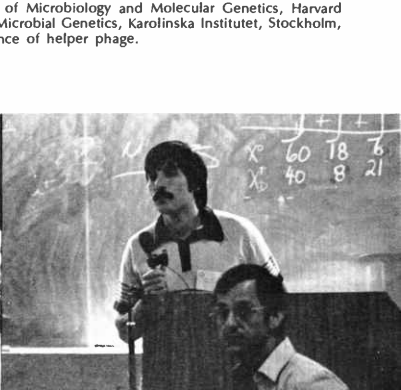
B. Lange-Gustafson and H. A. Nash, NIMH, National Institutes of Health, Bethesda, Maryland: λ Integrative recombination—Analysis of mutant *int* gene products.

D. K. Chattoraj and R. A. Weisberg, NICHHD, National Institutes of Health, Bethesda, Maryland: New attachment-site mutants of phage λ.

K. Abremski[1] and S. Gottesman,[2] [1]Cancer Biology Program, Frederick Cancer Research Center, Maryland; [2]NCI, National Institutes of Health, Bethesda, Maryland: Excisive recombination of bacteriophage λ—Properties of the in vitro reaction.

R. Goldstein,[1] J. Sedivy,[1] and E. Ljungquist,[2] [1]Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Microbial Genetics, Karolinska Institutet, Stockholm, Sweden: P4 maintenance as a plasmid in the absence of helper phage.





Session 3: Regulation of P22

R. T. SAUER,[1] P. HOPPER,[1] K. KING,[1] F. WINSTON,[1] and A. R. POTEETE,[2] [1]Biology Dept., Massachusetts Institute of Technology, Cambridge; [2]The Biological Laboratories, Harvard University, Cambridge, Massachusetts: c2 protein of phage P22.

A. R. POTEETE, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Control of overlapping promoters by P22 repressor.

P. YOUDERIAN, S. CHADWICK, and M. M. SUSSKIND, Dept. of Microbiology, University of Massachusetts Medical School, Worcester: Autogenous regulation by the P22 *arc* gene product.

S. BOUVIER, P. YOUDERIAN, and M. M. SUSSKIND, Dept of Microbiology, University of Massachusetts Medical School, Worcester: Characterization of the P22 *Pant* promoter and transcript.

J. E. RUTILA and E. JACKSON, Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: Regulation in the *imm*I region of P22—An analysis of *sieA* and *mnt* expression.

E. JACKSON and T. WEIGHOUS, Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: Control of transcription in the late region of the P22 chromosome.

M. STRAUCH,[1] S. ZEIGLER,[1] B. BIGELOW,[1] L. BARON,[2] and D. FRIEDMAN,[1] [1]Dept. of Microbiology, University of Michigan, Ann Arbor; [2]Dept. of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, DC: An *E. coli* mutation affecting the growth of *λimm*-P22 hybrid phage.

M. J. ORBACH and E. JACKSON, Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: P22-mediated transduction of plasmid DNA by homologous recombination.

B. WIGGINS, M. SCHLEIN, and S. HILLIKER, Dept. of Biology, Bucknell University, Lewisburg, Pennsylvania: DNA heteroduplex mapping of the early gene regions of *Salmonella* phages P22 and L.

Session 4: T4 and N4

P. R. TEMPEST and L. S. RIPLEY, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Mutagenic properties of hydroxylamine derivatives of hydroxymethylcytosine and cytosine in T4.

N. B. SHOEMAKER and L. S. RIPLEY, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: The effects of DNA polymerase mutants on the reversion of frameshift mutants in the *rII* region of T4.

P. M. MACDONALD and D. H. HALL, School of Biology, Georgia Institute of Technology, Atlanta: Mutations in bacteriophage T4 genes 41 and 61 cause folate analog resistance.

J. R. ALLEN, G. W. LASSER, and C. K. MATHEWS, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: The deoxyribonucleoside-triphosphate-synthesizing complex in phage-T4 infected bacteria.

J. KARAM,[1] L. GOLD,[2] M. DAWSON,[1] B. SINGER,[2] and L. HUNTER,[2] [1]Dept. of Biochemistry, Medical University of South Carolina, Charleston; [2]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Identification of a translational control site on a T4 mRNA.

L. HAYNES, C. MALONE, and L. B. ROTHMAN-DENES, Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: Specificity of transcription of the N4 virion-associated RNA polymerase.

W. ZEHRING[1] and L. B. ROTHMAN-DENES,[2] Depts. of [1]Microbiology and [2]Biophysics and Theoretical Biology, University of Chicago, Illinois: Transcription of coliphage N4 middle (class II) RNAs.

D. GUINTA, R. ZIVIN, and L. B. ROTHMAN-DENES, Depts. of Microbiology and Biophysics and Theoretical Biology, University of Chicago, Illinois: In vivo requirements for bacteriophage N4 late RNA synthesis.

D. MARCHETTI and L. B. ROTHMAN-DENES, Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: A DNA-dependent ADPase activity in bacteriophage N4 virions.

Session 5: Regulation of λ Lysogenization

M. HOYT, J. ABRAHAM, F. STEPHENSON, and H. ECHOLS, Molecular Biology and Virus Laboratory, University of California, Berkeley: Regulation of lysogenization by bacteriophage λ—Role of cII protein.

H. I. MILLER, University of California, Berkeley: Multi-level regulation of λ lysogeny by the *E. coli himA* gene.

M. BENEDIK, D. MASCARENHAS, and A. CAMPBELL, Dept. of Biological Sciences, Stanford University, California: Probing the regulation of PI using λ-*trp-lac* fusions.

D. MASCARENHAS, M. BENEDIK, and A. CAMPBELL, Dept. of Biological Sciences, Stanford University, California: Effect of the *b2* region on integrase synthesis in λ.

D. SCHINDLER and H. ECHOLS, University of California, Berkeley: Regulation of lysogenization by bacteriophage λ—Role of the *b* region.

U. SCHMEISSNER, D. COURT, and M. ROSENBERG, Laboratories of Molecular Biology and Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: Transcription of gene *int* in phage λ.

H. SHIMATAKE and M. ROSENBERG, Laboratory of Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: Regulation of lysogenization by bacteriophage λ. I. Cloning and purification of λcII gene product.

H. SHIMATAKE and M. ROSENBERG, Laboratory of Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: Regulation of lysogenization by bacteriophage λ. II. Reconstruction of P_{RE} and P_I activation in vitro.

H. SHIMATAKE and M. ROSENBERG, Laboratory of Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: Regulation of lysogenization by bacteriophage λ. III. Characterization of *cy*, cII-independent (*cin*) promoter.

Session 6: DNA Recombination

D. W. SCHULTZ, J. SWINDLE, and G. R. SMITH, Institute of Molecular Biology, University of Oregon, Eugene: Characterization of pseudorevertants of Chi recombinational hotspots.

I. KOBAYASHI,[1] F. W. STAHL,[1] M. M. STAHL,[1] J. M. CRASEMANN,[1] L. YOUNG,[1] and H. MURIALDO,[2] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]Dept. of Medical Genetics, University of Toronto, Ontario, Canada: Why do Chi sequences promote recombination in only one orientation in λ?

N. A. DOWER and F. W. STAHL, Institute of Molecular Biology, University of Oregon, Eugene: Chi activity in λ lysogens.

B. E. KORBA and J. B. HAYS, Dept. of Chemistry, University of Maryland, Catonsville: Recombinogenicity and S-1 sites in DNA isolated from *E. coli arl* mutants.

M. LIEB, Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: An insertion sequence (IS5) increases recombination in adjacent regions of the repressor gene of λ.

E. M. PHIZICKY, N. L. CRAIG, C. W. ROBERTS, and J. W. ROBERTS, Dept. of Biochemistry, Cornell University, Ithaca, New York: Regulation of *recA* function.

R. BRENT, Harvard University, Cambridge, Massachusetts: Control of *recA*, *lexA*, and SOS functions by the *lexA* gene product.

N. E. MELECHEN and G. GO, Dept. of Microbiology, St. Louis University School of Medicine, Missouri: Sulfate deprivation joins the list of adverse states known to induce λ lysogens.

Session 7: T-Phage Development–Morphogenesis

J. M. RUNNELS and L. R. SNYDER, Dept. of Microbiology and Public Health, Michigan State University, East Lansing: The physiological effect of RNA ligase deficiency in T4 development—Evidence for an in vivo interaction between the T4-induced RNA ligase and polynucleotide kinase.

W. COOLEY,[1] K. SIROTKIN,[2] and L. SNYDER,[1] [1]Dept. of Microbiology and Public Health, Michigan State University, East Lansing; [2]Division of Biochemistry, California Institute of Technology, Pasadena: A site on T4 DNA which affects expression of all T4 late genes.

G. MOSIG, D. GHOSAL, and S. BOCK, Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Interactions between replication, recombination, and maturation genes during growth of bacteriophage T4.

D. J. MCCORQUODALE and R. WOYCHIK, Dept. of Biochemistry, Medical College of Ohio, Toledo. A putative pre-early modification of RNA polymerase from *E. coli* by bacteriophage T5.

S. H. SAHNBLATT, M. P. ONTELL, and D. NAKADA, Dept. of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: Involvement of *E. coli* RNA polymerase in the development of T7 bacteriophage.

P. B. BERGET and M. CHIDAMBARAM, Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: In vitro assembly of the first intermediate in T4 base-plate 1/6th arm assembly.

D. GOLDENBERG, D. SMITH, and J. KING, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Use of ts mutations to probe the subunit composition and assembly of the P22 tail spike protein.

F. T. FERRUCCI and H. MURIALDO, Dept. of Medical Genetics, University of Toronto, Ontario, Canada: Evidence for an early gpB-gpNu3 interaction in λ prohead assembly.

A. OKABE and D. B. WILSON, Dept. of Biochemistry, Cornell University, Ithaca, New York: Identification of the λ*S* gene product as one of six λ-coded inner membrane proteins.

R. A. GRANT and R. E. WEBSTER, Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Localization of the minor coat proteins of the filamentous bacteriophage f1 to the ends of the phage particle.

Session 8: Transposition and Inversion

E. R. OLSON, K. R. LEASON, and D. I. FRIEDMAN, Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: λ*alt*SF—A phage variant that has the ability to substitute sets of genes at high frequency.

R. R. ISBERG and M. SYVANEN, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Tn*5*-promoted replicon fusions between phage λ and pBR322.

C. KAISER and M. SYVANEN, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Transposition of Tn*5* onto nonreplicating λ DNA.

D. Y. KWOH and D. ZIPSER, Cold Spring Harbor Laboratory, New York: Analysis of mutations in the G-loop *gin* region of Mu.

J. W. SCHUMM and M. M. HOWE, Dept. of Bacteriology, University of Wisconsin, Madison: λ-Mu clones and their use in the analysis of Mu transposition.

R. M. HARSHEY and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: Studies on Mu transposition.

G. CHACONAS, N. SARVETNICK, R. M. HARSHEY, and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: The role of cointegrate structures in the transposition of Mu DNA.

W. Schumann, C. A. Barron, C. Lœogl, and E. G. Bade, Fakultät für Biologie, Universität Konstanz, Federal Republic of Germany: Gene expression during Mu development.

L. H. Westphal, R. Clayton, and E. G. Bade, Fakultät für Biologie, Universität Konstanz, Federal Republic of Germany: Early replication of phage Mu DNA.

J. Teifel and H. Schmieger, Genetisches Institut der Universität Munich, Federal Republic of Germany: The structure of transducing Mu DNA.

Session 9: Regulation of λ Transcription

D. Drahos[1] and R. Hendrix,[2] [1]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; [2]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: λ-Directed regulation of the synthesis of gp*groE*, and several other important host proteins.

J. Kochan and H. Murialdo, Dept. of Medical Genetics, University of Toronto, Ontario, Canada: The mechanism of gp*groE* induction in λ-infected cells.

R. W. Hendrix, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Purification and characterization of λ early protein Ea10.

E. L. Flamm and D. I. Friedman, University of Michigan, Ann Arbor: Characterization of a λ*nutR* mutant.

A. T. Schauer,[1] L. S. Baron,[2] M. F. Baumann,[1] E. J. Mashni,[1] L. C. Plante-faber,[1] M. Strauch,[1] and D. I. Friedman,[1] [1]Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor; [2]Dept. of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, DC: Host factors involved in antitermination by λN product.

D. Ward and M. Gottesman, NCI, National Institutes of Health, Bethesda, Maryland: *E. coli* mutants affecting λ*N* function.

K. McKenney, C. Brady, and M. Rosenberg, Laboratory of Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: The effect of translation on transcription termination.

J. N. Alegre and J. W. Roberts, Dept. of Biochemistry, Cornell University, Ithaca, New York: Late-gene regulatory regions of λ, φ*82*, and P22.

E. J. Grayhack and J. W. Roberts, Dept. of Biochemistry, Cornell University, Ithaca, New York: The λQ protein mediates increased transcription of the λ late genes in vitro.

Session 10: Bacterial Proteins–Phage Genome Structure

H. Aiba and J. S. Krakow, Dept. of Biological Sciences, Hunter College, City University of New York, New York: Isolation and characterization of the amino and carboxyl proximal fragments of the *E. coli* cAMP receptor protein.

J. S. Heilig and R. Calendar, Dept. of Molecular Biology, University of California, Berkeley: Suppression of a *ts* mutation of the sigma subunit of *E. coli* RNA polymerase by a second site event.

T. Edlind and G. Ihler, Dept. of Medical Biochemistry, Texas A&M University College of Medicine, College Station: Long-range base pairing in φX174 single-stranded DNA.

P. B. Moses, J. D. Boeke, K. Horiuchi, and N. D. Zinder, Rockefeller University, New York, New York: Restructuring the f1 genome—Expression of gene VIII in the intergenic space.

T. S. B. Yen, R. A. Grant, and R. E. Webster, Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Identification of f1 X and gene II proteins in vivo.

F. Brunel and J. Davison, Unit of Molecular Biology, Institute of Cellular Pathology, Brussels, Belgium: Expression of cloned T5 DNA fragments in *E. coli* mini-cells.

K. Haberer,[1] A. I. Hallerer,[1] and J. Maniloff,[2] [1]Abteilung Mikrobiologie, Universität Ulm, Federal Republic of Germany; [2]Dept. of Microbiology, University of Rochester Medical Center, New York: Isolation and partial characterization of ts mutants of mycoplasmavirus MVL3.

C. F. Marrs and M. M. Howe, Dept. of Bacteriology, University of Wisconsin, Madison: *Ava*II and *Bgl*I restriction maps of bacteriophage Mu.

Herpesviruses, August 26 – August 31

Arranged by
Gary S. Hayward, *Johns Hopkins University, Baltimore, Maryland*
William C. Summers, *Yale University, New Haven, Connecticut*

228 participants

Session 1: Plenary Lectures

A. Levine, State University of New York, Stony Brook
G. Vande Woude, NCI, National Institutes of Health, Bethesda, Maryland
J. Clements, Johns Hopkins University, Baltimore, Maryland

Session 2: Virus Proteins

Chairperson: R. Courtney, University of Tennessee, Knoxville

A. Buchan, L. Tomkins, L. Harper, and A. Fuller, Dept. of Medical Microbiology, University of Birmingham Medical School, England: Serological comparison of the intracellular polypeptides synthesized by various herpesviruses.

C. J. Heilman, Jr.,[1] M. Zweig,[1] and B. Hampar,[2] [1]Frederick Cancer Research Center, Maryland; [2]NCI, National Institutes of Health, Frederick, Maryland: Immunoprecipitation of antigenically type-specific and cross-reactive peptides from digests of HSV-1 and HSV-2 protein p40.

V. Bibor-Hardy, M. Suh, and R. Simard, Institut du Cancer de Montrèal, Centre Hospitalier Notre-Dame, Quebec, Canada: Analysis of proteins in the nuclear envelope of BHK cells infected with HSV-1.

W. Gibson, D. Weiner, K.-T. Jeang, and C. Roby, Dept. of Pharmacology and Experimental Therapy, Johns Hopkins School of Medicine, Baltimore, Maryland: Comparisons of CMV proteins—Human and simian isolates.

J. E. Shaw, C. Moore, and J. Griffith, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Polypeptides associated with the EBV DNA of superinfected Raji cells.

G. J. Bayliss and H. Wolf, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: The control of protein synthesis in EBV-superinfected Raji cells.

R. W. Honess and R. E. Randall, National Institute for Medical Research, London, England: Proteins of herpesvirus saimiri—Location and functions of virus-specified polypeptides in productively infected cells.

S. Modrow and H. Wolf, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: Herpesvirus saimiri-induced proteins in lytically infected cells.

Workshops: CMV DNA Structure and Cloning; Immunology; Proteins of HSV and Related Viruses

Session 3: Poster Session

J. F. Baskar, I. Boldogh, and E.-S. Huang, Cancer Research Center, University of North Carolina, Chapel Hill: Persistent infection of murine CMV in murine carcinoma F9 cells.

R. Blanton and M. J. Tevethia, Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Immunoprecipitation of immediate early and early polypeptides from cells lytically infected with CMV strain AD169.

Y. M. Centifanto-Fitzgerald and H. Kaufman, Eye Center, Louisiana State University Medical Center School of Medicine, New Orleans: Avirulent HSV strains in the rabbit trigeminal ganglia.

G. Darai, R. M. Flügel, L. Zöller, B. Matz, H. Gelderblom, H. Delius, and R.H. Leach, Institut für Medizinische Virologie, Universität Heidelberg, Robert-Koch Institut, Berlin; Institut für Virusforschung am DKFZ, Heidelberg; European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; Mycoplasma Reference Laboratory, Norwich, England: The plaque-inducing factor for mink lung cells present in cytomegalo- and herpes zoster virus identified as a mycoplasma.

N. DeLuca, D. Bzik, S. Person, A. Keith, and W. Snipes, Biophysics Program, Pennsylvania State University, University Park: Photodynamic-induced crosslinking of HSV-1 glycoproteins and the study of glycoprotein-mediated viral functions.

R. D. Dix, J. G. Steele, and J. R. Baringer, Dept. of Neurology, Veterans Administration Medical Center, and University of California School of Medicine, San Francisco: Isolation of HSV-1 from the cerebrospinal fluid of a patient with recurrent aseptic meningitis (Mollaret's meningitis).

R. J. Feighny, B. E. Henry, and J. S. Pagano, Cancer Research Center, University of North Carolina, Chapel Hill: EBV early polypeptides—Identification, synthesis, glycosylation, and phosphorylation.

C. Glaubiger and L. F. Velicer, Michigan State University, East Lansing: Molecular characterization of Marek's disease herpesvirus antigens.

M. Hilty and D. Mann, Children's Hospital, Ohio State University, Columbus: Identification and characterization of HSV-1 and 2 polypeptides complexed with HSV-1 and 2 specific sera.

J. H. Joncas,[1] C. Alfieri,[1] M. Leyritz,[1] I. Boldogh,[2] and E. S. Huang,[2] [1]Pediatric Research Center, Ste.-Justine's Hospital, Montreal, Quebec, Canada;[2] [2]Cancer Research Center, University of North Carolina, Chapel Hill: Dual congenital infection with EBV and CMV.

K. G. Kousoulas and S. Person, Biophysics Program, Pennsylvania State University, University Park: NH_4Cl inhibits cell fusion and the accumulation of mature HSV-1 glycoproteins.

B. F. Ladin, M. L. Blankenship, and T. Ben-Porat, Dept. of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee: Processing of a capsid protein (35K) and assembly of the capsid of pseudorabies (PR) virus are interrelated.

W. Lawrence,[1] L. Kastrukoff,[2] D. Gilden,[2] and N. W. Fraser,[2] [1]School of Veterinary Medicine, University of Pennsylvania; [2]Wistar Institute, Philadelphia, Pennsylvania: HSV-1 sequences in brain.

E.-C. Mar and E.-S. Huang, Dept. of Medicine and Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Virus-specific structural proteins, DNA-binding proteins and phosphorylated proteins in CMV human virions and virus-infected WI-38 cells.

S. Modrow and H. Wolf, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: Strain comparison of various HVS- and HVA-isolates.

P. O'Hare, R. W. Honess, and R. E. Randall, National Institute for Medical Research, Mill Hill, London, England: Proteins of herpesvirus saimiri—Regulation of virus protein synthesis in productively infected cells.

D. Purtilo,[1] K. Sakamoto,[1] J. L. Sullivan,[2] L. Paquin,[1] A. Saemundson,[3] A. C. Synnerholm,[3] and G. Klein,[3] Depts. of [1]Pathology and [2]Pediatrics, University of Massachusetts Medical School, Worcester; [3]Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Documentation of EBV-induced lymphoproliferative disease in the X-linked lymphoproliferative syndrome.

Y. Suzuki and H. A. Blough, Scheie Eye Institute, University of Pennsylvania, Philadelphia: Effect of herpesvirus infection and 2-deoxy-D-glucose on the synthesis of ceramide monohexosides in vitro.

L. Turtinen and G. Allen, Dept. of Veterinary Sciences, University of Kentucky, Lexington: Identification of the glycoproteins of equine herpesvirus type 1.

A. Vahlne, E. Nilheden, and B. Svennerholm, Institute of Medical Microbiology, University of Göteborg, Sweden: Multiplicity activation of HSV in mouse neuroblastoma (C1300) cells.

E. Wenske, R. Dix, R. Eberle, and R. Courtney, Dept. of Microbiology, University of Tennessee, Knoxville: Intermediates of HSV-1 glycoproteins synthesized in the presence of inhibitors of glycosylation.

H. Wolf and E. Wilmes, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: Evidence for the persistence of EBV in the parotid gland.

Session 4: The S Region of HSV DNA and Genome Replication

Chairperson: G.S. Hayward, Johns Hopkins Medical School, Baltimore, Maryland

V. G. Preston, A. J. Davison, and M.-J. Murchie, Institute of Virology, Glasgow, Scotland: Location of temperature-sensitive mutations within the short repeat sequences of the HSV-1 genome.

S. P. Little[1] and P. A. Schaffer,[2] [1]Sidney Farber Cancer Institute, [2]Harvard Medical School, Boston, Massachusetts: A new essential gene of HSV-1 in the repeated sequences in S.

D. J. McGeoch, M. J. Murchie, and A. Dolan, Institute of Virology, Glasgow, Scotland: Nucleotide sequence analysis of portions of the genome of HSV-1.

R. J. Watson and G. F. Vande Woude, NCI, National Institutes of Health, Bethesda, Maryland: Structures of HSV-1 immediate-early mRNAs mapping in the virus DNA S component.

F. J. Rixon, A. J. Easton, and J. B. Clements, Institute of Virology, Glasgow, Scotland: Fine structure of HSV immediate-early mRNA.

D. Ciufo, P. Mounts, K. Peden, G. Reyes, and G. S. Hayward, Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins Medical School, Baltimore, Maryland: The HSV genome—L-segment defectives and cross homology with mammalian cell DNA.

R. J. Jacob,[1] L. S. Morse,[2] and B. Roizman,[2] [1]Dept. of Pathology, University of Kentucky, Lexington; [2]Kovler Viral Oncology Laboratory, University of Chicago, Illinois: Restricted synthesis of HSV DNA in cells treated with phosphonoacetate (PA) and its application for locating the origin of DNA synthesis.

C. V. Jongeneel and S. L. Bachenheimer, Dept. of Bacteriology and Immunology, University of North Carolina, Chapel Hill: Replicative intermediates of HSV-1 DNA.

T. Ben-Porat, B. F. Ladin, R. A. Veach, and L. Brown, Dept. of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee: Genetic analysis of the genome of pseudorabies (PR) virus indicates a circular map.

A. J. Davison and N. M. Wilkie, Institute of Virology, Glasgow, Scotland: Nucleotide sequences of the joint between the L and S segments of HSV-1 (strain 17) and HSV-2 (strain HG52).

Session 5: Glycoproteins and Immunology

Chairperson: J. S. Pagano, University of North Carolina, Chapel Hill

T. C. Holland, R. M. Sandri, J. C. Glorioso, and M. Levine, University of Michigan, Ann Arbor: HSV-1 (KOS) mutants altered in the expression of viral cell-surface glycoproteins and susceptibility to complement-mediated immune cytolysis.

B. A. Pancake and P. A. Schaffer, Sidney Farber Cancer Institute, Boston, Massachusetts: Genetic and biochemical characterization of temperature-sensitive mutants of HSV-1 resistant to immune cytolysis.

K. Rosenthal[1] and H. Shapiro,[2] [1]Northeastern Ohio Universities College of Medicine, Rootstown, Ohio; [2]Sidney Farber Cancer Institute, Boston, Massachusetts: Changes in membrane potential induced by herpesvirus.

G. J. Bayliss and H. Wolf, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: EBV induces cell fusion.

L. Pereira, D. Gallo, D. Dondero, and N. Schmidt, California Dept. of Health Services, Berkeley: Monoclonal antibody to HSV—An immunologic tool for typing of virus isolates.

N. Balachandran, R. A. Killington, L. Newhook, W. E. Rawls, and S. Bacchetti, Dept. of Pathology, McMaster University, Hamilton, Ontario, Canada: Production and characterization of hybridoma lines secreting antibodies against HSV.

L. Loh and E.-S. Huang, Cancer Research Center, University of North Carolina, Chapel Hill: Production of monoclonal antibodies against human CMV structural antigens.

D. A. Thorley-Lawson and C. M. Edson, Sidney Farber Cancer Institute, Boston, Massachusetts: EB virion antigens — Characterization of strain differences.

H. Rabin, B. C. Strnad, R. H. Neubauer, R. F. Hopkins, III, and T. J. Witmer, Biological Carcinogenesis Program, Frederick Cancer Research Center, Maryland: Antigenic heterogeneity of EBV nuclear antigen (EBNA).

S. L. Silverman and C. L. Miller, Dept. of Medicine, University of Pennsylvania, Philadelphia: Heterogeneous patterns of nuclear antigen expression in WI_2, an EBV-positive cell line, following differential fixation and staining.

D. J. Volsky, G. Klein, and I. M. Shapiro, Dept. of Tumor Biology, Karolinska Institutet, Stockholm, Sweden: Implantation of EBV receptors into membranes of receptor-negative cells—Virus binding, penetration, and viral antigen expression.

Workshops: Glycoproteins; Proteins of Lymphotropic Viruses; Transcription

Session 6: Poster Session

G. Allen and L. Turtinen, Dept. of Veterinary Science, University of Kentucky, Lexington: Cloning in *E. coli* of the *Bam*HI fragment of equine herpesvirus 1 (EHV-1) DNA that carries an active thymidine kinase (TK) gene.

S. S. Atherton, R. A. Robinson, and D. J. O'Callaghan, Dept. of Microbiology, University of Mississippi Medical Center, Jackson: Equine herpesvirus type 3 (EHV-3)—Genomic structure and transforming potential.

S. Bogger-Goren and P. L. Ogra, Children's Hospital, State University of New York, Buffalo: Characteristics of antibody response to varicella zoster virus (VZV) in the nasopharyngeal secretions after naturally acquired chicken pox.

H. Delius,[1] B. Matz,[2] R. M. Flugel,[3] and G. Darai,[2] [1]European Molecular Biology Laboratory; [2]Institute für Medizinische Virologie der Universität Heidelberg; [3]Institut für Virusforschung am Deutschen Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: Properties of DNA of tupaia herpesviruses.

K. Denniston-Thompson, L. W. Enquist, and G. F. Vande Woude, NCI, National Institutes of Health, Bethesda, Maryland: Three structural groups of *Eco*RI fragments from HSV-1 patton-defective DNA molecules cloned into bacteriophage λ.

A. D. Donnenberg and L. Aurelian, Johns Hopkins Medical Institutions, Baltimore, Maryland: Cell-mediated immunity in recurrent HSV-2 disease.

J. R. Ecker and R. W. Hyman, Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Analysis of interruptions in the phosphodiester backbone of HSV DNA.

M. Engels,[1,3] H. Ludwig,[2] and C. Mulder,[3] [1]Institut für Virologie, Universität Zurich, Switzerland; [2]Institut für Virologie, Freie Universität Berlin, Federal Republic of Germany; [3]Depts. of Pharmacology and Microbiology, University of Massachusetts Medical School, Worcester: Mapping of genomes from attenuated strains of infectious bovine rhinotracheitis virus and infectious pustular vulvovaginitis virus.

J. D. Hall and K. Scherer, Dept. of Cellular and Developmental Biology, University of Arizona, Tucson: HSV as a probe for repair of DNA damaged by 4,5',8-trimethylpsoralen plus light.

L. HUTT-FLETCHER,[1] E. FOWLER,[1] B. SULLIVAN,[1] R. FEIGHNY,[1] J. D. LAMBRIS,[2] and G. D. ROSS,[2] [1]Cancer Research Center and [2]Division of Rheumatology and Immunology, University of North Carolina School of Medicine, Chapel Hill: EBV receptor—Partial purification and relationship to the C3d receptor.

F. J. JENKINS, M. K. HOWETT, and F. RAPP, Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Cloning of HSV-2 (333) DNA fragments in the bacteriophage vector Charon 4A.

H. C. KAERNER, A. OTT-HARTMANN, and R. SCHATTEN, Institute of Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Multiple insertions of a short nucleotide sequence into the repeat units of three different classes of defective HSV-1 ANG DNA.

Y.-S. LEE and M. NONOYAMA, Life Sciences Biomedical Research Institute, St. Petersburg, Florida: Structural analysis of MDV DNA.

P. C. PATEL and J. MENEZES, Ste.-Justine Hospital, University of Montreal, Quebec, Canada: EBV replication cycle determines the type of cellular effector mechanism utilized to destroy EBV-infected cells.

M. ROGGENDORF, G. J. BAYLISS, D. WAGNER, and H. WOLF, Max von Pettenkofer Institute, Munich, Federal Republic of Germany: New approaches in the development of enzyme-linked immunosorbant assay (ELISA) for antibodies to EBV.

R. M. SANDRI, A. L. GOLDIN, M. LEVINE, and J. C. GLORIOSO, University of Michigan, Ann Arbor: Cloning of HSV-1 DNA.

C. H. SCHRÖDER, R. AUGUSTIN, and B. HENNES-STEGMANN, Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Processing of progeny DNA in the infectious cycle of HSV and its control by interfering particles.

I. M. SHAPIRO, G. KLEIN, and D. J. VOLSKY, Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Reconstitution of EBV with Sendai virus envelopes and EBV-receptor transplantation as a means for overcoming cellular resistance to the virus.

S. E. STRAUS,[1] H. S. AULAKH,[1] W. T. RUYECHAN,[3] J. A. HAY,[3] G. F. VANDE WOUDE,[2] J. OWENS,[1] and H. A. SMITH,[1] [1]Laboratory of Clinical Investigation, NIAID, [2]Laboratory of Molecular Virology, NCI, National Institutes of Health; [3]Depts. of Biochemistry and Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland: Structural studies of varicella zoster virus DNA.

R. SZIGETI, G. KLEIN, J. LUKA, D. PURTILO, and D. J. VOLSKY, Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Leukocyte migration inhibition induced by EBV-associated antigens.

Session 7: Thymidine Kinase I

Chairperson: J. SUBAK-SHARPE, Institute of Virology, Glasgow, Scotland

M. J. WAGNER, J. A. SHARP, and W. C. SUMMERS, Yale University School of Medicine, New Haven, Connecticut: Nucleotide sequence analysis and control of expression of HSV-1 thymidine kinase.

S. L. MCKNIGHT, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Studies on the structure and expression of the HSV-1 thymidine kinase gene.

A. MINSON and K. BASTOW, Dept. of Pathology, University of Cambridge, England: Properties and virus DNA sequence content of cells carrying the HSV-2 thymidine kinase gene.

C. M. PRESTON, Institute of Virology, Glasgow, Scotland: Analysis of HSV-1 *Bam*HI *p*-specific mRNAs by hybrid-selected and hybrid-arrested translation.

W.-C. LEUNG,[1] B. FONG,[1] S. BACCHETTI,[1] P. E. BRANTON,[1] and R. COURTNEY,[2] [1]Dept. of Pathology, McMaster University, Hamilton, Ontario, Canada; [2]Dept. of Microbiology, University of Tennessee, Knoxville: Function and in vitro phosphorylation of HSV α polypeptides.

S. KIT, H. OTSUKA, H. QAVI, and M. HAZEN, Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas: Expression of HSV thymidine kinase (TK) in TK$^-$ *E. coli* K12 mutant transformed by hybrid plasmids.

F. COLBÈRE-GARAPIN,[1] M. COHEN-SOLAL,[2] A. GARAPIN,[1] and P. KOURILSKY,[1] [1]Institut Pasteur, Paris, [2]Unité INSERM, Créteil, France: Structure of the 5' end of the HSV-1 thymidine kinase gene and expression of the cloned gene in *E. coli*.

J. R. SMILEY and J. CAMPIONE-PICCARDO, Dept. of Pathology, McMaster University, Hamilton, Ontario, Canada: In vitro mutagenesis of the tk region of HSV-1 DNA.

L. LIPSICH, M. WIGLER, and D. ZIPSER, Cold Spring Harbor Laboratory, New York: Mapping the operator for cell resident HSV TK induction by herpes infection.

D. M. COEN,[1] M. H. ST. CLAIR,[2] P. A. FURMAN,[2] and P. A. SCHAFFER,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]Burroughs Wellcome Company, Research Triangle Park, North Carolina: Mutations in the HSV DNA polymerase and thymidine kinase genes and resistance to acyclovir.

Session 8: Thymidine Kinase II and Latency

Chairperson: B. HAMPAR, NCI, National Institutes of Health, Frederick, Maryland

B. M. COLBY,[1] P. A. FURMAN,[2] J. E. SHAW,[1] G. B. ELION,[2] and J. S. PAGANO,[1] [1]Cancer Research Center, University of North Carolina Medical School, Chapel Hill; [2]Burroughs Wellcome Company, Research Triangle Park, North Carolina: Phosphorylation of acyclovir in EBV-infected lymphoblastoid cell lines.

A. Calender,[1] T. Ooka,[1] M. de Turenne,[1] G. Lenoir,[2] and J. Daillie,[1] [1]University Claude Bernard, [2]International Agency for Research on Cancer, Lyon, France: Effect of 1-β-D arabinofuranosylthymine on EBV replication and relationship with a virally induced thymidine kinase activity.

H. Otsuka, M. Michalak, and S. Kit, Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas: Resistance of cells biochemically transformed by HSV DNA fragments to thymidine and deoxyadenosine cytotoxicity.

R. B. Tenser, M. Dunstan, and S. Ressel, Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Trigeminal ganglion infection by thymidine kinase positive, negative, and intermediate HSV and in vivo complementation.

A. A. Nash, H. J. Field, P. G. H. Gell, and P. Wildy, Dept. of Pathology, Cambridge, England: Cellular immune phenomena in relation to herpes simplex latency in the mouse.

R. L. Thompson, J. G. Stevens, and M. L. Cook, Dept. of Microbiology and Immunology, University of California School of Medicine, Los Angeles: Mapping of the gene(s) responsible for HSV neurovirulence in mice.

A. L. Goldin, R. M. Sandri, M. Levine, and J. C. Glorioso, University of Michigan, Ann Arbor: Persistence of viral genes in neuronal cells surviving HSV-1 infection.

J. K. McDougall,[1] D. A. Galloway,[1] and C. M. Fenoglio,[2] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]College of Physicians and Surgeons, Columbia University, New York, New York: Expression of HSV in latent infection and in neoplastic cells.

M. J. K. Selgrade,[1] J. G. Nedrud,[2] A. Collier,[3] and J. S. Pagano,[2,4] [1]Environmental Protection Agency, Research Triangle Park, [2]Cancer Research Center, Depts. of [3]Pediatrics and [4]Medicine, University of North Carolina, Chapel Hill: Attenuation of murine cytomegalovirus (MCMV) in vitro and in vivo.

K. Sakamoto,[1] M. Aiba,[1] I. Katayama,[2] R. Humphreys,[3] J. Sullivan,[4] and D. Purtilo,[1] Depts. of [1]Pathology, [3]Pharmacology, and [4]Pediatrics, University of Massachusetts Medical School, Worcester; [2]Dept. of Pathology, Saitama Medical School, Japan: EBV antibody responses in patients with hairy cell leukemia.

Workshops: HSV DNA, Genetics and Cloning; Acycloguanosine; Latency and Persistence

Session 9: Poster Session

T. Beck, A. Sesko, and R. Millette, Dept. of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan: In vitro transcription of HSV-1 DNA by RNA polymerase II from HEp-2 cells.

W. Bodemer, E. Knust, C. Kaschka-Dierich, and B. Fleckenstein, Institut für Klinische Virusforschung, University of Erlangen-Nürnberg, Federal Republic of Germany: Viral gene expression in lymphoid tumor cell lines transformed by oncogenic primate herpesviruses.

D. R. Bone,[1] R. Torczynski,[1] H. Maguire,[1] J. Harris,[1] and B. Francke,[2] [1]Depts. of Botany and Microbiology, University of Oklahoma, Norman; [2]Dept. of Human Genetics, Yale University Medical School, New Haven, Connecticut: Selective isolation and initial characterization of temperature-sensitive mutants of HSV-1.

B. M. Colby, J. E. Shaw, A. K. Datta, and J. S. Pagano, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Characterization of P3HR-1 cells following prolonged exposure to acyclovir.

C. Crumpacker[1,2] and K. W. Knopf,[1,3] [1]Medical Research Council Virology Unit, Glasgow, Scotland; [2]Division of Infectious Diseases, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts; [3]Institute for Virusforshung, Deutsche Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: Physical mapping of mutations within the viral DNA polymerase locus of HSV resulting in an altered HSV DNA polymerase activity conferring resistance to three antiviral drugs.

A. K. Datta and J. S. Pagano, Cancer Research Center, University of North Carolina, Chapel Hill: Mode of inhibition of EBV DNA polymerase by acyclovir triphosphate.

B. Fleckenstein,[1] I. Muller,[1] E. Knust,[1] and J. Collins,[2] [1]Institut für Klinische Virologie, University of Erlangen-Nürnberg; [2]Gesellschaft für Biotechnologische Forschung, Braunschweig, Federal Republic of Germany: Cosmids as vectors for gene banks of herpesviruses.

A. Garcia, B. Jacquemont, A. Epstein, and A. Niveleau, Unité de Virologie Fondamentale et Appliquee INSERM U51, Groupe de Recherche CNRS, Lyon, France: Effect of N-6 methyl adenosine analogues on maturation of viral RNA in HSV-1-infected cells.

D. Grossberger and W. Clough, Dept. of Molecular Biology, University of Southern California, Los Angeles: Dependence of the phosphonoacetic acid sensitivity of the EBV-induced DNA polymerase upon availability of DNA 3'-OH ends and upon accessory proteins.

E. R. Kaufman, R. L. Davidson, and L. E. Schnipper, Harvard Medical School, Boston, Massachusetts: Genetic and biochemical studies of the antiviral nucleoside analog acycloguanosine.

D. Knipe and A. Spang, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Genetic analysis of linked mutations conferring resistance to phosphonoacetate and acycloguanosine.

K.-W. Knopf[1,2] and C. C. Crumpacker,[1,3] [1]Institute of Virology, Glasgow, Scotland; [2]Institute für Virusforschung, Deutsche Krebforschungzentrum, Heidelberg, Federal Republic of Germany; [3]Dept. of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts: Resistance of HSV-1/HSV-2 intertypic recombinants both to ACG and to PAA is due to altered viral DNA polymerase.

R. LaFemina and G. S. Hayward, Dept. of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Physical mapping of the human CMV DNA molecule.

R. Y. Lau,[1] M. Nonoyama,[1] and G. Klein,[2] [1]Laboratory of Molecular Virology, Life Sciences Biomedical Research Institute, St. Petersburg, Florida; [2]Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Resident viral genome structure and control of viral genome transcription in two EBV-DNA-containing somatic hybrid cell lines.

Y.-S. Lee,[1] A. Tanaka,[1] R. Y. Lau,[1] M. Nonoyama,[1] and H. Rabin,[2] [1]Life Sciences Biomedical Research Institute, St. Petersburg, Florida; [2]Frederick Cancer Research Center, Maryland: The linkage map of herpesvirus papio (HVP) and its relation to that of EBV DNA.

D. S. Parris and J. E. Harrington, Dept. of Medical Microbiology and Comprehensive Cancer Center, Ohio State University College of Medicine, Columbus: Characterization of acycloguanosine resistance in HSV encephalitis strains.

J. Skare,[1] J. Farley,[1] J. Strominger,[1] and K. Fresen,[2] [1]Sidney Farber Cancer Institute, Boston, Massachusetts; [2]Institute for Virology, Freiburg, Federal Republic of Germany: Restriction mapping of EBV recombinant viruses.

D. H. Spector, J. Tamashiro, and L. Hock, University of California, San Diego: Molecular cloning and characterization of human CMV DNA.

D. R. Thomsen and M. F. Stinski, Dept. of Microbiology, University of Iowa, Iowa City: Cloning human CMV DNA *Xba*I restriction endonuclease fragments into bacterial plasmid pACYC184.

M. W. Westrate and J. L. M. Geelen, Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, The Netherlands: Structural organization of the DNA of HCMV—Comparison of the physical maps of strain AD169 and SG.

D. R. Yager and S. L. Bachenheimer, Dept. of Bacteriology and Immunology, University of North Carolina, Chapel Hill: Transcription of HSV-1 DNA in infected permeabilized cells.

Y. Yajima and R. Glaser, Dept. of Medical Microbiology and Immunology, and Comprehensive Cancer Center, Ohio State University College of Medicine, Columbus: Transcription of the EBV genome in superinfected Raji cells.

Session 10: Transformation

Chairperson: D. J. O'Callaghan, University of Mississippi Medical Center, Jackson

I. R. Cameron, N. M. Wilkie, and J. C. M. Macnab, Institute of Virology, University of Glasgow, Scotland: Transformation studies using defined fragments of HSV-2.

D. A. Galloway and J. K. McDougall, Dept. of Tumor Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington: Transformation of rodent cells by a cloned fragment of the HSV-2 genome.

F. M. van den Berg and J. M. M. Walbooners, Laboratory of Pathological Anatomy, University of Amsterdam, The Netherlands: Oncogenic transformation of hamster embryo cells with DNA of HSV-2.

G. R. Reyes and G. S. Hayward, Dept. of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Biochemical manipulation of cloned HSV DNA fragments and their subsequent introduction into mammalian cells.

A. Boyd[1] and B. Hampar,[2] [1]Frederick Cancer Research Center, [2]NCI, National Institutes of Health, Frederick, Maryland: Activation of endogenous retroviruses from BALB/c mouse cells by herpesvirus DNA.

I. Boldogh, J. F. Baskar, E. C. Mar, and E. S. Huang, Cancer Research Center, University of North Carolina, Chapel Hill: Transformation of human embryonic lung cells by restriction endonuclease (*Xba*) fragmented DNA of CMV.

R. A. Robinson, P. W. Tucker, and D. J. O'Callaghan, University of Mississippi Medical Center, Jackson: Mapping and molecular cloning of host genomic and herpesvirus oncogenic DNA sequences.

B. Hampar,[1] S. D. Showalter,[1] and J. G. Derge,[2] [1]NCI, National Institutes of Health; [2]Frederick Cancer Research Center, Maryland: Biochemical and morphological transformation by HSV-1 are independent and unrelated events.

J. Lewis,[1] L. Kucera,[2] R. Eberle,[1] and R. Courtney,[1] [1]Dept. of Microbiology, University of Tennessee, Knoxville; [2]Dept. of Microbiology and Immunology, Bowman Gray School of Medicine, Winston-Salem, North Carolina: Identification of specific HSV-2 glycoproteins expressed in HSV-2-transformed cells.

M. M. Manak, M. McKinlay, C. C. Smith, L. Aurelian, and P. O. P. Ts'o, Baltimore City Hospital, Johns Hopkins Medical Institutions, Maryland: Phenotypic properties and viral expression in HSV-2-transformed Syrian hamster embryo cells.

G. Darai,[1] L. Zoller,[1] and R. M. Flügel,[2] [1]Institut für medizinische Virologie der Universität Heidelberg; [2]Institut für Virusforschung, Heidelberg, Federal Republic of Germany: Attempts to immortalize human umbilical-cord-blood leukocytes by HSV and its DNA.

T. Aya,[1] O. Shiratori,[2] and T. Osato,[1] [1]Cancer Institute, Hokkaido University School of Medicine, Sapporo; [2]Shionogi Research Laboratories, Osaka, Japan: Isolation of rapidly growing lymphocytes in semisolid agar medium in primary infection with EBV.

R. A. Respess, L. S. Kucera, and M. Waite, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: Aminoacyl-fucosides as in vitro markers of tumorigenic potential of tetradecanoyl phorbol acetate (TPA)-treated and untreated HSV-2-transformed rat cells.

L. S. Kucera, I. Edwards, and R. A. Respess, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: The tumor promoter (tetradecanoyl-phorbol-13-acetate) enhances the malignant potential of HSV-2-transformed cells.

Session 11: EBV Molecular Biology

Chairperson: W. C. Summers, Yale University, New Haven, Connecticut

G. N. Buell and D. Reisman, McArdle Laboratory, University of Wisconsin, Madison: Analysis of EBV genome structure using λ recombinants.

M. Heller, T. Dambaugh, and E. Kieff, Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Organization of the DNA of EBV—Variation in the viral DNAs in producer and nonproducer cell lines from various origins.

S. Lazarowitz,[1] G. Hayward,[2] and S. D. Hayward,[2] Depts. of [1]Microbiology and [2]Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Studies on the structure of EBV DNA in and around the internal repeats.

V. L. van Stanten, W. King, A. Cheung, and E. Kieff, Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Cytoplasmic EBV-specific RNAs in an EBV-transformed continuous lymphoblastoid cell line.

J. R. Arrand[1] and L. Rymo,[2] [1]Imperial Cancer Research Fund, London, England; [2]Dept. of Clinical Chemistry, University of Gothenburg, Sweden: The expression of EBV-related sequences in various lymphoblastoid cell lines.

M. Lerner,[1] N. Andrews,[1] G. Miller,[2] and J. A. Steitz,[1] Depts. of [1]Molecular Biophysics and Biochemistry, [2]Pediatrics, Yale University, New Haven, Connecticut: Two new EBV-encoded RNAs are found in the form of small ribonucleoproteins.

G. Miller,[1] L. Gradoville,[1] L. Heston,[1] E. Grogan,[1] J. Brandsma,[1] M. Westergaard,[2] and W. Maris,[2] [1]Dept. of Pediatrics, Yale University School of Medicine, New Haven, Connecticut; [2]University of Amsterdam, Holland: Structure and infectivity of the genome of an EBV derived from saliva of a patient with infectious mononucleosis.

A. Tanaka, S. Yano, and M. Nonoyama, Life Sciences Biomedical Research Institute, St. Petersburg, Florida: A study on probing possible integration of EB viral genome in transformed cells by Southern blot hybridization.

J. Brandsma and G. Miller, Dept. of Pediatrics, Yale University School of Medicine, New Haven, Connecticut: Nucleic acid spot hybridization—Rapid quantitative screening of large numbers of samples for EB viral DNA.

D. Larocca and W. Clough, Dept. of Molecular Biology, University of Southern California, Los Angeles: Methylation of viral and cellular DNA in EBV-infected cells.

Session 12: Nucleoproteins, Transcription, and Enzymes

Chairperson: S. Kit, Baylor College of Medicine, Houston, Texas

I. W. Halliburton, Dept. of Microbiology, University of Leeds, England: Isolation and characterization of recombinants between herpessimplex and bovine mammillitis viruses.

Y.-R. Roth and S. L. Bachenheimer, Dept. of Bacteriology and Immunology, and Curriculum in Genetics, University of North Carolina, Chapel Hill: Encapsidation of HSV DNA.

S. St. Jeor, C. Hall, C. McGaw, and M. Hall, Dept of Microbiology, University of Nevada School of Medicine, Reno: Characterization of human CMV nucleoprotein complexes.

J. M. DeMarchi and A. S. Kaplan, Dept. of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee: Analysis of the human cytomegalovirus (HCMV) transcripts accumulation in permissively and nonpermissively infected cells.

K. P. Anderson, R. J. Frink, B. H. Gaylord, R. H. Costa, G. B. Devi, and E. K. Wagner, Dept. of Molecular Biology and Biochemistry, University of California, Irvine: High resolution mapping of HSV-1 mRNA.

F. Wohlrab and B. Francke, Dept. of Human Genetics, Yale University, New Haven, Connecticut: Control of expression of the HSV-1-specific deoxypyrimidine triphosphatase in cells infected with temperature-sensitive mutants of HSV-1 and HSV-2 and interytpic recombinants.

P. J. Hoffmann, Dept. of Medical Microbiology and Comprehensive Cancer Center, Ohio State University, Columbus: On the mechanism of action of the deoxyribonuclease specified by HSV.

B. E. Henry, R. J. Feighny, and J. S. Pagano, Cancer Research Center, University of North Carolina, Chapel Hill: An EBV-associated DNase activity appearing in superinfected Raji cells.

W. Clough and J. McMahon, Dept. of Molecular Biology, University of Southern California, Los Angeles: Biological role of the DNA polymerase activity associated with EB virions.

D. Huszar and S. Bacchetti, Dept. of Pathology, McMaster University, Hamilton, Canada: Ribonucleotide reductase induced by HSV infection of mammalian cells—Properties of the enzyme from crude and partially purified extracts.

Session 13: Reports from Workshops

Summary: P. Wildy, University of Cambridge, England

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8th Cold Spring Harbor Conference on Cell Proliferation
Protein Phosphorylation, September 2 — September 7

Arranged by
Ora M. Rosen, *Albert Einstein College of Medicine, New York, New York*
Edwin G. Krebs, *Howard Hughes Medical Institute, University of Washington, Seattle*

267 participants

Session 1: Protein Kinases and Phosphoprotein Phosphatases. I.

Chairperson: E. G. KREBS, Howard Hughes Medical Institute, University of Washington, Seattle

S. S. TAYLOR, A. R. KERLAVAGE, M. J. ZOLLER, N. C. NELSON, and R. L. POTTER, Dept. of Chemistry, University of California, San Diego: The nucleotide binding sites and structural domains of cAMP-dependent protein kinases.

K. TITANI,[1,2] S. SHOJI,[2] L. H. ERICSSON,[2] J. G. DEMAILLE,[4] K. A. WALSH,[2] H. NEURATH,[2] E. H. FISCHER,[2] K. TAKIO,[2] S. B. SMITH,[1,3] and E. G. KREBS,[1,3] [1]Howard Hughes Medical Institute, Depts. of [2]Biochemistry and [3]Pharmacology, University of Washington, Seattle; [4]Faculté de Médicine and CNRS, Montpellier, France: Primary structure of cAMP-dependent protein kinase type II from bovine cardiac muscle.

S. E. BUILDER,[1,2] J. S. BEAVO,[3] and E. G. KREBS,[2] [1]Merck, Sharpe & Dohme Research Laboratories, Rahway, New Jersey; [2]Howard Hughes Medical Institute, [3]Dept. of Pharmacology, University of Washington, Seattle: The mechanism of activation and inactivation of cAMP-dependent protein kinase—Several lines of evidence.

J. D. CORBIN, S. R. RANNELS, and D. A. FLOCKHART, Howard Hughes Medical Institute and Dept. of Physiology, Vanderbilt University, Nashville, Tennessee: Structure-function relationships of cAMP-dependent protein kinases.

S. B. SMITH, H. D. WHITE, J. B. SIEGEL, and E. G. KREBS, Howard Hughes Medical Institute, University of Washington, Seattle: cAMP-dependent protein kinase—Cyclic nucleotide binding, structural changes, and release of the catalytic subunits.

E. T. KAISER,[1] H. N. BRAMSON,[1] R. N. ARMSTRONG,[1] H. KONDO,[1] J. STINGELIN,[1] N. THOMAS,[1] J. GRANOT,[2] and A. S. MILDVAN,[2] [1]Depts. of Chemistry and Biochemistry, University of Chicago, Illinois; [2]Institute for Cancer Research, Philadelphia, Pennsylvania: Mechanistic studies on the catalytic action of bovine cardiac muscle cAMP-dependent protein kinase.

S. SHALTIEL, E. ALHANATY, R. CESLA, J. S. JIMENEZ, A. KUPFER, and Y. ZICK, Dept. of Chemical Immunology, Weizmann Institute of Sciences, Rehovot, Israel: cAMP-dependent protein kinase—Biorecognition and bioregulation.

Session 2: Protein Kinases and Phosphoprotein Phosphatases. II.

Chairperson: E. G. KREBS, Howard Hughes Medical Institute, University of Washington, Seattle

J. A. BEAVO and M. C. MUMBY, Dept. of Pharmacology, University of Washington, Seattle: Use of monoclonal antibodies to cyclic nucleotide-dependent protein kinases as site-specific reagents.

H. HILZ, W. WEBER, H. SCHRÖDER, and G. SCHWOCH, Institut für Physiologische Chemie, Universität Hamburg, Federal Republic of Germany: Analysis of cAMP-dependent protein kinases by immunotitration—Multiple forms—multiple functions?

U. WALTER,[1] P. DECAMILLI,[2] S. M. LOHMANN,[3] P. MILLER,[3] and P. GREENGARD,[3] [1]Depts. of Physiological Chemistry and Medicine, University of Würzburg, Federal Republic of Germany; [2]Section of Cell Biology and [3]Dept. of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: Regulation and cellular localization of cAMP- and cGMP-dependent protein kinases.

N. PRASHAD, Dept. of Neurology, University of Texas Health Science Center, Dallas: Induction of free cAMP binding protein in neuroblastoma cells.

R. A. Steinberg, Biological Sciences Group, University of Connecticut, Storrs: cAMP-dependent protein phosphorylations in intact cultured cells.

M. M. Gottesman, T. Singh, A. LeCam, C. Roth, F. Cabral, J.-C. Nicolas, and I. Pastan, NCI, National Institutes of Health, Bethesda, Maryland: cAMP-dependent phosphorylations in cultured fibroblasts—A genetic approach.

B. P. Schimmer, J. Tsao, and P. Doherty, Banting and Best Dept. of Medical Research, University of Toronto, Ontario, Canada: cAMP-dependent protein kinase and regulation of adrenocortical functions—A genetic evaluation.

S. E. Mayer,[1] L. L. Brunton,[1] and J. S. Hayes,[2] [1]Division of Pharmacology, University of California, San Diego; [2]Eli Lilly Laboratories, Indianapolis, Indiana: Selective activation of subcellular fractions of cAMP-dependent protein kinase in heart by isoproterenol and prostaglandin E_1.

Y. Nishizuka and Y. Takai, Dept. of Biochemistry, Kobe University School of Medicine, Japan: Calcium and phospholipid in a new receptor mechanism for protein phosphorylation.

Session 3: Protein Kinases and Phosphoprotein Phosphatases. III.

Chairperson: E. G. Krebs, Howard Hughes Medical Institute, University of Washington, Seattle

G. N. Gill, University of California School of Medicine, San Diego: cGMP-dependent protein kinase.

D. B. Glass and L. H. McFann, Dept. of Pharmacology, Emory University School of Medicine, Atlanta, Georgia: Initial velocity kinetics of the cGMP-dependent protein kinase using histone H2B or a synthetic peptide as substrate.

D. J. Graves, R. Uhing, J. Miller, and M. Kobayashi, Depts. of Biochemistry and Biophysics, Iowa State University, Ames: Phosphorylation and activation of glycogen phosphorylase.

L. M. G. Heilmeyer, Jr., U. Jahnke, M. W. Kilimann, K. P. Kohse, and J. E. Sperling, Institut für Physiological Chemistry, Ruhr-Universität, Bochum, Federal Republic of Germany: Intramolecular regulatory cascades in phosphorylase kinase and troponin, interrelationship between Mg^{2+} or Ca^{2+} binding and phosphorylation.

B. E. Kemp and M. J. John, Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria, Australia: Synthetic peptide substrates for protein kinases.

H. S. Sul, R. H. Cooper, T. E. McCullough, C. A. Pickett-Gies, K. L. Angelos, and D. A. Walsh, Dept. of Biological Chemistry, University of California School of Medicine, Davis: Mechanism of regulation of cardiac phosphorylase kinase.

K.-P. Huang, NICHHD, National Institutes of Health, Bethesda, Maryland: Phosphorylation of rabbit skeletal muscle glycogen synthase by cAMP-dependent and -independent protein kinases.

K. Schlender,[1] S. J. Beebe,[1] and E. M. Reiman,[2] Depts. of [1]Pharmacology and [2]Biochemistry, Medical College of Ohio, Toledo: Isolation of cAMP-independent glycogen synthase kinase from renal cortex and medulla.

Session 4: Protein Kinases and Phosphoprotein Phosphatases. IV.

Chairperson: E. G. Krebs, Howard Hughes Medical Institute, University of Washington, Seattle

J. W. Mitchell,[1] W. Lau,[2] and J. A. Thomas,[2] [1]Monsanto Corp., St. Louis, Missouri; [2]Dept. of Biochemistry, Iowa State University, Ames: Covalent modification and regulation of bovine cardiac glycogen synthase.

T. R. Soderling and M. E. Payne, Howard Hughes Medical Institute and Dept. of Physiology, Vanderbilt Medical School, Nashville, Tennessee: Liver-calmodulin-dependent glycogen synthase kinase.

E. Y. C. Lee, S. R. Silberman, H. Paris, M. Ganapathi, and S. Petrovic, Dept. of Biochemistry, University of Miami School of Medicine, Florida: Phosphorylase phosphatase—Isolation and properties of the enzymes from rabbit muscle.

H.-C. Li, Dept. of Biochemistry, Mount Sinai School of Medicine, City University of New York, New York: Properties of cardiac muscle phosphorylase phosphatase, glycogen synthase phosphatase, and alkaline phosphatase isozymes.

D. L. Brautigan, L. M. Ballou, and E. H. Fischer, Dept. of Biochemistry, University of Washington Medical School, Seattle: Formation of protein:glutathione disulfides in the regulation of sarcoplasmic protein phosphatase.

R. L. Khandelwal, S. K. Sloan, and D. J. Craw, Dept. of Oral Biology, University of Manitoba, Winnipeg, Canada: Characterization and interaction of heat-stable inhibitor 2 and an activator with phosphoprotein phosphatase from liver.

N. B. Madsen, R. J. Fletterick, and P. J. Kasvinsky, Dept. of Biochemistry, University of Alberta, Edmonton, Canada: Regulation of protein phosphatase 1 via glycogen phosphorylase.

J. R. Vandenheede, S.-D. Yang, J. Goris, and W. Merlevede, Afdeling Biochemie Dept. Humane Biologie, Faculteit der Geneeskunde, Katholieke Universiteit te Leuven, Belgium: Regulation of rabbit muscle ATP-Mg-dependent protein phosphatase by cAMP- and Ca^{2+}-independent synthase kinase.

J. H. Wang, S. T. Tam, W. G. Lewis, and R. K. Sharma, Dept. of Biochemistry, University of Manitoba, Winnipeg, Canada: The regulation of protein phosphorylation by calmodulin in skeletal muscle.

Session 5: Metabolism. I.

Chairperson: J. Exton, Vanderbilt University, Nashville, Tennessee

T. D. Chrisman and J. H. Exton, Howard Hughes Medical Institute and Dept. of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee: Hormonal regulation of liver glycogenolysis.

J. Garrison, Dept. of Pharmacology, University of Virginia Medical School, Charlottesville: The role of Ca^{2+} ion and cyclic-nucleotide-independent protein kinases in the control of hepatic carbohydrate metabolism.

S. J. Pilkis, T. H. Claus, and R. El-Maghrabi, Dept. of Physiology, Vanderbilt University, Nashville, Tennessee: Modulation of hepatic pyruvate kinase and phosphofructokinase activity.

L. Engström,[1] U. Ragnarsson,[2] and Ö. Zetterqvist,[1] [1]Institute of Medical and Physiological Chemistry; [2]Institute of Biochemistry, Biomedical Center, University of Uppsala, Sweden: Regulation of pyruvate kinase by phosphorylation and proteolysis.

J. B. Blair and R. F. Kletzien, Dept. of Biochemistry, West Virginia University Medical Center, Morgantown: Glucocorticoids and the phosphorylation of liver pyruvate kinase.

M.-S. Shiao, R. F. Drong, R. E. Dugan, T. A. Baker, and J. W. Porter, William S. Middleton Memorial Veterans Administration Hospital, and Dept. of Physiological Chemistry, University of Wisconsin, Madison: Activators and inactivators of rat liver acetyl-CoA carboxylase and HMG-CoA reductase activities.

R. A. Parker,[1] T. S. Ingebristen,[1] M. J. H. Geelen,[2] and D. M. Gibson,[1] [1]Dept. of Biochemistry, Indiana University School of Medicine, Indianapolis; [2]Laboratory of Veterinary Biochemistry, State University of Utrecht, The Netherlands: Short-term modulation of HMG CoA reductase activity in rat hepatocytes in response to insulin and glucagon.

H. B. Brewer, Jr. and Z. H. Beg, NHLBI, National Institutes of Health, Bethesda, Maryland: Short-term regulation of hepatic cholesterol synthesis.

Session 6: Metabolism. II.

Chairperson: J. Exton, Vanderbilt University, Nashville, Tennessee

K.-H. Kim, Dept. of Biochemistry, Purdue University, West Lafayette, Indiana: Regulation of acetyl-CoA carboxylase by covalent modification.

K. Uyeda, E. Furuya, and T. Kagimoto, Veterans Administration Medical Center, and Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Regulation of liver phosphofructokinase by phosphorylation.

J. C. Khoo and D. Steinberg, Dept. of Medicine, University of California, San Diego: Hormone-sensitive neutral triglyceride lipase in adipose tissue and in 3T3-L1 cells.

A. L. Kerbey, P. J. Randle, and G. J. Sale, Dept. of Clinical Biochemistry, University of Oxford, England: Regulation of pyruvate dehydrogenase complex by phosphorylation and dephosphorylation.

L. Reed and F. Pettit, Clayton Foundation Biochemical Institute and Dept. of Chemistry, University of Texas, Austin: Phosphorylation and dephosphorylation of pyruvate dehydrogenase.

Session 7: Insulin and Growth Factors

Chairperson: O. M. Rosen, Albert Einstein College of Medicine, New York, New York

L. Jarett,[1] F. L. Kiechle,[1] D. A. Popp,[2] and N. Kotagal,[2] [1]Dept. of Pathology and Laboratory Medicine, University of Pennsylvania Medical School, Philadelphia; [2]Washington University School of Medicine, St. Louis, Missouri: The role of a chemical mediator of insulin action in the control of phosphorylation.

K. Cheng, G. Galasko, L. Huang, J. Kellogg, and J. Larner, Dept. of Pharmacology, University of Virginia, Charlottesville: Mediators of insulin action on protein phosphorylation and dephosphorylation.

N. S. Ranganathan, T. C. Linn, and P. A. Srere, Pre-Clinical Science Unit, Veterans Administration Center; Dept. of Biochemistry, University of Texas Health Science Center, Dallas: A phosphopeptide isolated from phospho ATP citrate lyase.

S. Ramakrishna and W. B. Benjamin, Dept. of Physiology and Biophysics, State University of New York, Stony Brook: ATP-citrate lyase phosphorylation by a cyclic AMP-independent protein kinase.

M. C. Alexander, J. L. Palmer, and J. Avruch, Howard Hughes Medical Institute Laboratory, Harvard Medical School; Diabetes Unit, Massachusetts General Hospital, Boston: Hormonally stimulated phosphorylation of ATP-citrate lyase.

C. J. Smith, C. S. Rubin, and O. M. Rosen, Dept. of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York: Insulin-stimulated phosphorylation of ribosomal protein S6 in differentiated 3T3-L1 cells.

G. Thomas and M. Siegmann, Dept. of Cell Biology, Friedrich Miescher-Institut, Basel, Switzerland: Increased multiple phosphorylation of ribosomal protein S6 following serum-stimulation of quiescent 3T3 cells.

S. Cohen, Dept. of Biochemistry, Vanderbilt University, Nashville, Tennessee: Epidermal growth factor-receptor-protein kinase interactions.

Session 8: Contractile Proteins

Chairperson: R. Adelstein, NHLBI, National Institutes of Health, Bethesda, Maryland

R. S. Adelstein, M. D. Pato, J. R. Sellers, and M. A. Conti, NHLBI, National Institutes of Health, Bethesda, Maryland: Regulation of the contractile proteins in muscle and nonmuscle cells by phosphorylation-dephosphorylation.

J. T. Stull, Moss Heart Center and Dept. of Pharmacology, University of Texas Health Science Center, Dallas: Phosphorylation of myosin in different types of muscles.

F. Hofmann and H. Wolf, Pharmakologisches Institut der Universität, Heidelberg, Federal Republic of Germany: Regulatory protein kinases of cardiac muscle.

C. J. LePeuch, M. P. Walsh, J.-C. Cavadore, A. Molla, and J. G. Demaille, Faculté de Médecine and CNRS, Montpellier, France: The concerted control of contraction by cyclic nucleotide and calcium-calmodulin dependent phosphorylation. Modulation of myosin phosphorylation and of sarcoplasmic reticulum calcium uptake.

A. M. Katz, University of Connecticut, Farmington: Regulation of calcium transport in cardiac sarcoplasmic reticulum by cAMP-dependent protein kinase.

J. Y. J. Wang and D. E. Koshland, Jr., Dept. of Biochemistry, University of California, Berkeley: Protein phosphorylation as a regulatory mechanism in procaryotes.

P. de Lanerolle,[1] J. R. Feramisco,[2] K. Burridge,[2] and R. S. Adelstein,[1] [1]NHLBI, National Institutes of Health, Bethesda, Maryland; [2]Cold Spring Harbor Laboratory, New York: Localization of myosin light chain kinase in nonmuscle cells.

M. Bárány,[1] K. Bárány,[1] J. T. Barron,[1] S. J. Kopp,[1] R. A. Janis,[2] D. D. Doyle,[1] S. R. Hager,[1] F. Homa,[1] and S. T. Sayers,[1] [1]University of Illinois Medical Center; [2]Northwestern University Medical Center, Chicago, Illinois: Protein phosphorylation in live muscle.

W. G. L. Kerrick, P. E. Hoar, and P. S. Cassidy, Dept. of Physiology and Biophysics, University of Washington, Seattle: Skinned muscle fibers—The functional significance of phosphorylation in Ca^{2+}-activated tension.

R. J. Solaro, M. J. Holroyde, T. H. Crouch, S. Robertson, J. D. Johnson, and J. D. Potter, Depts. of Physiology, Pharmacology, and Cell Biophysics, University of Cincinnati College of Medicine, Ohio: Myofilament protein phosphorylation in striated muscle.

J. L. Daniel, I. R. Molish, H. Holmsen, and L. Salganicoff, Thrombosis Research Center, Temple University, Philadelphia, Pennsylvania: Phosphorylation of myosin light chain in intact platelets.

Session 9: Protein Synthesis

Chairperson: I. M. London, Harvard-MIT Division of Health, Science, and Technology, Cambridge, Massachusetts

S. Ochoa, C. de Haro, J. Siekierka, and H. Grosfeld, Roche Institute of Molecular Biology, Nutley, New Jersey: Protein phosphorylation and translational control.

I. M. London, V. Ernst, R. Fagard, A. Leroux, D. H. Levin, and R. Petryshyn, Massachusetts Institute of Technology, Cambridge: Regulation of protein synthesis by phosphorylation and heme.

B. Hardesty and G. Kramer, Clayton Foundation Biochemical Institute, University of Texas, Austin: Regulation of peptide initiation by phosphorylation and dephosphorylation of eIF-2.

B. Safer, R. Jagus, and D. Crouch, NHLBI, National Institutes of Health, Bethesda, Maryland: Both the redox and phosphorylation states of eIF-2 regulate protein synthesis initiation.

T. Hunt, Dept. of Biochemistry, University of Cambridge, England: Protein phosphorylation and the control of protein synthesis.

J. A. Traugh, P. T. Tuazon, and R. W. Del Grande, Dept. of Biochemistry, University of California, Riverside: Phosphorylation of translational components.

M. K. Haddox and D. H. Russell, Dept. of Pharmacology, University of Arizona Health Sciences Center, Tucson: cAMP-dependent protein kinase implicated in the transcriptional induction of ornithine decarboxylase.

Session 10: Nuclear and Cytoskeletal Proteins

Chairperson: T. Langan, University of Colorado, Boulder

T. A. Langan, C. Zeilig, and B. Leichtling, Dept. of Pharmacology, University of Colorado School of Medicine, Denver: Characterization of multiple-site phosphorylation of H1 histone in proliferating cells.

J. A. D'Anna,[1] L. R. Gurley,[1] R. R. Becker,[2] S. S. Barham,[3] R. A. Walters,[1] and R. A. Tobey,[1] [1]Los Alamos Scientific Laboratory, New Mexico; [2]Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis; [3]Dept. of Cell Biology, Mayo Clinic, Rochester, Minnesota: Phosphorylation of the butyrate-enhanced protein, histone $H1^0$, in Chinese hamster cells.

L. R. Gurley, R. A. Tobey, J. A. D'Anna, M. S. Halleck, R. A. Walters, S. S. Barham, and J. J. Jett, Los Alamos Scientific Laboratory, University of California, New Mexico: Relationships between histone phosphorylation and cell proliferation.

H. Baydoun, J. Hoppe, and K. G. Wagner, Gesellschaft für Biotechnologische Forschung mbH, Braunschweig-Stöckheim, Federal Republic of Germany: The quarternary structure and ligand specificity of two messenger-independent protein kinases from porcine liver nuclei—A comparison with cAMP-dependent protein kinases I.

R. A. JUNGMANN and M. S. LAKS, Cancer Center, Northwestern University Medical School, Chicago, Illinois: Modulation of nuclear cAMP-dependent protein kinase activity at times of gene activity.
J. L. MALLER, Dept. of Pharmacology, University of Colorado School of Medicine, Denver: Control of cell division by protein phosphorylation.
D. L. PURICH, B. J. TERRY, H. D. WHITE, B. A. COUGHLIN, T. L. KARR, and D. KRISTOFFERSON, Dept. of Chemistry, University of California, Santa Barbara: Microtubule associated protein phosphorylation and calcium ion regulation of bovine brain microtubule self-assembly.
C. M. O'CONNOR, D. L. GARD, D. J. ASAI, and E. LAZARIDES, California Institute of Technology, Pasadena: Phosphorylation of the intermediate filament proteins, desmin and vimentin.

Session 11: Viruses and Cell Transformation. I.

Chairperson: T. HUNTER, Salk Institute, San Diego, California

T. HUNTER, B. SEFTON, and J. COOPER, Salk Institute, San Diego, California: Phosphorylation of tyrosine—Its importance in normal cell metabolism and viral transformation.
A. F. PURCHIO,[1] E. ERIKSON,[1] M. S. COLLETT,[2] and R. L. ERIKSON,[1] [1]Dept. of Pathology, University of Colorado Health Sciences Center, Denver; [2]Dept. of Microbiology, University of Minnesota, Minneapolis: Comparison of the Rous sarcoma virus transforming gene product, $pp60^{src}$, and its homologue, $pp60^{src}$, from normal cells.
Y.-S. E. CHENG, C.-Y. CHENG, and L. B. CHEN, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Alteration in protein phosphorylations in RSV transformed cells.
J. G. BURR, S. R. LEE, and J. M. BUCHANAN, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Association of the RSV *src* gene product with the cytoskeleton and identification of a possible cellular substrate protein.
K. SHRIVER and L. R. ROHRSCHNEIDER, Fred Hutchinson Cancer Research Center, Seattle, Washington: Spatial and enzymatic interaction of the Rous sarcoma virus transforming protein with components of the cellular cytoskeleton.

Session 12: Viruses and Cell Transformation. II.

Chairperson: T. HUNTER, Salk Institute, San Diego, California

A. LEVINSON,[1] S. COURTNEIDGE,[2] H. VARMUS,[2] and J. M. BISHOP,[2] [1]Genentech, Inc., South San Francisco; [2]Dept. of Microbiology, University of California, San Francisco: Functional domains and substrate specificity of the transforming protein of avian sarcoma virus.
D. LIVINGSTON, M. BRADLEY, and J. GRIFFIN, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Enzymatic and DNA binding properties of the large SV40 T antigen.
K.-H. SCHEIDTMANN, A. KAISER, and G. WALTER, Institut für Immunbiologie der Universität Freiburg, Federal Republic of Germany: The localization of phosphothreonine in the sequence of SV40 large T antigen.
B. SCHAFFHAUSEN and T. BENJAMIN, Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Phosphorylation of polyoma virus T antigens.

Session 13: Membranes and Neural Function

Chairperson: C. RUBIN, Albert Einstein College of Medicine, New York, New York

P. GREENGARD, Dept. of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: Protein phosphorylation and brain function.
M. TAO, R. CONWAY, S. CHETA, H. C. CHIANG, and T. F. YAN, Dept. of Biological Chemistry, University of Illinois Medical Center, Chicago: Role of protein kinases in erythrocyte membrane phosphorylation.
H. R. DE JONGE and F. S. VAN DOMMELEN, Dept. of Biochemistry I, Erasmus University, Rotterdam, The Netherlands: Cyclic GMP-dependent phosphorylation and ion transport in intestinal microvilli.
C. S. RUBIN,[1] D. SARKAR,[1] N. FLEISCHER,[2] and J. ERLICHMAN,[2] Depts. of [1]Molecular Pharmacology, [2]Medicine, Albert Einstein College of Medicine, Bronx, New York: Immunological characterization of a unique subclass of type II cAMP-dependent protein kinases—Neural-specific protein kinase.
J. HERMOLIN and M. D. BOWNDS, Laboratory of Molecular Biology, University of Wisconsin, Madison: Light-influenced outer segment protein phosphorylation in frog retina—Effects of cyclic nucleotides and calcium.
C. G. PARIS, E. R. KANDEL, and J. H. SCHWARTZ, Division of Neurobiology and Behavior, Columbia University College of Physicians and Surgeons, New York, New York: Serotonin stimulates phosphorylation of a 137,000 dalton membrane protein in the abdominal ganglion of *Aplysia*.
N. WEINER, J. MELIGENI, and P. R. VULLIET, Dept. of Pharmacology, University of Colorado School of Medicine, Denver: Regulation of tyrosine hydroxylase activity and alteration in the properties of the enzyme following phosphorylation by protein kinases.
S. KAUFMAN, H. HASEGAWA, H. WILGUS, and M. PARNIAK, NIMH, National Institutes of Health, Bethesda, Maryland: The regulation of hepatic phenylalanine hydroxylase activity by phosphorylation and dephosphorylation.

P. E. Branton,[1] S.-P. Yee,[1] J. F. Downey,[2] S. Mak,[2] F. L. Graham,[1,2] and S. T. Bayley,[2] Depts. of [1]Pathology, [2]Biology, McMaster University, Hamilton, Ontario, Canada: Protein kinase activity associated with the tumor antigens of human adenoviruses.

C. G. Davis, D. Milfay, I. Diamond, and A. S. Gordon, University of California, San Francisco: Acetylcholine receptor phosphorylation—Kinase localization and ligand regulation.

S. Halegoua and J. Patrick, Neurobiology Laboratory, Salk Institute, San Diego, California: Nerve growth factor mediates phosphorylation of specific proteins.

M. M. Hosey and F. Marcus, Dept. of Biochemistry, University of Health Sciences/Chicago Medical School, Illinois: Analysis of fructose-1, 6-bisphosphatases as substrates for the cAMP-dependent protein kinase.

S.-Y. Kao and W. R. Bauer, Dept. of Microbiology, State University of New York, Stony Brook: Purification and characterization of a superhelix binding protein from vaccinia virus.

R. G. Kemp, S. E. Bazaes, L. G. Foe, and S. P. Latshaw, Dept. of Biochemistry, University of Health Sciences/Chicago Medical School, Illinois: Phosphorylation of muscle phosphofructokinase in vivo and in vitro.

M. M. King and G. M. Carlson, Dept. of Chemistry, University of South Florida, Tampa: Synergistic effect of Ca^{2+} and Mg^{2+} in promoting a Ca^{2+}-independent activity of phosphorylase kinase.

E. G. Kranias, L. M. Bilezikjian, F. Mandel, J. D. Potter, and A. Schwartz, Dept. of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Ohio: cAMP and calmodulin dependent phosphorylation of cardiac sarcoplasmic reticulum.

M. D. Pato and R. S. Adelstein, NHLBI, National Institutes of Health, Bethesda, Maryland: Properties of myosin phosphatases from turkey gizzard.

K. M. Rose, D. A. Stetler, and S. T. Jacob, Hershey Medical Center, Penn State University, Hershey, Pennsylvania: Protein kinase activity of purified RNA polymerase I—Probable function of 42,000 and 24,600 molecular weight polypeptides.

J. R. Sellers and R. S. Adelstein, NHLBI, National Institutes of Health, Bethesda, Maryland: Preparation of phosphorylatable subfragments from *Limulus* and gizzard myosin.

This meeting was supported in part by the National Institute of General Medical Sciences; National Institute of Arthritis, Metabolism, and Digestive Diseases; National Cancer Institute; National Heart, Lung, and Blood Institute; and Fogarty International Center, National Institutes of Health.

Poxvirus-Iridovirus, September 15–September 18

Arranged by
Bernard Moss, *National Institutes of Health, Bethesda, Maryland*
David C. Kelly, *University of Oxford, England*

65 participants

Session 1: DNA Structure

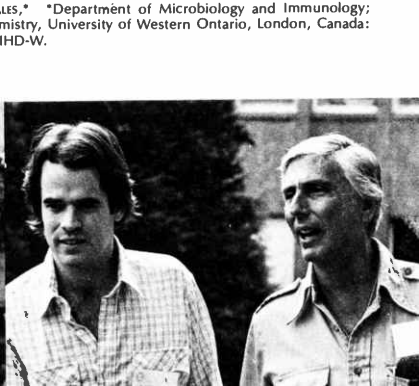
Chairperson: E. Paoletti, New York State Department of Health, Albany

R. Wittek and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: Tandem repeats within the inverted terminal repetition of vaccinia virus DNA.
B. Moss and E. Winters, NIAID, National Institutes of Health, Bethesda, Maryland: Novel variants of vaccinia virus that rapidly reiterate and delete terminal sequences.
R. W. Moyer and R. L. Graves, Department of Microbiology, Vanderbilt University, Nashville, Tennessee: Transpositions and deletions lead to hypervariable poxvirus DNA termini.
L. C. Archard, M. Mackett, and K. R. Dumbell, Department of Virology, St. Mary's Hospital Medical School, London, England: Symmetrical, terminal aberration in orthopox virus genome structure.
J. Esposito, C. Cabradilla, J. Nakano, and J. Obijeski, Virology Division, Center for Disease Control, Atlanta, Georgia: Intragenomic sequence transposition in monkeypox virus.
D. Wing and A. Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey: Cloning of *Hin*dIII restricted vaccinia DNA fragments.
A. Talavera, J. Almendral, V. Ley, and E. Viñuela, Centro de Biologia Molecular (CSIC-UAM), Canto Blanco, Madrid, Spain: Order and cloning of DNA fragments from African swine fever virus genome.
R. Goorha and K. G. Murti, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Structure of the frog virus 3 genome.
M. H. Lee, D. B. Willis, and A. Granoff, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Restriction endonuclease mapping of the frog virus 3 genome.
D. Willis and A. Granoff, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Frog virus 3 DNA is heavily methylated at CpG sequences.

Session 2: DNA Replication and Genetics

Chairperson: J. Holowczak, Rutgers Medical School, Piscataway, New Jersey

R. Goorha, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Replication of frog virus 3 DNA.
S. J. Keller,* B. Jasny,† and W. Bauer,† *Department of Biology, University of Cincinnati, Ohio; †Department of Microbiology, State University of New York, Stony Brook: Location of the replication origin in vaccinia virus WR.
C. K. Sam and K.R. Dumbell, Department of Virology, St. Mary's Hospital Medical School, London, England: Rescue of poxvirus DNA.
E. Nakano, D. Panicali, and E. Paoletti, Division of Laboratories and Research, New York State Department of Health, Albany: Rescue of unique L-variant DNA sequences by S-variant vaccinia virus.
A. Pellicer* and M. Esteban,† *Institute of Cancer Research, Columbia University, New York, New York; †Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn: Gene transfer of vaccinia DNA into animal cells.
G. McFadden,* E. Faust,† G. Mackie,‡ and S. Dales,* *Department of Microbiology and Immunology; †Cancer Research Unit; ‡Department of Biochemistry, University of Western Ontario, London, Canada: Cloning of *Eco*RI fragment D of vaccinia virus IHD-W.



G. D. Brown and R. W. Moyer, Department of Microbiology, Vanderbilt University, Nashville, Tennessee: The behavior of "early" host range mutants of rabbit poxvirus (RPμhr) mutants under permissive and nonpermissive conditions.

K. Essani and S. Dales, Department of Microbiology and Immunology, University of Western Ontario, London, Canada: Further studies on recombination in group E mutants.

R. Drillien, D. Spehner, and A. Kirn, INSERM U74 and Laboratory of Virology, Strasbourg, France: Complementation and recombination between vaccinia virus temperature-sensitive mutants.

R. C. Condit and A. Motyczka, Biochemistry Department, State University of New York, Buffalo: Isolation and characterization of temperature-sensitive mutants of vaccinia virus.

Session 3: Transcription

Chairperson: W. K. Joklik, Duke University Medical Center, Durham, North Carolina

J. Morgan and B. Roberts, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Viral gene expression in vaccinia infected L-cells.

A. Mahr and B. Roberts, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Organization and expression of some cloned *Eco*RI vaccinia virus fragments.

H. Belle Isle, S. Venkatesan, and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: Mapping of vaccinia virus early and late polypeptides by cell-free translation of mRNA selected by hybridization to cloned DNA fragments.

R. Wittek, J. A. Cooper, and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: Expression of the vaccinia virus genome: Analysis and mapping of mRNAS and polypeptides encoded within the left 21 KB of the genome.

S. Venkatesan and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: In vitro transcription of the inverted terminal repetition of the vaccinia virus genome.

D. E. Hruby and L. A. Ball, Biophysics Laboratory, University of Wisconsin, Madison: Studies concerning the temporal regulation of vaccinia virus thymidine kinase activity during infection.

E. Paoletti, C. Whitkop, and B. R. Lipinskas, Division of Laboratories and Research, New York State Department of Health, Albany: Two-dimensional analysis of ribonuclease T_1 resistant 5'-terminal oligonucleotides derived from vaccinia virus RNAs.

S. Shuman and J. Hurwitz, Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: GTP-pyrophosphate exchange activity associated with vaccinia capping enzyme: Implications for the mechanism of transguanylylation.

H. S. Caplen and J. A. Holowczak, College of Medicine and Dentistry, Rutgers Medical School, Piscataway, New Jersey: Transcription in parapoxviruses.

Session 4: Transcription–Effects on Host Cells

Chairperson: J. Kates, Scripps Clinic, La Jolla, California

M. L. Salas, J. Kuznar, and E. Viñuela, Centro de Biologia Molecular (CSIC-UAM), Canto Blanco, Madrid, Spain: Characterization of the RNAs synthesized in vitro by the RNA polymerase associated to African swine fever virus.

R. Raghow and A. Granoff, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Heterogeneity of methylated nucleotide sequences in frog virus 3 messenger RNAs.

B. G. T. Pogo, P. Freimuth, and A. Stein, Center for Experimental Cell Biology, Mount Sinai School of Medicine, New York, New York: Differences in structure and expression of two strains of Shope fibroma virus.

A. Rice and B. Roberts, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Degradation of L cell messenger RNA during vaccinia infection.

G. Beaud, F. Ben-Hamida, R. Lemieux, and A. Person, Institut de Recherche en Biologie Moléculaire du CNRS et de l'Université Paris, France: Shutoff of host protein synthesis by vaccinia virus.

A. M. Aubertin, P. Descamps, L. Tondre, and A. Kirn, INSERM U74 and Laboratory of Virology, Strasbourg, France: Modification of the permeability of the plasma membrane by frog virus 3 soluble antigens—Relation to the inhibition of the host cell metabolism.

M. Cerutti, J. Attias, N. Balange, C. Monnier, and G. Devauchelle, Laboratoire de Microbiologie, Centre de Recherches de Biochimie et Physiologie Cellulaires, Mont-Saint-Aignan, France: Effects of reconstituted vesicles from *Chilo* iridescent virus on invertebrate cell cultures.

Session 5: Biology

Chairperson: S. Dales, University of Western Ontario, London, Canada

V. Zaslavsky* and P. H. Hofschneider,† *Kimron Veterinary Institute, Bet Dagan, Israel; †Max-Planck-Institute of Biochemistry, Martinsried, Federal Republic of Germany: Uncoating of vaccinia virus by cells in an inhibited state.

M. Silver and S. Dales, University of Western Ontario, London, Canada: Biogenesis of vaccinia—Carbohydrate of the hemagglutin molecule.

H. Shida and S. Dales, Department of Microbiology and Immunology, University of Western Ontario, London, Canada: Biogenesis of vaccinia—Carbohydrate of the hemagglutinin molecule.

L. G. Payne, Department of Virology, Karolinska Institute School of Medicine, Stockholm, Sweden: The effect of glycosylation inhibitors on the release of enveloped vaccinia virus.

J. Byrne and J. A. Holowczak, Rutgers Medical School, Piscataway, New Jersey: Immune response in DBA/2 mice challenged with vaccinia virus—Immunotherapy of murine melanoma tumors.

A. Cole,* G. O. Poinar,† R. Hess,† and T. J. Morris,* *Department of Plant Pathology and †Department of Entomology, University of California, Berkeley: A new iridovirus of two species of terrestrial isopods also infects their nematode parasite.

BANBURY CENTER
1980 Activities

The third year of the Banbury Center program on the environmental health risks that are an urgent concern of biological science and public policy was a period of both fulfillment and difficulty.

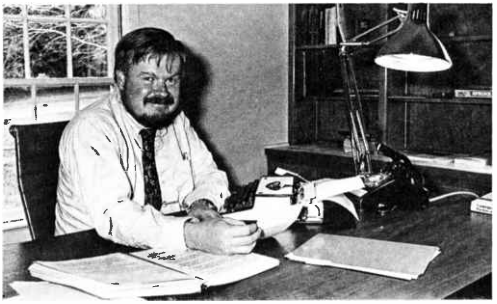
The sense of fulfillment came from several sources. Reports continued to emerge from meetings that participants found both focused and stimulating. There was continued praise from participants for the facilities at Banbury and the arrangements made by many people, most notably by Beatrice Toliver, the Banbury administrative assistant, and by Katya Davey, our hostess and cook in Robertson House. Further meetings were held in 1980 on topics likely to produce reports of even wider interest than those published previously. Response was excellent to an appeal for conference ideas made to leaders in research on environmental health risks. Small meetings at Banbury on rapidly developing fields of fundamental biology continued to proliferate. Planning for meetings in 1981 and 1982, several involving collaboration with other institutions including government agencies, pushed forward.

The difficulty came because early expectations of funding the Banbury program entirely from sources outside the Laboratory were not met. About 30 percent of the program's cost so far has come from appropriations of Laboratory funds by a Board of Trustees that vigorously endorses the public service goals of the Banbury program but insists, along with the Laboratory and Banbury administrations, that the deficits must be removed promptly through vigorous fund raising for both short-term and long-term needs.

Environmental Health Risks

The three years since the start of the Banbury program have been a time of intensifying concern about the possibility that agricultural and industrial chemicals, or factors in the diet, or such pleasurable habits as smoking—things that other people do to us or that we do to ourselves—may contribute to variations in cancer rates between different localities or over time. In the same period, scientific studies of the linkages between the environment and cancer have intensified, in the hope of better understanding of how cancers may be prevented.

But as the studies continue, the goal of cancer prevention is clouded by complications. Writing in *Nature* in January 1981, John Cairns, former Director of Cold Spring Harbor Laboratory, pointed out that few types of cancer fit a simple model of causation by damage to the genetic material in the nuclei of cells, as would be caused by



Victor McElheny, Banbury Center Director

mutagenic chemicals in the workplace or the general environment. Cairns, now at the Harvard School of Public Health and one of Banbury Center's scientific advisors, noted that more complex models, involving several stages, drastic rearrangements of chromosomes, the action of the body's metabolism and the action of so-called promoters, are thrusting themselves forward. The new complexity is not discouraging. Instead, it becomes clearer than ever that biology and genetics at the molecular level—the subjects that Cold Spring Harbor Laboratory works on—have a multi-faceted relevance to the understanding of the onset of cancer. Also clearer than ever is the relevance of an environmental health-risk program, focused on cancer prevention, at a fundamental biology laboratory.

Four New Books

Publication of the first two Banbury Reports in 1979, covering environmental mutagens and mammalian-cell mutagenesis tests, was followed in 1980 by Banbury Reports 3 through 6, covering low-tar cigarettes, populations with low cancer rates, ethylene dichloride, and the labeling of dangers. The four books explore a wide range of issues: whether cigarette-linked lung cancer, the largest single environmental cancer cause, can be reduced; how we can improve our knowledge of such risks as dietary factors by studying defined populations with low cancer rates; how to resolve conflicts between two different cancer tests of the same substance; and whether labeling by itself can be heavily relied on for public health protection strategies.

The production of the four books was made smoother and faster by good relations with authors, typesetters, and printers, achieved by Lynda Moran and Judith Cuddihy, the Banbury editors, and Kathleen Kennedy, the Banbury editorial assistant. Conferences held in October and November of 1979 resulted in books published in May, June, and August of 1980. The conference on labeling in May 1980 produced a book in December. Our book on cancer risks in defined populations, appearing in June 1980, came out five months earlier than the proceedings of a conference involving many of the same people—held one year before our conference.

The availability of a professional transcript of each conference allowed us to increase the interest of each book by including lively discussion of the formal papers. Through strong sales, including those to the National Cancer Institute, the books came very close to meeting all publication expenses, including marketing and overhead.

Conferences

The first 1980 conference, in May, on environmental health risks, represented a first foray into the social-science aspects of this field. The conference concluded that labeling could not handle the regulatory burden of public health protection single-handedly. The conference on gastrointestinal cancer in October focused new attention on substances formed inside the body, rather than those taken in, that may be important in causing cancer of the colon. The conference on hormones and breast cancer, also in October, explored the notion of a close linkage between total estrogen exposure and the risk of contracting breast cancer.

The uses of the Banbury estate continued to expand. At the beginning of March, James Hicks of the yeast group at the Laboratory, organized a workshop on the molecular biology of plants. In May, two members of the Laboratory's Neurobiology Advisory Committee organized a small conference on the molluscan nerve cell. To edit the book resulting from this conference, which received a very favorable review in the journal

Katya Davey

Judith Cuddihy, Lynda Moran, Beatrice Toliver

Science, on May 15, 1981, Judith Cuddihy of our staff temporarily shifted to the editorial group in Nichols. In November, Ron McKay of the scientific staff organized a workshop on monoclonal antibodies against neural antigens, which is expected to result in a book in 1981. In December, the Banbury staff worked with Joe Sambrook in organizing the conference on construction and use of mammalian vectors, which was summarized in the British journal, *Nature*, on March 5, 1981.

Planning continued for what has been undoubtedly the most difficult and controversial of the Banbury risk assessment conferences so far. This was the conference at the end of March 1981 on the quantification of occupational cancer. Elaborate consultations were held up to the last minute with all sides of the complex arguments about the true size of the cancer risk in the workplace, to assure that major viewpoints were represented. The 55 participants heard estimates of cancer attributable to occupation that were smaller than the most alarmist figures, but larger than those put forward by opponents of government regulation of workplace conditions.

In 1980, planning began for many meetings in 1981–82, including the conferences on gene amplification and aberrant chromosomal structures, scheduled for October, patenting of life forms, also scheduled for October, and environmental risks for developing organisms (including children), scheduled for Novemeber.

Funds were requested for conferences in February and March 1982 on the possible role of nitrosamines in human cancer and new techniques of chemical dosimetry.

Courses

Occupying Banbury facilities almost continuously from the end of the spring conference season in early June to the beginning of the late-summer conference season in mid-August was a series of courses. The first of these, on the Neurobiology of Behavior, was taught by Eric Kandel and John Koester of the Columbia University College of Physicians and Surgeons and Keir Pearson of the University of Alberta. The second, called The Synapse: Cellular and Molecular Neurobiology, was taught by Rami Rahamimoff of the Hebrew University Medical School in Jerusalem, Jack McMahon of Stanford University, Bernard Katz of University College, London, Charles Stevens of Yale University and Doju Yoshikami of the University of Utah. The third course, on Principles of Neural Development, was taught by Dale Purves of Washington University in St. Louis and P. H. Patterson of Harvard Medical School. After these courses, Arnold J. Levine of the State University of New York organized, along with D. P. Lane of Imperial College, London, a former member of the Laboratory scientific staff, a workshop on Tumor and Development Antigens. Details on attendance and programs are printed in the

section on post-graduate training programs. Both Dr. Stevens and Dr. Levine are scientific trustees of Cold Spring Harbor Laboratory.

Support

Through the year, plans were made for the first of our workshops for journalists under the $100,000 grant from the Alfred P. Sloan Foundation (noted in the report for 1979). This workshop, for the editorial staff of *Newsday*, was held in January 1981, and concerned issues of ground water pollution, the disposal of chemical wastes, and the influence of diet on cancer rates. The success of that workshop triggered planning for several more during 1981, including a highly successful workshop on DNA held for leading editorial managers and writers of Time Inc. magazines on May 3 and 4, 1981.

Our fund raising in 1980 was greatly assisted by the purchase of many books from four Banbury conferences by the National Cancer Institute, for wide distribution to institute grantees. Approaches to each United States government agency with a mission in the field of environmental health risks, aimed at joint sponsorship of conferences, bore fruit in the scheduling of two conferences in 1981. Gratifying support was received from the Exxon Education Foundation, the International Life Sciences Institute, and the Environmental Protection Agency for the labeling conference, and from Hoffmann-LaRoche, Inc. and Merck Sharp and Dohme Laboratories for the conference on gastrointestinal cancer. The conference on mammalian vectors drew contributions from nearly a dozen companies, including Abbott Laboratories, Cetus Corporation, Collaborative Genetics Inc., E.I. duPont de Nemours & Co., Genex Corporation, Genentech Inc., Lilly Research Laboratories, Molecular Genetics Inc., Monsanto Company, and New England BioLabs. Very welcome sustaining support was received from New York Life Insurance Company and the Bristol-Myers Foundation.

Product Labeling and Health Risks, May 21–May 23

Because of increasing public discussion of the use of labeling as a substitute for banning products which might be hazardous to at least some people, Louis A. Morris of the Food and Drug Administration, Michael B. Mazis of the American University and Ivan Barofsky of the Johns Hopkins Medical Institutions were asked to organize a conference on the efficacy of labeling as the major reliance in schemes of warning. This sixth of the Banbury conferences on the assessment of environmental health risks brought a large number of behavioral scientists together with representatives of consumer-protection groups, regulatory agencies, industry and the legal profession. The conclusion of the conference was that it would be a mistake to rely wholly on labeling and voluntary action to handle a clearly identified risk.

Session 1: Labeling Case Studies

M. Mazis, The American University, Washington, DC: Introduction, classification, integration.
D. Murphy, Federal Trade Commission, Washington, DC: Cigarette warning labels.
L. Morris, Food and Drug Administration, Rockville, Maryland: Estrogenic drugs—PPIs.
A. Reich, Occupational Safety and Health Administration, Washington, DC: Carcinogens at the work place.
R. Staelin, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Food, product safety, and performance labeling.
R. C. Stokes, Food and Drug Administration, Washington, DC: FDA's food labeling research program.

Session 2: Labeling as a Communications Device

W. McGuire, Yale University, New Haven, Connecticut: Communications model.
J. C. Olson, Pennsylvania State University, University Park: Attention/memory factors.
P. Ley, Plymouth Polytechnic, Devon, England: Practical methods of improving communication.
D. Kanouse, Rand Corporation, Santa Monica, California: Critical aspects of communication.
P. Slovic, Decision Research, Eugene, Oregon: Convening risk information.

Session 3: Roundtable—Labeling Alcohol Bottles with Pregnancy Warnings

Chairperson: P. White, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland

H. Breslawec, Food and Drug Administration, Rockville, Maryland
M. Dressler, Bureau of Alcohol, Tobacco and Firearms, Washington, DC
S. Fabro, George Washington University Medical Center, Washington, DC
P. Gavaghan, Distilled Spirits Council of the United States, Inc. (DISCUS), Washington, DC

Session 4: Labeling as a Social Policy

W. Schultz, Public Citizens Litigation, Washington, DC: Labeling, consumer needs, consumer rights.
J. Walden, The Proprietary Association, Washington, DC: From the producers' perspective.
H. Beales, Federal Trade Commission, Washington, DC: Cost/benefit; economic perspectives.
I. Barofsky, Johns Hopkins Medical Institutions, Baltimore, Maryland: Psychosocial consequences.
P. Rheinstein, Food and Drug Administration, Rockville, Maryland: Labeling and the health environment.
R. Cooper, Williams & Connolly, Washington, DC: Labeling as a regulatory alternative.

Carcinogen and Mutagen Formation in the Gastrointestinal Tract, October 12–October 15

New interest in the relationship between diet and gastrointestinal cancer has been stirred by the discovery of a mutagenic factor in human feces, possibly a bacterial product, and by the discovery that mutagenic substances such as nitrosamines can be formed in the gastrointestinal tract. To summarize current knowledge and to focus additional attention on the role that endogenous factors may play in gastrointestinal cancer, W. Robert Bruce of the Ludwig Institute (then of the Ontario Cancer Institute), Pelayo Correa of the Louisiana State University Medical Center, Martin Lipkin of the Memorial Sloan-Kettering Cancer Center, Steven R. Tannenbaum of the Massachusetts Institute of Technology, and Tracy Wilkins of the Virginia Polytechnic Institute, organized a conference of some 40 specialists to consider such topics as the chemical structure of the mutagen found in feces, and epidemiological studies indicating that, in Japan, daily meat consumption reduces the risk of colon and rectal cancer while increasing the risk of pancreatic cancer, and that daily intake of yellow and green vegetables, containing vitamin A, is protective against several types of cancer. The book reporting on this conference was published in April 1981, exactly six months after the meeting.

Session 1: Gastrointestinal Microbiology

Chairperson: T. D. WILKINS, Virginia Polytechnic Institute and State University, Blacksburg

T. D. WILKINS, Virginia Polytechnic Institute and State University, Blacksburg: Microbiological considerations in interpretation of data obtained with experimental animals.
W. E. C. MOORE, Virginia Polytechnic Institute and State University, Blacksburg: The effect of diet on the human intestinal flora.
P. GOLDMAN, Harvard Medical School, Boston, Massachusetts: Metabolism of xenobiotics by the gastrointestinal flora.
B. GOLDIN, Tufts University School of Medicine, Boston, Massachusetts: Factors that affect intestinal bacterial activity—Implications for colon carcinogenesis.

Session 2: Fiber

Chairperson: P. J. VAN SOEST, Cornell University, Ithaca, New York

P. J. VAN SOEST, Cornell University, Ithaca, New York: Some factors influencing the ecology of gut fermentation in man.
J. H. CUMMINGS, MRC Dunn Clinical Nutrition Centre, Cambridge, England: Implications of dietary fiber breakdown in the human colon.
N. D. NIGRO, Wayne State University School of Medicine, Detroit, Michigan: Fat, fiber, and other modifiers of intestinal carcinogenesis—A strategy for prevention.

Session 3: Host Response to Carcinogens

Chairperson: M. LIPKIN, Memorial Sloan-Kettering Cancer Center, New York, New York

M. LIPKIN, Memorial Sloan-Kettering Cancer Center, New York, New York: Susceptibility of high-risk populations.
P. CORREA, Louisiana State University Medical Center, New Orleans: Mutagenesis and adenomatous polyps of the colon.
M. E. BALIS, Sloan-Kettering Institute for Cancer Research, New York, New York: Enzymes in gastrointestinal cells in cancer.
H. W. STROBEL, University of Texas Health Science Center, Medical School, Houston: Role of cytochrome P-450 in the response of the colon to xenobiotics.
L. W. WATTENBERG, University of Minnesota Medical School, Minneapolis: Inhibitors of gastrointestinal neoplasia.
G. N. WOGAN, Massachusetts Institute of Technology, Cambridge, and others: Discussion.

Session 4: Mutagens

Chairperson: W. R. BRUCE, Ontario Cancer Institute, Toronto, Canada

G. M. STEMMERMANN, Japan-Hawaii Cancer Study, Honolulu, Hawaii: Mutagens in extracts of gastrointestinal mucosa.
T. D. WILKINS, Virginia Polytechnic Institute and State University, Blacksburg: Isolation of a mutagen produced in the human colon by microbial action.
D. G. I. KINGSTON, Virginia Polytechnic Institute and State University, Blacksburg: Structural studies on a mutagenic bacterial product from human feces.
W. R. BRUCE, Ontario Cancer Institute, Toronto, Canada: Studies of a mutagen from human feces.
S. VENITT, Institute of Cancer Research, Chalfont St. Giles, Buckinghamshire, England: Detection of mutagens in feces.
H. F. STICH, British Columbia Cancer Research Centre, Vancouver, Canada: Intake formation and release of mutagens by man.

Session 5: N-nitroso Compounds

Chairperson: S. R. TANNENBAUM, Massachusetts Institute of Technology, Cambridge

S. R. TANNENBAUM, Massachusetts Institute of Technology, Cambridge: Metabolism of nitrates.
S. R. TANNENBAUM, Massachusetts Institute of Technology, Cambridge: Endogenous formation of *N*-nitroso compounds.
G. EISENBRAND, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: Assessment of exposure.
H. NEWMARK and W. J. MERGENS, Hoffmann-La Roche Inc., Nutley, New Jersey: Blocking nitrosamine formation using ascorbic acid and alpha-tocopherol.
E. BALISH, University of Wisconsin Medical School, Madison: Distribution of metabolism of nitrate and nitrite in rodents.

M. C. Archer, Ontario Cancer Institute, Toronto, Canada: Nitrate, nitrite, and *N*-nitroso compounds in the human intestine.
D. H. Fine, New England Institute of Life Sciences, Waltham, Massachusetts; L. K. Keefer, National Cancer Institute, Bethesda, Maryland; P. N. Magee, Temple University School of Medicine, Philadephia, Pennsylvania: Discussion.

Session 6: Bile Acids and Other Lipids

Chairperson: B. S. Reddy, American Health Foundation, Valhalla, New York

B. S. Reddy, American Health Foundation, Valhalla, New York: Bile salts and other constituents of the colon as tumor promoters.
M. J. Hill, Central Public Health Laboratory, London, England: Bile acids and colorectal cancer.
S. Hecht, Naylor Dana Institute for Disease Prevention, Valhalla, New York: Analysis of feces for benzo[a]pyrene after consumption of charcoal-broiled meat.

Session 7: Epidemiology and Design of Future Studies

Chairperson: P. Correa, Louisiana State University Medical Center, New Orleans

S. Graham, State University of New York at Buffalo: Epidemiologic tests of hypotheses of diet and cancer.
H. F. Mower, University of Hawaii, Manoa, Honolulu: Identification of mutagens found in gastric mucosa.
T. Hirayama, National Cancer Center Research Institute, Tokyo, Japan: A cohort study of life-style variables in gastrointestinal cancer in Japan.
W. Haenszel, University of Illinois, Chicago: The methodology for analysis of the relationship between mutagens and gastrointestinal cancer.

Hormones and Breast Cancer, October 26–October 28

Breast cancer, which tragically accounted for 19% of the 183,000 female cancer deaths recorded in the United States in 1978, presents some of the most tangled puzzles faced by the researchers attempting to sort out the influences of genetic endowment and the environment in cancer causation. Still unidentified environmental factors apparently influence the sharp differences in breast cancer rates between Japan and the United States. Work by epidemiologists and experiments on rodents have underlined the importance of total exposure to estrogens in influencing the risk of breast cancer. But the organizers of the conference, Malcolm Pike of the University of Southern California, Pentti K. Siiteri of the University of California at San Francisco, and Clifford Welsch of Michigan State University, felt there was a strong need for a joint review of what is known about the etiology of breast cancer by epidemiologists, endocrinologists, and animal experimenters. They and the other conference participants surveyed the exogenous and endogenous factors affecting breast cancer incidence, summarized the underlying mechanisms by which hormones and carcinogens induce and promote tumor growth, and projected future directions for epidemiological and clinical research in the field.

Session 1: Review of Epidemiology of Breast Cancer

M. C. Pike, University of Southern California Medical School, Los Angeles: Epidemiology of breast cancer as it relates to menarche, pregnancy, and menopause.
F. de Waard, University of Utrecht, The Netherlands: Body size as a risk factor for breast cancer.

Session 2: Endocrinology of Women at Risk to Breast Cancer

J. B. Brown, University of Melbourne, Victoria, Australia: Hormone profiles in young women at risk.
R. Vihko, University of Oulu, Finland: Endocrine maturation in the course of female puberty.
S. S. Korenman, University of California, Los Angeles School of Medicine, Sepulveda: Abnormal ovarian function and breast cancer risk.
P. K. Siiteri, University of California, San Francisco: Estrogen production and transport following the menopause.

Session 3: Review of Studies Attempting to Establish Endogenous Hormones as Important in Human Breast Cancer

P. Cole, University of Alabama, Birmingham: Estrogens and progesterone.
B. E. Henderson, University of Southern California Medical School, Los Angeles: Prolactin.

R. D. Bulbrook, Imperial Cancer Research Fund Laboratories, London, England: Androgens and thyroid.
B. Zumoff, Montefiore Hospital and Medical Center, Bronx, New York: Hormonal studies in women with breast cancer.

Session 4: In Vitro Studies of Human Breast Tissue

M. E. Lippman, National Cancer Institute, Bethesda, Maryland: Hormonal regulation of breast cancer cells.
R. P. C. Shiu, University of Manitoba Faculty of Medicine, Winnipeg, Canada: Prolactin regulation of breast cancer cells.
R. Ottman, University of California, Berkeley, and P. K. Siiteri, University of California, San Francisco: Analysis of estrogen receptor assay data.

Session 5: Exogenous Hormones and Breast Cancer

J. L. Kelsey, Yale University School of Medicine, New Haven, Connecticut: Epidemiological studies of exogenous estrogens.
A. Segaloff, Alton Ochsner Medical Foundation, New Orleans, Louisiana: Hormonal therapy of breast cancer.

Session 6: Other Exogenous Factors and Breast Cancer

N. L. Petrakis, University of California Medical School, San Francisco: Epidemiological studies of mutagenicity of breast fluids—Relevance to breast cancer risk.
P. Hill, American Health Foundation, Valhalla, New York: Diet and hormone levels.

Session 7: Hormones and the Genesis and Progression of Murine Mammary Tumors

T. L. Dao, Roswell Park Memorial Institute, Buffalo, New York: Role of ovarian and adrenal steroids in mammary carcinogenesis.
C. W. Welsch, Michigan State University, East Lansing: Prolactin and growth hormone in murine mammary tumorigenesis.
R. Hilf, University of Rochester Medical Center, New York: Insulin.
C. J. Shellabarger, Brookhaven National Laboratory, Upton, New York: Pituitary and steroid hormones in radiation-induced mammary tumors.
R. C. Moon, Illinois Institute of Technology Research Institute, Chicago: Pregnancy, lactation, and thyroid hormones.
J. Meites, Michigan State University, East Lansing: Relation of neuroleptic drugs to mammary tumorigenesis.
Y. N. Sinha, Scripps Clinic and Research Foundation, La Jolla, California: Plasma prolactin analysis as a potential predictor of murine mammary tumorigenesis.

Session 8: Mechanism of Hormone Action

J. Rosen, Baylor College of Medicine, Houston, Texas: Regulation of casein gene expression in hormone-dependent mammary cancer.
D. A. Sirbasku, University of Texas Medical School, Health Science Center, Houston: Mechanism of estrogen action—Estrogen-induced growth factors.
S. Nandi, University of California, Berkeley: Role of hormones in carcinogenesis.

Construction and Use of Mammalian Vectors
December 10–December 13

Expression of foreign genes in mammalian cells was the focus of this early example of what are expected to be many small conferences on molecular aspects of biology to be held at Banbury Center. Organized with support from a dozen industrial companies by Joseph Sambrook, the Laboratory's Assistant Director for Research, the conference heard reports from academic, industrial, and government scientists on progress with complementary methods of introducing foreign genes into cultured mammalian cells. These methods include 1) microinjection; 2) attachment of the genes to segments of viral DNA; and 3) the simultaneous transformation of the cells with two separate pieces of DNA, one carrying the gene of interest and the other carrying a genetic marker, such as the gene for the enzyme thymidine kinase, which confers a selective advantage on cells receiving the foreign genes. Successes were reported in obtaining constitutive expression of microinjected genes and in obtaining expression through viral transmission of such foreign genes as human growth hormone, hepatitis surface antigen and human genomic globin sequences. The conference heard that some disadvantages with the most commonly employed virus vector, simian virus 40, such as a sometimes-small proportion of an SV40 population carrying the foreign genes and limits on how much foreign DNA can be packed into SV40 particles, are being overcome. New selective markers were discussed, along with the possibility that cotransformed genes can continue to respond to hormones.

Session 1: Delivery Systems

Chairperson: W. C. Summers, Radiobiology Laboratories, New Haven, Connecticut

W. C. Summers, Radiobiology Laboratories, New Haven, Connecticut: Structure and expression of the HSV-TK locus.

F. Ruddle, Yale University, New Haven, Connecticut: Gene transfer in mammalian cells.

M. Wigler, Cold Spring Harbor Laboratory, New York: The stable transformation of animal cells with biochemically selectable vectors.

G. Milman, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland: Efficient DNA transfection and rapid detection of DNA expression.

M. L. Pearson, Frederick Cancer Research Center, Maryland: Clonal analysis of the efficiency of *tk* and *oua* DNA-mediated gene transfer in mouse L cell recipients.

M. Capecchi, University of Utah, Salt Lake City: The expression and stable integration of EDN injected into nuclei of cultured mammalian cells.

C. Lo, University of Pennsylvania, Philadelphia: Iontophoretic injection and integration of DNA into mouse L cells.

W. F. Anderson, National Heart, Lung, & Blood Institute, Bethesda, Maryland: Fate of eukaryotic genes microinjected into mouse L cells.

Session 2: Papovaviruses as Vectors

(i) Replication/Integration/Excision

Chairperson: C. Basilico, New York University Medical Center, New York

C. Basilico, New York University Medical Center, New York, New York: Requirements for integration, excision, and amplification of polyoma virus genomes.
Y. Gluzman, Cold Spring Harbor Laboratory, New York: An SV40 host-vector system for carrying pure population of recombinant viruses.
M. Botchan, University of California, Berkeley: The stable transformation of animal cells with biochemically selectable vectors.
W. Jelinek, Rockefeller University, New York, New York: Interspersed repetitious sequences in mammalian DNA.
J. T. Elder, Yale University School of Medicine, New Haven, Connecticut: Structural and transcriptional analysis of interspersed repetitive polymerase III transcription units in human DNA.
J. Hassell, McGill University, Montreal, Canada: The organization, expression, and recovery of transfected genes—The polyoma transforming gene as a model.
M. Fried, Imperial Cancer Research Fund Laboratories, London, England: Polyoma virus expression.
C. Rogers, Oregon State University, Corvallis: The construction of recombinant DNA molecules containing the yeast leucine gene for use as eukaryotic vectors.
R. Breathnach, Faculté de Médecine, Strasbourg, France: SV40 and polyoma based vectors.
A. Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey: The construction of recombinant DNA molecules containing the yeast leucine gene for use as eukaryotic vectors.

(ii) Expression of Cloned Genes

Chairperson: T. Shenk, State University of New York, Stony Brook

P. Gruss and G. Khoury, National Cancer Institute, Bethesda, Maryland: Expression of preproinsulin genes carried in SV40.
P. T. Lomedico, Hoffmann-La Roche Inc., Nutley, New Jersey: Programming mammalian cells to synthesize insulin.
P. J. Southern, Stanford University Medical Center, California: SV40 vector systems.
S. Subramani, Stanford University Medical Center, California: Expression of the mouse dihydrofolate reductase cDNA in SV40-based vectors.
P. Mellon, California Institute of Technology, Pasadena: Two assays correlating available structural and genetic information with in vivo expression of globin genes.
G. Pavlakis, National Cancer Institute, Bethesda, Maryland: Expression of eukaryotic genes cloned in SV40 vectors.

Session 3: Adenoviruses

Chairperson: T. Grodzicker, Cold Spring Harbor Laboratory, New York

D. Solnick, Cold Spring Harbor Laboratory, New York: Adenovirus recombinants containing an ectopic copy of the major late promoter which directs the expression of downstream genes.
C. S. Thummel, University of California, Berkeley: An adenovirus vector system for the expression of foreign eukaryotic proteins.
M. Rossini, Cold Spring Harbor Laboratory, New York: A study of expression and regulation of the adenovirus genome using microinjection of mammalian cells.

Session 4: Retroviruses

Chairperson: G. Vande Woude, National Cancer Institute, Bethesda, Maryland

J. Sorge and S. Hughes, Cold Spring Harbor Laboratory, New York: Uses of an avian sarcoma virus vector.
W. McClements, National Cancer Institute, Bethesda, Maryland: Long terminal repeat (LTR) of Moloney sarcoma virus.
I. M. Verma, Salk Institute, San Diego, California: Retroviral DNAs as eukaryotic cloning vectors.

Session 5: Specialized Systems

Chairperson: G. Khoury, National Cancer Institute, Bethesda, Maryland

M. J. Cline, University of California School of Medicine, Los Angeles: Use of a mutant dihydrofolate reductase gene to transform bone marrow cells of intact mice.
M. J. Gething, Imperial Cancer Research Fund Laboratories, London, England: Expression of influenza virus hemagglutinin in eukaryotic cells.
A. Pellicer, Columbia University College of Physicians & Surgeons, New York, New York: Control of expression of transforming genes.
D. T. Kurtz, Cold Spring Harbor Laboratory, New York: Hormonal control of the expression of the rat α_{2u} globin genes.
P. Howley, National Institutes of Health, Bethesda, Maryland: Bovine papilloma virus DNA—A novel eukaryotic cloning vector.

Marie H. Robertson Fund for Neurobiology

Crucial support for Cold Spring Harbor Laboratory's program of conferences, courses and year-round research in the neurosciences is provided by the Marie H. Robertson Fund for Neurobiology. This fund, established in 1976 through the Banbury Foundation by the family of Mr. Charles S. Robertson, honors the memory of Mr. Robertson's wife, who died in 1972. At first the fund, which provides $75,000 annually, was used largely to support the summer teaching program in neurobiology, which includes laboratory courses given on the main Laboratory grounds in the village of Laurel Hollow and lecture courses given at Banbury Center in the village of Lloyd Harbor. But in 1979, after receipt of a substantial training grant from the National Institutes of Health, and added support from the National Science Foundation, it was decided to use some of the Marie H. Robertson funds to support specific summer workshops and to make possible one or two specialized meetings each year at Banbury Center. The first two of these Marie H. Robertson meetings were held in 1980. The programs are listed below.

Lessons from the Study of Molluscan Nerve Cells
May 18—May 20

To stimulate interaction between investigators of membrane biophysics and of neural control of behavior, Eric Kandel of the College of Physicians and Surgeons of Columbia University and Charles Stevens of the Yale University School of Medicine, both members of the Neurobiology Advisory Committee of Cold Spring Harbor Laboratory, organized a three-day conference of 20 specialists and five graduate-student rapporteurs who summarized the presentations and discussion for what became, a few months later, the first of the Cold Spring Harbor Reports in the Neurosciences. The meeting was designed to summarize what now appears to be a revolution in knowledge that allows neuroscience researchers "to relate the biophysical properties of individual neurons to the features of the behavior that they mediate," as John Koester of Columbia Physicians and Surgeons and John H. Byrne of the University of Pittsburgh School of Medicine, the editors of the conference report, wrote in their introduction.

Arranged by
Charles F. Stevens, *Yale University, New Haven, Connecticut*
Eric R. Kandel, *Columbia University, College of Physicians & Surgeons, New York, New York*

27 participants

Session 1

Chairperson: C. F. Stevens, Yale University, New Haven, Connecticut

C. F. Stevens, Dept. of Physiology, Yale University, New Haven, Connecticut: Introduction.
R. C. Thomas, Dept. of Physiology, Yale University School of Medicine, New Haven, Connecticut: Ion pumps in nerve cells.

S. THOMPSON, Dept. of Biology, and Hopkins Marine Station, Stanford University, California: The delayed K^+ channel and its inactivation.
J. CONNOR, Dept. of Physiology and Biophysics, University of Illinois, Urbana: The fast K^+ channel and repetitive firing.

Session 2

Chairperson: S. HAGIWARA, University of California School of Medicine, Los Angeles

R. W. MEECH, Dept. of Physiology, University of Utah Medical School, Salt Lake City: The calcium-dependent K^+ channel.
H. D. LUX, Max-Planck-Institut für Psychiatrie, Munich, Federal Republic of Germany: The calcium-dependent K^+ channel.

Session 3

Chairperson: A. M. BROWN, University of Texas Medical Branch, Galveston

S. HAGIWARA, Dept. of Physiology, University of California School of Medicine, Los Angeles: The calcium channel—Introduction.
D. TILLOTSON, Dept. of Physiology, Boston University Medical School, Massachusetts: Inactivation of the calcium channel.

Session 4

Chairperson: F. J. BRINLEY, NINCDS, National Institutes of Health, Bethesda, Maryland

A. M. BROWN, Dept. of Physiology, University of Texas Medical Branch, Galveston: Noise analysis.
F. J. BRINLEY, Neurological Disorders Program, NINCDS, National Institutes of Health, Bethesda, Maryland: Transmembrane flux and buffering of Ca^{++}—Axon.
S. SMITH, Dept. of Physiology and Anatomy, University of California, Berkeley: Transmembrane flux and buffering of Ca^{++}—Cell body.
T. G. SMITH, JR., National Institutes of Health, Bethesda, Maryland: Ionic channels in burst-generating cells.

Session 5

Chairperson: R. LLINAS, New York University, New York

W. A. WILSON, Epilepsy Center, Veterans Administration Hospital, Durham, North Carolina: Synaptic transmission—Postsynaptic channels: Voltage sensitivity.
A MARTY, Laboratoire de Neurobiologie, Ecole Normale Superieure, Paris, France: Synaptic transmission—Postsynaptic channels: Noise analysis.
R. LLINAS, Dept. of Physiology, New York University, New York: Synaptic transmission—Presynaptic channels: Ca^{++} channels and transmitter release.

Session 6

Chairperson: E. R. KANDEL, Columbia University College of Physicians & Surgeons, New York, New York

E. SHAPIRO, Division of Neurobiology and Behavior, Columbia University College of Physicians & Surgeons, New York, New York: Modulation of presynaptic calcium channels.
M. KLEIN, Division of Neurobiology and Behavior, Columbia University College of Physicians & Surgeons, New York, New York: Biophysics of behavior—Molecular mechanisms of habituation and sensitization.
J. KOESTER, Division of Neurobiology and Behavior, Columbia University College of Physicians & Surgeons, New York, New York: Biophysics of behavior—Control of inking in *Aplysia I.*
J. BYRNE, Dept. of Medicine, University of Pittsburgh School of Medicine, Pennsylvania: Biophysics of behavior—Control of inking in *Aplysia II.*
R. LLINAS, Dept. of Physiology, New York University, New York: Applicability of channel analyses in molluscs to vertebrate central neurons.

Usage of Monoclonal Antibodies in Neurobiology
November 5—November 8

Our ability to generate specific immunoglobulin and nucleic acid probes allows quite new questions to be answered in many complex biological systems. The nervous system is particularly open to study with these new molecular tools. This meeting was the first gathering of scientists studying the nervous system with monoclonal antibodies. We heard antibodies described which distinguish central and peripheral neurons, subtypes of neurons in the neural crest, antigenic gradients in the retina, many tens of neuronal types in the leech, the synaptic sites at the neuromuscular junction, neurotransmitters and their enzymes.

Arranged by
Ron McKay, *Cold Spring Harbor Laboratory, New York*
Martin Raff, *University College London, England*
Louis F. Reichardt, *University of California, San Francisco*

39 participants

Session 1

M. Raff, University College London, England: Introduction.

Session 2: Defining Cell Types and Cell Lines

Chairperson: H. Karten, State University of New York, Stony Brook

M. Schachner, Dept. of Neurobiology, University of Heidelburg, Federal Republic of Germany: Monoclonal antibodies recognizing subpopulations of glial cells in mouse cerebellum.

K. Fields, Dept. of Neurology, Albert Einstein College of Medicine, Bronx, New York: Indication for the use of monoclonal antibodies against brain filament proteins.

Y. Berwald-Netter,[1] F. Couraud,[2] and A. Koulakoff,[2] [1]College of France, Paris; [2]Faculty of Medicine, Marseille, France: Specific surface membrane markers as probes for neuronal evolution in vivo and in vitro.

W. Stallcup, J. Levine, and W. Raschke, Salk Institute, San Diego, California: Monoclonal antibody against the NG2 marker.

G. Giotta, J. Heitzmann, and M. Cohen, Developmental Biology Laboratory, Salk Institute, San Diego, California: Monoclonal antibodies and the identification of cerebellar cell.

J. Cohen, R. Mirsky, S. Selvendren, and T. Vulliamy, Dept. of Zoology, University College London, England: Monoclonal antibodies which define neuron-specific cell surface molecules in the mammalian central and peripheral nervous system.

L. Lampson, Dept. of Anatomy, University of Pennsylvania, Philadelphia: Expression of the major histocompatibility antigens in the human nervous system.

Session 3: The Synapse

Chairperson: C. F. Stevens, Yale University School of Medicine, New Haven, Connecticut

R. Kelly, Dept. of Biochemistry, University of California, San Francisco: Antibodies to cholinergic synaptic vesicles.

W. Matthew, Dept. of Biochemistry, University of California, San Francisco: Monoclonal antibodies to synaptic membranes and vesicles.
A. de Blas, N. Busis, and M. Nirenberg, NHLBI, National Institutes of Health, Bethesda, Maryland: Monoclonal antibodies to synaptic membrane molecules.
S. Fuchs, D. Mochly-Rosen, M. Souroujon, and Z. Eshhar, Dept. of Chemical Immunology, Weizmann Institute, Rehovot, Israel: Monoclonal antibodies against the nicotinic acetylcholine receptor.

Session 4: The Retina

Chairperson: M. Nirenberg, NHLBI, National Institutes of Health, Bethesda, Maryland

H. Karten and N. Brecha, State University of New York, Stony Brook: Biochemical and morphological specificity of retinal amacrine cells—Immunohistochemical findings.
G. Eisenbarth, K. Shimizu, M. Conn, B. Mittler, and S. Wells, Duke University Medical Center, Durham, North Carolina: Monoclonal antibody F12A2B5-reaction with a plasma membrane antigen of vertebrate neurons and peptide secreting endocrine cells.
C. Barnstable, Dept. of Neurobiology, Harvard Medical School, Boston, Massachusetts: Developmental studies of rat retina cells using cell-type specific monoclonal antibodies.
D. Trisler, M. Schneider, and M. Nirenberg, NHLBI, National Institutes of Health, Bethesda, Maryland: A gradient of molecules in avian retina with dorsoventral polarity.

Session 5: Defined Antigens

Chairperson: L. F. Reichardt, University of California, San Francisco

M. E. Ross, E. E. Baetge, D. J. Reis, and T. H. Joh, Dept. of Neurobiology, Cornell University Medical College, New York, New York: Monoclonal antibodies against catecholamine neurotransmitter synthesizing enzymes can be used for immunochemistry and immunocytochemistry.
A. C. Cuello[1] and C. Milstein,[2] [1]Dept. of Pharmacology and Human Anatomy, University of Oxford, England; [2]Medical Research Council Molecular Biology Unit, Cambridge, England: Monoclonal antibodies against neurotransmitter substances.
R. Akeson, J. S. Radman, K. Graham, and A. Roberts, Children's Hospital Research Foundation, Cincinnati, Ohio: Identification of a rat nervous system specific polypeptide.
R. Pruss,[1] R. Mirsky,[1] M. C. Raff,[1] R. Thorpe,[2] and B. H. Anderton,[2] [1]University College London, England; [2]St. George's Hospital Medical School, London, England: A monoclonal antibody recognizes a determinant present on common as well as class-specific intermediate filament subunits.
G. E. Lemke and J. P. Brockes, Dept. of Biology, California Institute of Technology, Pasadena: An immunochemical approach to the purification and characterization of glial growth factor.
E. Yavin,[1,2] Z. Yavin,[1] M. D. Schneider,[3] and L. D. Kohn,[1] [1]NIAMDD, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel; [3]NHLBI, National Institutes of Health, Bethesda, Maryland: Monoclonal antibodies to the thyrotropin receptor—Implications for receptor structure and the action of autoantibodies in Graves' disease.

Session 6: The Neuromuscular Junction

Chairperson: M. Raff, University College London, England

S. Burden, Dept. of Anatomy, Harvard Medical School, Boston, Massachusetts: Monoclonal antibodies directed against the frog nerve-muscle synapse.
E. Bayne, J. Gardner, and D. M. Fambrough, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Monoclonal antibodies against extracellular matrix antigens in chicken skeletal muscle.
D. Gottlieb and J. Greve, Department of Anatomy/Neurobiology, Washington University School of Medicine, St. Louis, Missouri: Effects of monoclonal antibodies to the cell surface on cultured myogenic cells.

Session 7: Ganglia

Chairperson: E. Jones, Washington University School of Medicine, St. Louis, Missouri

G. Ciment and J. Weston, Dept. of Biology, University of Oregon, Eugene: Immunochemical studies of avian peripheral neurogenesis.
B. Zipser, Cold Spring Harbor Laboratory, New York: Monoclonal antibodies specific for identifiable leech neurons.

POSTGRADUATE TRAINING PROGRAM

SUMMER 1980

The Postgraduate Training Program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects which are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Neurobiology of Behavior, June 6—June 20

INSTRUCTORS

Kandel, Eric R., M.D., Columbia University, New York, New York
Pearson, Keir, Ph.D., University of Alberta, Edmonton, Canada
Koester, John, Ph.D., Columbia University, New York, New York

This course was designed to introduce students to cellular approaches to the study of behavior and learning. The lectures provided an intensive coverage of four main areas: 1) general principles of behavior and cellular neurobiology; 2) simple forms of behavior, learning, and motivation; 3) initiation and maintenance of complex locomotor sequences, including voluntary movement and motor learning; 4) communication. To illustrate general principles, suitable systems for study were selected from both invertebrate and vertebrate behavior. To put the cellular work into perspective, selected examples were also taken from human behavior and its abnormalities.

Jones Laboratory (right) as viewed from gazebo terrace

PARTICIPANTS

Abrams, Thomas W., B.A., University of Alberta, Edmonton, Canada
Aletta, John, Columbia University Medical School, New York, New York
Bicker, Gerd, Ph.D., University of California, Santa Cruz
Evans, Randolph W., M.D., Baylor College of Medicine, Houston, Texas
Ganong, Alan H., B.S., University of California, Irvine
Gibson, Daniel J., B.S., Johns Hopkins University, Baltimore, Maryland
Haydon, Philip G., B.S., University of Leeds, England
Lupatkin, Joel F., B.A., Columbia University, New York, New York
MacDonald, Beth, B.A., University of Connecticut, Farmington
Mackey, Steve L., B.A., Columbia University, New York, New York
Masinovsky, Boris, M.S., University of Washington, Seattle
Murata, David N., B.S., University of Western Ontario, Canada
Murtaugh, Maryanne, M.S., Montefiore Hospital, Bronx, New York
Pardo, Francisco C., M.A., Johns Hopkins Hospital, Baltimore, Maryland
Randall, David C., Ph.D., University of Kentucky, Lexington
Tramo, Mark J., B.A., Cornell Medical College, Ithaca, New York
Wallen, Peter R., B.S., Karolinska Institute, Stockholm, Sweden
Warach, Steven J., B.S., Michigan State University, Ann Arbor
Zehring, William A., B.A., University of Chicago, Illinois

SEMINARS

Kandel, E.R., Columbia University. *Introduction to the cellular study of behavior.*
Koester, J., Columbia University. *Introduction to biophysics of behavior.*
———. *Repetitive firing properties and the control of behavior.*
Kandel, E.R., Columbia University. *Morphology and physiology of synaptic transmission.*
———. *Learning. I. Habituation.*
———. *Learning. II. Sensitization.*
Wine, J., Stanford University. *Nerve circuitry for simple behavioral acts and their control.*
Lynch, G., University of California. *Long-term plasticity in the vertebrate CNS.*
Truman, J., University of Washington. *Hormones and behavior. I.*
Kupfermann, I., Columbia University. *Hormones and behavior. II.*
———. *Motivation.*
Wurtz, R., National Institute of Mental Health. *Attention.*
Pearson, K., University of Alberta. *Introduction to motor sequences.*
———. *Central and reflex control of movements in motor systems.*
Getting, P., Stanford University. *Rhythm generation in invertebrate motor systems.*
Kristan, W., University of California. *Leech swimming.*
Pearson, K., University of Alberta. *Walking in the cat.*
Ghez, C., Columbia University. *Voluntary movements in mammals.*
———. *Pyramidal and extra-pyramidal systems in motor control.*
Thach, W.T., Jr., Washington University. *Does the cerebellum learn motor programs?*
Lisberger, S., National Institutes of Health. *Adaptive regulation in the oculomotor system.*
Marler, P., Rockefeller University. *Introduction to communication.*
———. *Communication in birds. I. Predispositions brought to the learning of a complex motor task.*
Sugo, N., Washington University. *Communication in bats.*
Arnold, A., University of California. *Communication in birds. II. Brain pathways for vocal learning.*
Gazzaniga, M., Cornell University Medical Center. *Lateralization of brain function.*
Ross, E., Southwestern Medical School. *Language and the brain.*
Zigmond, R., Harvard Medical School. *Neurotransmitters: Role in behavioral abnormalities.*

Molecular Biology and Developmental Genetics of *Drosophila*, June 8—June 28

INSTRUCTORS

Gelbart, William, Ph.D., Harvard University, Cambridge, Massachusetts
Pardue, Mary-Lou, Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANT

Ribolini, Ann, Ph.D., Harvard University, Cambridge, Massachusetts

Drosophila melanogaster is an especially favorable organism for use in studies of gene control in higher animals. The course on The Molecular Biology and Developmental Genetics of *Drosophila* consisted of laboratory work, lectures, and discussions on current problems and approaches to the study of the molecular basis of developmental phenomena in this organism. Emphasis was placed on integration of the classical genetic, cytogenetic, and developmental biological techniques with microtechniques for molecular analysis. Topics covered included: chromosome mechanics, chromosome structure, intragenic organization, gene-enzyme systems, neurogenetics, embryology and early determinative events, pattern formation and regulation, regulation in specific gene systems, genetics of mutable elements, and speciation in *Drosophila*. Students came from many parts of the United States as well as Germany, France, Belgium, and England. Some of the students were new to the field, having worked previously on bacteria or vertebrates. Other students were already working on some aspect of *Drosophila* development.

PARTICIPANTS

Beckers, Christoph, Ph.D., Technische Hochschule, Darmstadt, Federal Republic of Germany
Dambly-Chaudiere, C.M., Ph.D., University of Brussels, Belgium
Dunn-Coleman, E. Janet, B.S., University of Virginia, Charlottesville
Hall, Nick A., B.S., University of Sussex, England
Ireland, Robert C., M.S., Dartmouth College, Hanover, New Hampshire
Kale, Purushottam, Ph.D., Brookhaven National Laboratory, Upton, New York
Martin, Diana W., Ph.D., Rutgers University, New Brunswick, New Jersey
Pardo, Daniel, Ph.D., CNRS, Marseille, France
Spencer, Forrest A., B.A., Harvard University, Cambridge, Massachusetts
Vitek, Michael P., B.A., Dartmouth College, Hanover, New Hampshire
Wu, Ting, B.A., Sidney Farber Cancer Center, Harvard Medical School, Cambridge, Massachusetts
Yannoni, Claudine Z., B.A., Boston College, Massachusetts

SEMINARS

Spradling, A., Carnegie Institution of Washington. *Gene amplification in* Drosophila.
Cline, T., Princeton University. *Determination, gene regulation, and maternal effects in the* Drosophila *embryo.*
———. *A specific maternal-zygotic glue interaction controlling sex determination and X chromosome dosage compensation in* Drosophila.
———. *Micromanipulation of* Drosophila *embryos.*
Cherbas, P., Harvard University. *Insect endrochronology.*
———. *Hormonal control of chromosome puffing.*
———. *Ecdysteroid effects on cultured* Drosophila *cells.*
Ashburner, M., Cambridge University. *The fine structure of genes in* Drosophila.
———. *Hybrid dysgenesis and related phenomena in* Drosophila.
———. *Speciation in* Drosophila.
Young, M., Rockefeller University. *Organization of repeated DNA in* Drosophila.
———. *Transposable elements in* Drosophila.
Shearn, A., Johns Hopkins University. *Mutational analysis of imaginal development in* Drosophila.
———. *Indirect homeosis.*
Hall, J., Brandeis University. *Everything you ever wanted to know about behavioral genetics.*
Kankel, D., Yale University. *Developmental neurobiology.*
Garcia-Bellido, A., Universidad Autonomia de Madrid. *Compartments and development.*
———. *The bithorax locus.*
———. *The scute-achete locus.*

Nervous System of the Leech, June 9–June 29

INSTRUCTORS

Nicholls, John, Ph.D., Stanford Medical School, California
Muiler, Kenneth J., Ph.D., Carnegie Institution of Washington, D.C.
Parnas, Itzhak, Ph.D., Hebrew University, Jerusalem, Israel
Zipser, Birgit, Ph.D., Cold Spring Harbor Laboratory, New York

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

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

The final phase of the course consisted of devising and performing original experiments, some of which proved to be of sufficient interest to be pursued in greater detail. For example, the nervous systems of various hitherto unexplored leeches were studied. In addition, the properties of a new fluorescent dye, Lucifer Yellow, were tested by intracellular injection into identified cells, and individual cells were killed by injection of proteotype enzymes.

PARTICIPANTS

Cline, Hollis T., B.A., University of California, Berkeley
Cohen, Ilan, B.S., Hebrew University, Jerusalem
Flanagan, Thomas R.J., M.S., Wesleyan University, Middletown, Connecticut
Marshall, Cameron G., B.S., Brown University, Providence, Rhode Island
Peterson, Eric L., Ph.D., Unversity of Sussex, England
Simonneau, Marc, M.D., CNRS, Gif-sur-Yvette, France
Small, Rochelle, Ph.D., Columbia University, New York, New York
Wenning, Angela, Ph.D., University of Konstanz, Federal Republic of Germany
Young, Steven R., B.S., University of Virginia, Charlottesville
Zimering, Mark B., B.S., Albert Einstein College, New York, New York

SEMINARS

Nicholls, J., Stanford Medical School. *Introduction to the leech.*
———. *Sensory and motor cells.*
———. *Conduction block and potassium accumulation.*
———. *Regeneration, cultured ganglia, and individual cells.*
Muller, K.J., Carnegie Institution of Washington. *Circuitry and recording techniques.*
———. *Structure of synapses.*
———. *The S cell.*
Weisblat, D., University of California, Berkeley. *Biology of leeches.*
———. *Leech development.*
Parnas, I., Hebrew University. *Killing single cells.*
Blackshaw, S., Stanford Medical School. *Sensory neurons and the structure of their nerve endings.*
Stent, G., University of California, Berkeley. *Development.*
———. *Generation of rhythmic behavior.*
Kristan, W., University of California, San Diego. *Swimming in the leech.*
———. *Demonstration of leech swimming.*
Calabrese, R., Harvard University. *Control of heartbeat.*
Zipser, B., Cold Spring Harbor Laboratory. *Synaptic interactions between sex neurons.*
———. *A leu-enkephalin neuron.*
———. *Monoclonal antibodies binding to specific leech neurons.*

The Mammalian Central Nervous System, June 9–July 2

INSTRUCTORS

Hubel, David, M.D., Harvard Medical School, Boston, Massachusetts
Kirkwood, Peter, Ph.D., Institute of Neurology, London, England
Malpeli, Joseph, Ph.D., University of Illinois, Chicago
Sherk, Helen, Ph.D., Harvard Medical School, Boston, Massachusetts
Rymer, William Z., M.D., Northwestern Medical School, Chicago, Illinois

ASSISTANT

Edwards, Frank, Ph.D., Northwestern Medical School, Chicago, Illinois

This workshop offered laboratories and lectures on the mammalian central nervous system. It began with four days of lectures by Peter Kirkwood and David Hubel on the spinal cord, motor control, and the visual system. These were followed by a day of demonstrations of recording from cat spinal motorneurons and muscle afferents and lateral geniculate neurons. The remaining 18 days were devoted to four series of laboratory experiments on the cat CNS, with the eight participants working in pairs, spending four to five days on each series. In all labs, participants learned the appropriate surgical techniques, the preparation of microelectrodes, and histological procedures for reconstructing microelectrode tracks. In one lab participants studied the reflex activity of motorneurons in the spinal cord using both intracellular and extracellular recording techniques. Functional properties of the associated motor units were identified and synaptic connections from various cutaneous and muscle afferent systems were examined. In another lab, the participants recorded extracellularly from SIII of the somatosensory cortex, the medial geniculate nucleus, and the inferior colliculus. They observed single neuron responses to the appropriate stimuli and mapped their topographic organization. The other two labs focused on extracellular recording from visual cortex (areas 17, 18, 19, and the Clare-Bishop area) and subcortical visual structures (the lateral geniculate nucleus and the superior colliculus). The single-cell responses to visual stimuli, retinotopic organization, and functional cytoarchitecture of these structures were examined. A digital computer was used to generate poststimulus histograms. In the subcortical vision labs the participants studied orthodromic and antidromic shock-evoked driving, making use of the impulse collision technique. Retrograde transport of horseradish peroxidase was used in the somatosensory, auditory, and vision experiments to study the connections between cortical and subcortical structures.

PARTICIPANTS

Baker, Curtis L., B.S., University of California, San Diego
Edgley, Stephen A., B.S., University of Bristol, England
Holets, Vicky R., B.S., University of Minnesota, Minneapolis
Jeffrey, Glen, B.S., University of Oxford, England
Luini, Alberto, M.D., Weizmann Institute, Rehovot, Israel
Marcotte, Ronald R., M.S., Memphis State University, Tennessee
Petrides, Michael, Ph.D., McGill University, Montreal, Quebec, Canada
Schmidt, Marie Luise, Ph.D., Union College, Schenectady, New York

SEMINARS

Sparks, D.L., University of Alabama. *The role of the superior colliculus in control of eye movements.*
Suga, N., Washington University. *Bat echo-location system.*

McClintock Laboratory

Gilbert, C., Harvard Medical School. *Interval circuitry of visual cortex.*
Burke, R.E., National Institutes of Health. *Peripheral inputs to spinal motor neuron.*
Kitai, S.T., Michigan State University. *Physiology and morphology of cells in the basal ganglia.*

Animal Cell Culture in Serum-Free Medium, June 14–June 28

INSTRUCTORS

Sato, Gordon, Ph.D., University of California, San Diego
Barnes, David, Ph.D., University of Pittsburgh, Pennsylvania

Recent investigations into the role of serum in cell culture medium have shown that the serum requirement for many established cell lines can be replaced by specific mixtures of hormones and purified serum factors. Participants learned techniques for serum-free growth of cell lines of general interest, such as HeLa, and cell lines expressing differentiated functions, such as the GC rat pituitary line, B104 rat neuroblastoma line, and MDCK dog kidney line. Participants also designed and carried out experiments in the use of hormone-supplemented medium for the selection of specific cell types in primary cultures of normal and neoplastic tissues. Laboratory exercises were supplemented by lectures and discussions with invited speakers expert in various aspects of cell culture and hormonal control of cell proliferation and differentiation. Special thanks go to Dr. Coon, Dr. Mather and Dr. Reid for arranging laboratory exercises in specialized aspects of the use of serum-free, hormone-supplemented media.

PARTICIPANTS

Chepko, Gloria J., Ph.D., National Eye Institute, Bethesda, Maryland
Clark, Edward P., Ph.D., Stanford University, California
Christensen, Britta, M.S., Fibiger Laboratory, Copenhagen, Denmark
Coughlin, Shaun R., M.S., Massachusetts Institute of Technology, Cambridge
Dautry, Francois, Ph.D., Cancer Institute, Villejuif, France
Gordon, Carin R., Ph.D., University of Pennsylvania, Philadelphia
Harris, Wendelyn A., B.A., Vanderbilt University, Nashville, Tennessee
Iliakis, Georg, Ph.D., GSF, Frankfurt, Federal Republic of Germany
Kumbaraci, Nuran M., Ph.D., Stevens Institute of Technology, Hoboken, New Jersey
Markert, Claus, Ph.D., Goethe Universitat, Frankfurt, Federal Republic of Germany
Plouet, Jean, M.D., INSERM, Paris, France
Price, J. Michael, Ph.D., University of Massachusetts, Boston
Ross, Peter M., Ph.D., Yale University, New Haven, Connecticut
Shimada, Kazunori, Ph.D., Kyushu University, Japan
Taketo, Teruko, Ph.D., Rockefeller University, New York, New York
Van Tieghem, Nicole, University of Brussels, Belgium

SEMINARS

Sirbasku, D., University of Texas Medical School. *Estromedins.*
Coon, H., National Cancer Institute. *Thyroid and neurons in low serum culture.*
Sherman, M., Roche Institute. *Development of mouse embryos in culture.*
Lane, M.D., Johns Hopkins Medical School. *Differentiation of preadipocytes in culture.*
McKeehan, W., W.A. Jones Cell Science Center. *Control of cell proliferation by nutritional factors.*
Yamada, K., National Institutes of Health. *Fibronectin.*
Sato, G., University of California, San Diego. *A unifying hypothesis for cell culture.*
Lippman, M., National Institutes of Health. *Breast tumor cells in serum-free medium.*
Mather, J., Rockefeller University. *Serum-free media for primary culture.*
Varga, J., Yale University. *Melanoma cells in culture.*
Barnes, D., University of Pittsburgh. *Methods for serum-free cell culture.*
Reid, L., Albert Einstein College of Medicine. *Cell-substratum interactions.*
Larner, J., University of Virginia. *Mechanism of insulin action.*
Glass, J., Beth Israel Hospital. *Transferrin.*

The Synapse: Cellular and Molecular Neurobiology
June 22–July 12

INSTRUCTORS

Rahamimoff, Rami, M.D., Hebrew University Medical School, Jerusalem, Israel
McMahan, U. Jack, Ph.D., Stanford University, California
Katz, Bernard, Ph.D., University College London, England
Stevens, Charles F., M.D., Ph.D., Yale University, New Haven, Connecticut
Yoshikami, Doju, Ph.D., University of Utah, Salt Lake City

This course was designed for graduate students and research workers interested in the structure and function of synapses. It consisted of lectures; readings of papers; group discussions; presentations of selected topics by participants; seminars by instructors, faculty, and participants; and demonstrations. After the course ended, some of the participants stayed on for a workshop.

PARTICIPANTS

Angelides, Kimon J., Ph.D., McGill University, Montreal, Quebec, Canada
Anholt, Roger R.H., M.S., University of California, San Diego
Boyarsky, Gregory, B.A., University of Kentucky, Lexington
Braun, Jochen, M.S., University of California, Berkeley
Castellucci, Lise B., M.S., Columbia University, New York, New York
Chaillet, John R., B.A., Yale University, New Haven, Connecticut
Connor, Elizabeth A., B.S., University of Vermont, Burlington
Cooper, Carol L., B.S., Case Western Reserve University, Cleveland, Ohio
Farber, Ira C., M.S., Weizmann Institute, Rehovot, Israel
Fleshman, James W., B.S., University of Florida, Gainesville
Hoch, Daniel B., M.S., University of North Carolina, Chapel Hill
Kerr, Lynne M., B.A., University of Virginia, Charlottesville
Kolb, Hans Albert, Ph.D., University of Konstanz, Federal Republic of Germany
Kolton, Lihu, M.S., Hebrew University, Jerusalem
Kristbjarnarson, Helgi, M.D., Karolinska Institute, Stockholm, Sweden
Lewis, Richard S., B.S., California Institute of Technology, Pasadena
MacDonald, Beth, B.A., University of Connecticut, Farmington
Matsumoto, Mitsuhiko, B.S., Iwate Medical University, Japan
Nerbonne, Jeanne M., Ph.D., California Institute of Technology, Pasadena
Ranscht, Barbara E., Ph.D., Max-Planck-Institut, Tübingen, Federal Republic of Germany
Schehr, Robert S., B.A., Columbia University, New York, New York
Wallace, Jean M., B.S., University of Pennsylvania, Philadelphia

SEMINARS

Jack McMahan, Stanford University. *Structure of peripheral synapses.*
———. *Structure of central synapses.*
———. *Localization of the acetylcholine receptor and cholinesterase.*
———. *Structure of the presynaptic nerve terminal and vesicle recycling.*
———. *Histochemical demonstration of AChE and ACLR at the neuromuscular junction of the frog.*
———. *The effects of denervation on nerve and muscle.*
———. *Reinnervation.*
Rami Rahamimoff, Hebrew University. *Principles of signaling in the nervous system.*
———. *The ionic basis of membrane potentials: The forces.*
———. *Ionic fluxes through excitable membranes.*
———. *The resting membrane potential.*
———. *The action potential: Phenomenology.*
———. *Cable properties and the conductance of the action potential.*
———. *The ionic basis of the action potential: Currents and conductances.*
———. *The Hodgkin-Huxley model of the axonal membrane.*
———. *The role of extracellular and intracellular calcium in the regulation of transmitter release.*
———. *Frequency modulation of transmitter release.*
Doju Yoshikami, University of Utah. *The excitatory postsynaptic potential.*
———. *The ionic basis of the excitatory postsynaptic potential.*
———. *The inhibitory postsynaptic potential.*

———. *Excitatory electric synapses.*
———. *Inhibitory electrical synapses.*
———. *Microphysiology of the neuromuscular junction.*
John Nicholls, Stanford University. *Properties and formation of synapses in the nervous system of the leech.*
Dan Goodenough, Harvard University. *Properties of gap junctions.*
———. *Structure of gap junctions.*
———. *X-ray diffraction.*
Bernard Katz, University College London. *Quantal units of transmitter release.*
———. *Calcium and the vesicular hypothesis of transmitter release.*
———. *The molecular action of the transmitter at the motor endplate.*
Charles Stevens, Yale University. *Inferences about molecular mechanisms from studies of fluctuations.*
———. *Physical approaches to studying membrane channels.*
———. *Mechanisms of permeation and ion selectivity.*
———. *Molecular basis for channel gating.*
———. *Calculation of single channel conductance from noise measurements.*
Dale Purves, Washington University. *Quantitative aspects of synapse formation: Synapse elimination.*
———. *Qualitative aspects of synapse formation: Neural specificity.*
Alexander Mauro, Rockefeller University. *The action of black widow spider venom on the presynaptic nerve terminal.*
Y.N. Jan, University of California. *Substance P sensory transmitter.*
———. *Autonomic ganglia as a model system for study of peptide action.*
Edward Herbert, University of Oregon. *Neuropeptides.*
Birgit Zipser, Cold Spring Harbor Laboratory. *Analysis of a synaptic connection in the mating network in the leech.*
Douglas Fambrough, Carnegie Institution. *Structure of ACh receptors in membranes and in isolation.*
———. *ACh receptor metabolism and its regulation.*
———. *Structure and properties of acetylcholine cholinesterase.*
Ira Black, Cornell University Medical College. *The catecholamine synapses.*
———. *The development of the catecholamine synapses. I & II.*
———. *Pathophysiology of the catecholamine system.*
G. Albrecht-Buehler, Cold Spring Harbor Laboratory. *Control of tissue cell movement.*

Advanced Bacterial Genetics, June 30–July 20

INSTRUCTORS

Roth, John R., Ph.D., University of Utah, Salt Lake City
Davis, Ronald W., Ph.D., Stanford University School of Medicine, California
Botstein, David, Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANTS

Schmid, Molly, B.S., University of Utah, Salt Lake City
Carlson, John, A.B., Stanford University School of Medicine, California
Riggs, Paul D., B.A., Massachusetts Institute of Technology, Cambridge

Starting with a random pool of λ phages carrying cloned inserts of *Salmonella* DNA, students identified phages carrying portions of the histone operon. These phages were identified by lytic selection, lysogenic selection, and plaque hybridization. Phages were characterized by restriction analysis, complementation tests, and electron microscopy.

A series of deletion mutants of the *his* operon were mapped genetically by transductional crosses. The physical size of each of these same deletions was then determined by rfsh (restriction fragment spectrum hybridization). By this procedure it was also possible to map the position of restriction sites genetically.

Students performed a variety of genetic manipulations involving use of the Tn*10* insertion element. Auxotrophs were made by Tn*10* insertion, and Tn*10* insertions were selected near particular genes. Tn*10* was used as a selective marker to perform localized mutagenesis of a particular region of the chromosome.

Mutagenesis of cloned DNA was performed using the Tn3 β lactamase gene carried by phage λ. Students selected deletion and point mutations in this gene and characterized these mutants by genetic crosses, restriction analysis, and electron microscopy heteroduplex analysis.

PARTICIPANTS

Boos, Winfried, Ph.D., University of Konstanz, Federal Republic of Germany
Condra, Jon H., Ph.D., NLMB, National Institutes of Health, Bethesda, Maryland
Fox, J. Lawrence, Ph.D., University of Texas, Austin
Gennis, Robert B., Ph.D., University of Illinois, Urbana
Goff, Christopher G., Ph.D., Haverford College, Pennsylvania
Kirsch, Donald R., Ph.D., Rutgers Medical School, New Brunswick, New Jersey
Kustu, Sydney, Ph.D., University of California, Davis
Lin, Edmund C.C., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Mann, Barbara J., B.S., University of Virginia, Charlottesville
Newland, John W., B.A., University of Maryland, College Park
Oriel, Patrick J., Ph.D., Dow Chemical Co., Midland, Michigan
Plumbridge, Jacqueline A., Ph.D., Institut de Biologie Physico-Chimique, Paris, France
Price, Chester W., Ph.D., University of California, Berkeley
Purucker, Mary E., B.S., Albert Einstein College of Medicine, Bronx, New York
Steiner, Lisa A., M.D., Massachusetts Institute of Technology, Cambridge
Stewart, Valley, B.A., University of Virginia, Charlottesville

SEMINARS

Beckwith, J., Harvard Medical School. *New techniques in bacterial genetics.*
Walker, G., Massachusetts Institute of Technology. *Analysis of SOS functions* use Mu-lac *fusions.*
Campbell, A., Stanford University. *Divergent transcription of the biotin operon.*
Simon, M., University of California, San Diego. *Analysis of the mechanism of phase variation in* Salmonella typhimurium.
Ptashne, M., Harvard University. Molecular mechanisms of λ repressor function.
Friedman, D., University of Michigan Medical School. *Host functions involved in* λ *antitermination control.*
Stahl, F., University of Oregon. *Function of chi sequences in genetic recombination.*

Molecular Cloning of Eukaryotic Genes, June 30–July 20

INSTRUCTORS

Maniatis, Thomas, Ph.D., California Institute of Technology, Pasadena
Fritsch, Edward, Ph.D., California Institute of Technology, Pasadena
Hopkins, Nancy, Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANTS

Donis-Keller, Helen, Ph.D., Harvard Medical School, Cambridge, Massachusetts
O'Connell, Catherine D., B.A., Frederick Cancer Research Center, Maryland

This was a laboratory course in the application of recombinant DNA procedures to the study of eukaryotic genes with emphasis on the isolation of cellular structural genes. Among the topics covered were: construction of bacteriophage λ and cosmid libraries of high molecular weight eukaryotic DNA; screening DNA libraries with specific hybridization probes; purification and characterization of recombinant clones using restriction endonuclease and blot hybridization analyses; transfer of cloned DNA into cells in culture; and in vitro mutagenesis of cloned DNA to study regulatory sequences. Mammalian globin genes and the herpes virus thymidine kinase gene were utilized as model experimental systems.

PARTICIPANTS

Bernstine, Edward G., Ph.D., Oak Ridge National Laboratory, Tennessee
Brandsma, Janet L., M.S., Yale University, New Haven, Connecticut
Calva, Edmundo, Ph.D., National University of Mexico, Mexico City
Chan, Shu Jin, Ph.D., University of Chicago, Illinois

Fenton, Robert G., B.S., New York University, New York
Garel, Annie, Ph.D., Université Claude Bernard, Villeurbanne, France
Grindley, June N., Ph.D., University of Pittsburgh, Pennsylvania
Hayday, Adrian, B.A., Imperial Cancer Reserach Fund Laboratories, London, England
Krump-Konvalinkova, Vera, Ph.D., Radiobiological Institute, Rijswijk, The Netherlands
Lo, Cecilia W., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Manson, Katherine I., M.S., Fred Hutchinson Cancer Center, Seattle, Washington
Martin, Sandra L., B.A., University of California, Berkeley
Shimada, Kazunori, Ph.D., Kyushu University, Japan
Sloof, Paul, Ph.D., Jan Swammerdam Institute, Amsterdam, The Netherlands
Wasmuth, John J., Ph.D., University of California, Irvine
Waterston, Robert H., Ph.D., Washington University School of Medicine, Seattle

SEMINARS

Roberts, R., Cold Spring Harbor Laboratory. *Restriction enzymes.*
Hamer, D., National Institutes of Health. *Expression of β-globin sequences cloned in SV40.*
Meyerowitz, E., California Institute of Technology. Drosophila *and its genes.*
———. *Cloning large eukaryotic DNA segments using cosmid vectors.*
Davis, R., Stanford University. *Yeast transformation vectors for the functional analysis of yeast genes.*
———. *Electron microscopy of nucleic acids.*
Bishop, J.M., University of California School of Medicine, San Francisco. *The molecular biology of retroviruses.*
Roberts, B., Harvard Medical School. *Application of hybrid arrested translation.*
Vande Woude, G., National Institutes of Health. *sarc and sarc of murine sarcoma virus.*
Manley, J., Massachusetts Institute of Technology. *Transcription of mammalian genes in vitro.*
Wigler, M., Cold Spring Harbor Laboratory. *DNA transfection.*
Axel, R., Columbia University. *DNA transfection.*
Weinberg, R., Massachusetts Institute of Technology. *Transformation by DNA from chemically transformed cells.*
Lowy, D., National Institutes of Health. *Molecularly cloned retroviral genomes.*
Cooper, G., Sidney Farber Cancer Institute, Harvard Medical School. *Transformation by DNAs of normal and transformed cells.*
Ptashne, M., Harvard University. *Improved methods for maximizing expression of cloned eukaryotic genes in* E. coli.
DiMaio, D., Johns Hopkins University. *In vitro mutagenesis of DNA.*
Hood, L., California Institute of Technology. *New microchemical techniques.*
———. *Antibodies: Split genes and jumping genes.*
Sambrook, J., Cold Spring Harbor Laboratory. *Disintegration of DNA viral genomes.*
Feramisco, J., Cold Spring Harbor Laboratory. *Microinjection of antibodies, etc. into cultured cells.*

James Laboratory

Neuroanatomical Methods, July 8–July 28

INSTRUCTORS

Jones, Edward G., Ph.D., Washington University School of Medicine, St. Louis
Hartman, Boyd K., M.D., Washington University School of Medicine, St. Louis
Hand, Peter J., Ph.D., University of Pennsylvania, Philadelphia

ASSISTANTS

McClure, Bertha, Washington University School of Medicine, St. Louis
Steiner, Ron, B.A., Washington University School of Medicine, St. Louis
Kalmbach, Sandra, Washington University School of Medicine, St. Louis

This was primarily a laboratory course designed for research workers interested in learning basic neuroanatomical techniques. Participants carried out an extensive set of laboratory exercises involving most of the commonly used methods and there were lectures and discussions by the instructors and visiting speakers.

Classical methods covered included: perfusion fixation; embedding in different media; frozen, paraffin, celloidin, and plastic sectioning; conventional cell staining methods; lesion making; silver impregnation of normal and degenerating nerve fibers; brightfield, darkfield, and fluorescence microscopy, photomicrography, and electron microscopy.

Newly developed methods included histochemical, radiochemical, and immunocytochemical methods for tracing neuronal pathways in terms of axoplasmically transported material, neurotransmitter-related compounds, or metabolic activity.

PARTICIPANTS

Amico, Lonnie L., M.D., University of Chicago, Illinois
Carr, Catherine E., M.A., University of California, San Diego
Harry, Gaylia J., M.S., NIEHS, National Institutes of Health, Research Triangle Park, North Carolina
Huppenbaüer, Robert, B.A., University of California, Berkeley
Juliano, Sharon L., B.A., University of Pennsylvania, Philadelphia
Lydic, Ralph B., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Nahin, Richard L., B.A., University of California, Los Angeles
Schechter, Nisson, Ph.D., State University of New York, Stony Brook
Theriault, Elizabeth, B.A., McMaster University, Hamilton, Ontario, Canada
Wallace, N.M., B.S., University of Glasgow, England

SEMINARS

Pickel, V., Cornell Medical College. *Immunocytochemistry and the electron microscope.*
Raviola, E., Harvard Medical School. *Freeze fracture and related techniques.*
Swanson, L., Salk Institute. *Fluorescent dyes as retrograde tracers.*
Peters, A., Boston University. *The Golgi-EM technique.*
Hendrickson, A., University of Washington. *Autoradiographic techniques.*

Principles of Neural Development, July 14–July 29

INSTRUCTORS

Purves, Dale, M.D., Washington University School of Medicine, St. Louis, Missouri
Patterson, Paul H., Ph.D., Harvard Medical School, Boston, Massachusetts

The aim of this intensive two-week course was to expose students to the classical literature on neural development, with special emphasis on recent advances and controversies in this rapidly growing field. The selection of students was based primarily on recommendations and potential rather than background; thus this year's group of 21 students was composed of 7 postdoctoral fellows, 11 graduate students, and 3 fulltime faculty members involved in widely different research programs. The common denominator was an interest in pursuing research related to neural development.

The format of the course was to have invited lecturers present a detailed review of particular subjects in a three-hour morning session. The speaker then chose several important papers,

generally ones not discussed in the morning, for the students to read during the afternoon. In the late afternoon the speaker and instructors met with groups of 6–7 students to discuss the reading and prepare one of the students in each group to present the papers at an evening meeting of the whole class. The evening then consisted of several student presentations of aspects of the morning's subject not covered by the invited lecturer, as well as a general discussion of the issues raised during the day. Most evenings ended with one or two student research seminars in which an individual's work in progress was described for discussion and criticism by fellow students and instructors. In general, we attempted to have such presentations on an evening when the day's guest lecturer had a special interest in the student's field. An occasional variant of this format was to end the evening with a research seminar by an additional invited speaker whose material was deemed of special interest, but not broad enough to devote an entire day to.

PARTICIPANTS

Bastiani, Michael, B.A., University of California, Davis
Chiu, Arlene Y., M.S., California Institute of Technology, Pasadena
Cospito, Joseph A., Ph.D., University of California, Los Angeles
Denis-Donini, Suzanne, Ph.D., Laboratory of Molecular Embryology, Naples, Italy
di Porzio, Umberto, M.D., College of France, Paris
Doupe, Allison J., B.S., Harvard Medical School, Cambridge, Massachusetts
Fentress, John C.,¹Ph.D., Dalhousie University, Halifax, Nova Scotia, Canada
Hooghe-Peters, Elisabeth L., Ph.D., University of Geneva, Switzerland
Loring, Jeanne F., Ph.D., University of California, San Diego
Mailhammer, Reinhard, Ph.D., Rockefeller University, New York, New York
Moody, Sally A., M.S., University of Florida, Gainesville
Naujoks, Kurt W., B.S., Max-Planck-Institut, Martinsried, Federal Republic of Germany
O'Donovan, Michael J., Ph.D., Yale University, New Haven, Connecticut
Reh, Thomas A., B.S., University of Wisconsin, Madison
Rubin, Eric, B.A., Washington University School of Medicine, Seattle
Seeley, John, Ph.D., New York University, New York
Suburo, Angela Maria, M.D., IMBICE, La Plata, Argentina
Tapscott, Stephen J., B.A., University of Pennsylvania, Philadelphia
Vulliamy, Thomas J., Ph.D., University College London, England
Whitelaw, Virginia A., B.A., University of Chicago, Illinois
Yao, Yung-mae, B.A., University of North Carolina, Chapel Hill

SEMINARS

Cowan, W.M., Washington University School of Medicine. *Neural induction, neural proliferation, neural migration, and neural aggregation.*
Patterson, P.H., Harvard Medical School. *Epigenetic control of development: In vivo and in vitro studies of neural crest.*
———. *Nerve growth factor: Its biological role and its biochemistry.*
Brockes, J., California Institute of Technology. *Immunological approaches to neural development.*
Lawrence, P.A., Medical Research Council Laboratory of Molecular Biology. *Pattern formation and its genetic control.*
Bentley, D., University of California, Berkeley. *Invertebrate development.*
Rakic, P., Yale University School of Medicine. *Cell migration in the monkey CNS.*
———. *Differentiation of neuronal geometry.*
———. *Mutant mice as a tool for studying CNS development.*
Berg, D., University of California, San Diego. *A trophic factor secreted by skeletal muscle or eye.*
———. *Fundamentals of synapse formation.*
Landmesser, L.T., Yale University. *Axon guidance.*
Jaffe, L., Purdue University. *Role of ionic currents in development.*
Purves, D., Washington University School of Medicine. *Synapse elimination.*
———. *Sprouting of axons in maturity.*
Sanes, J., Washington University School of Medicine. *Denervation and reinnervation of muscle cells.*
Purves, D., Washington University School of Medicine. *Target/neuron interactions in maturity.*
———. *Specificity in the peripheral nervous system.*
Constantine-Paton, M., Princeton University. *Retinotopic mapping.*
Easter, S., University of Michigan. *Retino-tectal connectivity.*
Hubel, D.H., Harvard Medical School. *The mammalian visual system.*

Electrophysiological Methods for Cellular Neurobiology
July 21–August 10

INSTRUCTORS

Kehoe, JacSue, Ph.D., Ecole Normale Supérieure, Paris, France
Chiarandini, Dante, M.D., New York University School of Medicine, New York
Stefani, Enrique, M.D., Instituto Politecnico Nacinal, Mexico City, Mexico

GUEST LECTURER

Kado, Ray, Ph.D., Centre National de la Recherche Scientifique, Gif-sur-Yvette, France

In this neurobiology course the neuromuscular junction of the frog and the central ganglia of the mollusk *Aplysia* were used as experimental preparations for training students in basic electrophysiological methods for cellular neurobiology. Examination of certain characteristics of the resting, action and synaptic potentials of these two preparations served as a basis for introducing the following techniques: microdissection, fabrication of single and multibarreled capillary microelectrodes; intracellular recording of membrane voltage changes (in so-called current clamp) and membrane currents (using a slow voltage clamp); intracellular and extracellular application of ions and drugs (by ionophoresis and pressure injections); and intracellular staining of *Aplysia* neurons.

The first 3 days of the course were devoted to lectures and exercises on electronics for cellular neurobiologists given by Ray Kado. The last 2 days of the course were devoted to individual experimental projects chosen by the students. These projects permitted them to try techniques not taught in the course and to use biological preparations that they intended to study when they returned to their own laboratories.

PARTICIPANTS

Chaillet, John, B.A., Yale University, New Haven, Connecticut
Crean, Geraldine, M.S., University of Connecticut, Farmington
Covarrubias, Manuel, M.S., National University of Mexico, Mexico City
Hernandez, Norma, B.S., IVIC, Caracas, Venezuela
Horton, Jonathan C., B.A., Harvard Medical School, Cambridge, Massachusetts
Lautt, Ruth, B.A., Hunter College, City University of New York, New York
Role, Lorna W., B.A., Harvard Medical School, Cambridge, Massachusetts
Schlichter, Lyanne, M.S., University of Toronto, Canada
Tapparelli, Carlo, B.S., University of Basel, Switzerland
Taylor, P., B.S., University of Bristol, England

Transformed Cells and Teratocarcinomas, July 23–August 12

INSTRUCTORS

Hynes, Richard O., Ph.D., Massachusetts Institute of Technology, Cambridge
Martin, Gail R., Ph.D., University of California, San Francisco

ASSISTANTS

Destree, Antonia, B.S., Massachusetts Institute of Technology, Cambridge
Fujii, Joann, B.S., University of California, San Francisco

The course consisted of an integrated series of lectures, demonstrations, discussions, and laboratory experiments designed to introduce students to the concepts and techniques involved in the study of oncogenic transformation, teratocarcinomas, and mouse embryos. The lecture topics covered initiation of transformation by tumor viruses and chemicals, the nature of the transformed phenotype, establishment and properties of teratocarcinomas, and the study of early mouse embryos.

Established cell lines and primary cultures were transformed by SV40, by SV40 DNA, by avian sarcoma viruses, and by microinjection of SV40 DNA. Growth properties of transformed cells were analyzed and the transformed phenotype was studied by a variety of techniques: immunofluorescence for T antigen, cell-surface fibronectin, laminin and type IV collagen and several

cytoskeletal proteins; SDS-polyacrylamide gels; immunoprecipitation; plasminogen activator assays; and nutrient transport. Teratocarcinoma cells were cultivated for differentiation and analyzed by similar methods. Superovulated mice were used to prepare two-cell and blastocyst embryos for culture and immunosurgery.

PARTICIPANTS

Bach, Richard G., B.S., New York University, New York
Backer, Joseph M., Ph.D., Columbia University, New York, New York
Blikstad, Ingrid, B.S., Uppsala University, Sweden
Boecker, Joerg F., Ph.D., University Freiburg, Federal Republic of Germany
Carlsson, Lars, Ph.D., Uppsala University, Sweden
Daya-Grossjean, Leela, Ph.D., Institut de Recherches Scientifiques, Villejuif, France
Denny, John B., M.S., University of Florida, Gainesville
Kunz, Linda M., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Kwan, Sau-Wah, Ph.D., University of Texas, Galveston
Lennox, Ronald W., Ph.D., Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania
MacDonald, Raymond J., Ph.D., University of Texas, Dallas
Orida, Norman K., Ph.D., University of California, San Diego
Pourcel, Christine, B.S., Institut Pasteur, Paris, France
Reich, Nancy C., M.S., State University of New York, Stony Brook
Saborio, Jose L., Ph.D., CINVESTAV, Mexico City, Mexico

SEMINARS

Hynes, R., Massachusetts Institute of Technology. *Introduction to transformation.*
———. *Surface changes in transformation.*
Martin, G., University of California, San Francisco. *Introduction to teratocarcinoma.*
Hopkins, N., Massachusetts Institute of Technology. *Murine retroviruses.*
Benjamin, T., Harvard Medical School. *Small DNA tumor viruses.*
Fidler, J., Frederick Cancer Research Center. *Analysis of metastasis.*
Todaro, G., NCI, National Institutes of Health. *Growth factors and transformation.*
Quigley, J., Downstate Medical Center, State University of New York, Brooklyn. *Proteases in transformation and malignancy.*
Fisher, P., Columbia University. *Initiating and promoting carcinogens.*
Martin, S., University of California, Berkeley. *Transformation by avian retroviruses.*
Stevens, L., Jackson Laboratory. *Establishment of teratocarcinomas.*
Papaioiannou, G., University of Oxford. *Embryos and teratocarcinomas.*
Strickland, S., Rockefeller University. *Induction of endoderm formation.*
Stringer, J., Cold Spring Harbor Laboratory. *Viral integration sites.*
Zetter, B., Children's Hospital. *Tumor angiogenesis.*
Feramisco, J., Cold Spring Harbor Laboratory. *Microinjection studies on the cytoskeleton.*
Silver, L., Einstein University. *Cell surfaces and teratocarcinomas.*
———. *The T locus of mice.*
Willison, K., Cold Spring Harbor Laboratory. *Cell surfaces and teratocarcinomas.*

Molecular Biology and Genetics of Yeast, July 23–August 12

INSTRUCTORS

McLaughlin, Calvin S., Ph.D., University of California, Irvine
Cox, Brian S., Ph.D., University of Oxford, England

ASSISTANTS

Dobson, Melanie, Ph.D., University of Oxford, England
Tuite, Mick, Ph.D., University of California, Irvine

This program emphasized the major laboratory techniques used in the genetic analysis of yeast tetrad analysis, mitotic recombination, five-structure mapping, cytoduction, tetraploid analysis, complementation, and allelism. The isolation and characterization of both chromosomal and cytoplasmic mutants was undertaken. Biochemical studies including two-dimensional gel electrophoresis were performed with auxotrophic, radiation-sensitive, temperature-sensitive, and altered

mating type mutants. Recombinant DNA techniques including yeast transformation filter hybridization, gel electrophoresis mRNA isolation and in vitro protein synthesis were applied to cloning and genetic analysis of yeast DNA.

PARTICIPANTS

Belfort, Marlene, Ph.D., New York State Dept. of Health, New York
Cleveland, Don W., Ph.D., University of California, San Francisco
Cumsky, Michael G., B.A., University of California, Berkeley
Freese, Elisabeth B., Ph.D., NLMB, National Institutes of Health, Bethesda, Maryland
Gibson, Raymond K., Ph.D., Public Health Laboratory, Salisbury, England
Gill, Gurnam S., Ph.D., Upjohn Company, Kalamazoo, Michigan
Hillman, Richard E., M.D., Washington University, Seattle
Huberman, Joel A., Ph.D., Roswell Park Memorial Institute, Buffalo, New York
Huffaker, Tim C., B.A., Massachusetts Institute of Technology, Cambridge
Knapp, Gayle, Ph.D., University of California, San Diego
Orlowska, Gabriela, M.S., University of Wroclaw, Poland
Romesser, James A., Ph.D., E.I. duPont de Nemours & Co., Wilmington, Delaware
Spierenburg, Henk S., M.S., Gist-Brocades, Delft, The Netherlands
Toselli, Mauro, California Institute of Technology, Pasadena
Walder, Roxanne Y., B.A., University of Iowa, Iowa City
Walsh, J.M., B.S., Oregon State University, Eugene

SEMINARS

Cox, B., University of Oxford. *An introduction to yeast genetics.*
———. *Natural plasmids and cytoplasmic inheritance.*
Fogel, S., University of California, Berkeley. *Genetic recombination and gene conversion in yeast. I.*
———. *Genetic recombination and gene conversion in yeast. II.*
Coruzi, G., Columbia University. *Mitochondrial genetics.*
Haynes, R., York University. *Mutagenesis.*
Tuite, M., Univeristy of California, Irvine. *Translational suppression.*
———. *In vitro protein synthesis.*
Carbon, J., University of California, Santa Barbara. *Cloning techniques and concepts.*
———. *Cloning a centromere.*
Warner, J., Albert Einstein College of Medicine. *mRNA: Properties and translation.*
———. *RNA synthesis.*
Esposito, R., University of Chicago. *Genetic analysis of meiosis.*
McLaughlin, C., University of California, Irvine. *Genetic analysis of protein synthesis.*
———. *Analysis of the cell cycle.*

The original Davenport Laboratory (now part of Delbrück Laboratory), site of genetics courses since 1945

Knapp, G., University of California, San Diego. *tRNA: Processing of intervening regions.*
Broach, J., State University of New York, Stony Brook. *Properties of the 2μ plasmid.*
Hicks, J., Cold Spring Harbor Laboratory. *Cloning the mating type gene.*
McKay, V., Rutgers University. *Genetic analysis of mating in yeast.*
Klar, A. and J. Strathern, Cold Spring Harbor Laboratory. *Genetic and molecular analysis of mating type switching.*

Workshop on Tumor and Developmental Antigens
August 3–August 10

ORGANIZERS
Levine, A.J., Ph.D., State University of New York, Stony Brook
Lane, D.P., Ph.D., Imperial College, London, England

The advent of the monoclonal antibody technique has revolutionized our ability to examine the antigenic changes that accompany normal cellular differentiation and also tumor formation. The impact of the technique and the many new areas which it is opening up were discussed in this Workshop, which brought together a small group of immunologists, virologists, and developmental biologists.

The Workshop first considered cellular differentiation antigens, particularly with reference to the immune system. In this context, elegant studies using monoclonal antibodies to isolate novel H-2 mutants were described, as well as the extensive and intriguing cross reactions between histocompatibility antigens of different species. Markers for T cell and B cell lineages and the development of these cell types was discussed. Here the monoclonal antibody system has made it possible to subdivide the human and mouse T cell population and, in the human case, this is providing an exciting new way of studying the cell population of leukemia patients. The direct use of monoclonal antibodies in the therapy of leukemia was described in a mouse model system. The results were striking and very encouraging.

Studies on developmental antigens were described and discussed: in particular the T locus received a very fine and clear coverage. This session introduced the extensive description of tumor antigens, which was a major theme of the Workshop. These antigens often represented normal cellular components which in the tumor were rendered more abundant either by direct transposition placing them under the control of a viral promoter or more obliquely by the action of virus specific proteins or other transforming agents. The nature of the potential transforming genes themselves was reviewed and their exciting association with differentiation markers became apparent in the case of the defective leukemia viruses. The Workshop ended on a high note with a beautiful description of UV irradiation, tumorigenesis antigens and a review of the diagnostic use of monoclonal antibodies in certain human tumors. The interdisciplinary nature of the group and the skillful chairmanship led to an enormously exciting and productive meeting.

PRESENTATIONS
Mitchison, A., University College London, England. *The mechanisms of tumor recognition and rejection.*
———. *The major histocompatability locus.*
Rajan, T.V., Albert Einstein College of Medicine, Bronx, New York. *Genetics.*
Nathenson, S., Albert Einstein College of Medicine, Bronx, New York. *Biochemistry.*
McKearn, T., University of Pennsylvania, Pittsburgh. *Monoclonal antibodies.*
Cantor, H., Harvard Medical School, Boston, Massachusetts. *T-cell antigens.*
Schlossman, S., Harvard Medical School, Boston, Massachusetts. *T-cell antigens.*
Doherty, P., The Wistar Institute, Philadelphia, Pennsylvania. *T-cell functions and lineages.*
Ledbetter, J., Stanford University, California. *T-cell functions and lineages.*
Cooper, M., University of Alabama, Birmingham. *B-cell development and molecular biology.*
Seidman, J., National Institutes of Health, Bethesda, Maryland. *B-cell development and molecular biology.*
Wiley, L., University of Virginia, Charlottesville. *Development of the mouse.*
Silver, L., Sloan-Kettering Cancer Research Center. *The T-locus of mice.*
Lilly, F., Albert Einstein College of Medicine, Bronx, New York. *Genetic loci of the mouse involved in tumorigenesis.*
Nowinski, R., Fred Hutchinson Cancer Research Center, Seattle, Washington. *Immunotherapy of mouse leukemia with monoclonal antibodies.*

Risser, R., University of Wisconsin, Madison. *Murine and acute leukemia virus antigens.*
Hayman, M., Imperial Cancer Research Fund Laboratories, London, England. *Murine and acute leukemia virus antigens.*
Scolnick, E., NCI, National Institutes of Health, Bethesda, Maryland. *Murine and avian sarcoma virus antigens.*
Erikson, R., University of Colorado, Denver. *Murine and avian sarcoma virus antigens.*
Weinberg, R., Massachusetts Institute of Technology, Cambridge. *Cellular and viral transforming genes.*
Cooper, G., Harvard Medical School, Boston, Massachusetts. *Cellular and viral transforming genes.*
———. *Cellular tumor antigens.*
Jay, G., National Institutes of Health, Bethesda, Maryland. *Chemically transformed cells.*
Lane, D., Imperial College of Science and Technology, London, England. *SV40-cellular tumor antigens.*
———. *Cellular tumor antigens.*
Krepke, M., Frederick Cancer Reserach Center, Maryland. *Irradiation tumorigenesis antigens.*
Koprowski, H., The Wistar Institute, Philadelphia, Pennsylvania. *Melanoma antigens.*

UNDERGRADUATE SUMMER RESEARCH PROGRAM

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 216 students have completed the course, and many have gone on to creative careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology, (2) an increased awareness of major problem areas under investigation, (3) better understanding of the physical and intellectual tools for modern research and the pertinence of this information to future training, and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from a large number of applicants, took part in the program, which was supported by The Camille and Henry Dreyfus Foundation, Inc. They are listed below with their laboratory sponsors and topics of research.

Alexander Baxter, Haverford College *Research Advisor:* D. Kurtz	Characterization of the rat α_{2u} globulin gene.
Chris Corless, University of California, Berkeley *Research Advisor:* R. Roberts	Dideoxy sequencing of adenovirus-2 DNA using *Hin*dII restriction fragments as primers.
Andy Ellington, Michigan State University *Research Advisor:* J. Lewis	In vitro mutagenesis of Ad2 as a means of examining the significance of poly(A) tailing of mRNA.
Deborah S. Gibson, Rensselaer Polytechnic Institute *Research Advisor:* J. Garrels	Two-dimensional gel electrophoresis analysis of human fibroblast proteins: In quest of the genetic defect which causes cystic fibrosis.
Felicia M. Hendrickson, Harvard University *Research Advisors:* L. K. Drickamer, J. Garrels	Mitochondrial protein identification on two-dimensional gels.
Thomas M. Laton, LeMoyne College *Research Advisor:* J. Smart	(1) Does SV40 large T antigen exist in a multimeric complex? (2) Determination of the monoclonal antibody binding site of SV40 large T antigen.
Elizabeth McFarland, Northwestern University *Research Advisor:* D. Zipser	Sequence analysis of deletion and insertion mutations of the cloned HSV-1 thymidine kinase gene.
Allen B. Oser, Brown University *Research Advisor:* A. I. Bukhari	Restriction enzyme mapping of bacteriophage Mu DNA and use of various methods to make plasmids (pSC101 and pBR322) containing Mu wild-type repressor gene.
Barry S. Rosen, Wesleyan University *Research Advisor:* T. Broker	Nucleotide sequence evolution. Adenovirus: The determination of the sequence of gene IV_{a2} in Ad7 and comparison to Ad2 sequences.
Eric S. Schulze, University of California, Berkeley *Research Advisor:* S. Blose	Purification and characterization of the midbody of dividing HeLa cells.
Olney Fellow Leona Ling, University of California, Berkeley *Research Advisor:* Y. Gluzman	Sequencing the junctions of Ad2-SV40 defective hybrids.

IN-HOUSE SEMINARS

Kurt Drickamer conducting a seminar in James Laboratory

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

1979–1980

August

Marc van Montagu, Rijks University, Gent, Belgium: *The Ti plasmids of* Agrobacterium tumifaciens: *Possible vectors for genetic engineering in plants.*

December

Robert G. Roeder, Washington University, St. Louis, Missouri: *Transcription of purified eukaryotic genes in reconstituted systems.*

Magdelene So, University of California Medical Center, San Francisco: *The molecular biology of the* E. coli *heat stable toxin.*

Fred Heffron, University of California, San Francisco: *Structural and functional analysis of the bacterial transposon Tn3.*

Francine Eden, National Institutes of Health, Bethesda, Maryland: *Very large methylated repeated elements in chickens.*

Mitchell Smith, University of Edinburgh, Scotland: *Histone gene organization in yeast.*

January

James Manley, Massachusetts Institute of Technology, Cambridge: *Studies of adenovirus transcription in vitro.*

K. Wohlfarth-Botterman, University of Bonn, Federal Republic of Germany: *Motive force generation by cytoplasmic actomyosin for protoplasmic streaming in* Physarum.

Hans van Ormondt, University of Leiden, The Netherlands: *The nucleotide sequence of early region 1 of adenovirus DNAs.*

February

Benjamin Walcott, State University of New York, Stony Brook: *Magnetic field detection by specialized cells in pidgeons.*

John Condeelis, Albert Einstein College of Medicine, Bronx, New York: *Reciprocal interactions between the actin lattice and cell membrane.*

Brad Ozanne, University of Texas, Dallas: *Transforming factors produced by virally transformed cells.*

Arthur Levinson, University of California, San Francisco: *Characterization of the protein encoded by the transforming gene of avian sarcoma virus.*

Martin Chalfie, Medical Research Council Laboratories, Cambridge, England: *Microtubule cells of* C. elegans*; Genetics and development of identified neurons.*

George Vande Woude, NCI, National Institutes of Health, Bethesda, Maryland: *Properties of two regions of the leukemia virus genome that are required for transformation by* src/sarc.

March

Clark Tibbetts, University of Connecticut, Farmington: *Site-directed mutagenesis—An efficient and general approach.*

Margarita Salas, University of Madrid, Spain: *Protein p3, covalently linked to ϕ29 DNA, is involved in initiation of replication.*

April

Eric Gruenstein, University of Cincinnati Medical Center, Ohio: *Identification of lysosomal abnormalities in fibroblasts from individuals with Duchenne muscular dystrophy.*

Moshe Yaniv, Pasteur Institute, Paris, France: *Mutants of polyoma virus able to grow in teratocarcinoma cells.*

Fuyuhiko Tamanoi, Harvard University, Cambridge, Massachusetts: *Initiation of replication of bacteriophage T7 DNA.*

Mike Fried, Imperial Cancer Research Fund Laboratories, London, England: *Organization and expression of polyoma viral DNA sequences in transformed cells.*

Nancy Hogg, Imperial Cancer Research Fund Laboratories, London, England: *Heterogeneity of human macrophages detected by monoclonal antibodies.*

Gunther Schutz, Max-Planck-Institut für Molekulare Genetik, Bonn, Federal Republic of Germany: *Structure and expression of the chicken lysozyme gene.*

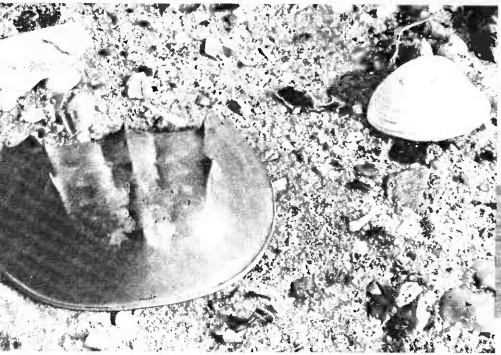
May

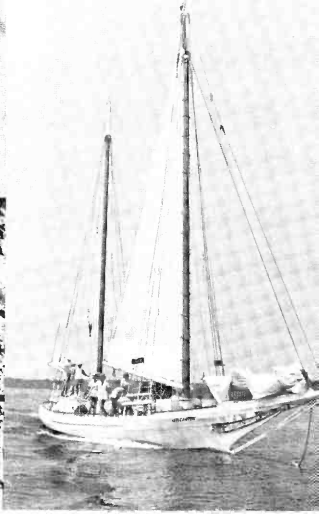
Cathy Laughlin, National Institutes of Health, Bethesda, Maryland: *Involvement of adenovirus early region genes in the AAV helper function.*

David Schubert, Salk Institute, San Diego, California: *Role of a 16S glycoprotein complex in cellular adhesion.*

Bill Welch, Salk Institute, San Diego, California: *Synthesis and orientation of viral membrane glycoproteins.*

Brian D. Crawford, Johns Hopkins University, Baltimore, Maryland: *Genetic aspects of neoplastic transformation in vitro.*





NATURE STUDY PROGRAM

The Nature Study Program is designed for elementary and high school students who wish to achieve a greater understanding of their environment. During Summer and Fall a total of 440 students participated in these activities. Most classes were held outdoors, when weather permitted, or at Uplands Farm Nature Preserve of the Nature Conservancy, where the Laboratory has equipped and maintains classroom laboratories for the study of field specimens collected by the students.

In addition to courses, a series of one-day Marine Biology Workshops was offered, with special workshops for adults. Studies were conducted on Long Island Sound aboard a 55-foot schooner chartered from Schooner, Inc. of New Haven, Connecticut. This vessel is equipped with a variety of instrumentation and is staffed by a captain, mate, and marine biologists. Students participated in biological studies and in the actual sailing of the vessel.

This summer a new course, Adventure Education II, was conducted at Watch Hill on Fire Island. Students studied the environment while camping on the barrier beach.

In the Fall a Long Island Field Geology course included trips to the Montauk Peninsula, North and South Shores of Long Island, and the State University of New York at Stony Brook.

PROGRAM DIRECTOR

Sanford Kaufman, M.S., M.P.A., science teacher, Hewlett High School

INSTRUCTORS

Kathryn Bott, M.S., science teacher, Friends Academy
Judith Calloway, M.S., biology teacher, East Islip High School
Don Dunn, M.S., art and photography teacher, Hewlett High School
Steven Englebright, M.S., curator of geology, State University of New York, Stony Brook
Carolyn Hess, M.S., Museum of Long Island Natural Sciences, State University of New York, Stony Brook
James Romansky, M.S., biology teacher, Bay Shore High School
Edward Tronolone, M.S., science teacher, Lynbrook North Middle School

COURSES

Nature Bugs
Nature Detectives
Advanced Nature Study
Pebble Pups
Rock Hounds
Long Island Field Geology
Bird Study
Seashore Life
Marine Biology
Fresh Water Life
Frogs, Flippers, and Fins
They Swim, Crawl, and Walk
Nature Photography I & II
Adventure Education I & II

LABORATORY STAFF

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J.D. Watson

ADMINISTRATIVE DIRECTOR
William R. Udry

ASSISTANT DIRECTOR FOR RESEARCH
Joseph Sambrook

DIRECTOR—BANBURY CENTER
Victor McElheny

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Stephen Blose
Thomas Broker
Ahmad Bukhari
Keith Burridge
Louise Chow
Jeffrey Engler
James Feramisco
John Fiddes
James Garrels
Thomas Gingeras
Yakov Gluzman
Terri Grodzicker
Fred Heffron
James Hicks
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Stephen Hughes
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Fuyuhiko Tamanoi
William Topp
Michael Wigler
Birgit Zipser
David Zipser

VISITING SCIENTISTS

Bingdong Jiang
Mara Rossini
Lee Silver

POSTDOCTORAL FELLOWS

Judith Abraham
Bruce Anderson
Marilyn Anderson
Frederick Asselbergs
Robert Blumenthal
Joan Brooks
George Chaconas
Kurt Drickamer
Hansjorg Engeser
Michele Francoeur
Richard Frisque
Marie George
Mitchell Goldfarb
Rasika Harshey
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Theodore Kwoh
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Kenji Shimizu
Joseph Sorge
Bruce Stillman
Nigel Stow
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Nikos Vamvakopoulos
Keith Willison
Clifford Yen

GRADUATE STUDENTS

Jane Danska
Douglas Hanahan
Richard Kostriken
Daniel Levy
Pamela Rosman
David Solnick

RESEARCH ASSISTANTS

Cara Adler
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Benjamin Urso
Mark VanBree
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Jeanne Wiggins
Jonathan Wood
Elizabeth Woodruff

Staff as of December 1980

ADMINISTRATIVE AND GENERAL STAFF

Charlaine Apsel
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Guy Cozza
Johanna DiGiovanni
Jeffrey Drum
Susan Gebhardt
Patrick Hickey
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Roberta Salant
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Jacqueline Terrence

SUPPORTING STAFF

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Cynthia Carpenter
Louisa Dalessandro
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Karen Herrmann
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Deborah Lukralle
Theodore Lukralle
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Madlyn Nathanson
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Warren Eddy
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Bruce Fahlbusch
Willie Hall
Douglas Haskett
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Susan Gensel
David Gibson
Laura Hyman
Glynnis Orloff

GENETICS RESEARCH UNIT CARNEGIE INSTITUTION OF WASHINGTON

Alfred Hershey
Barbara McClintock

First row: E. Chow, G. Binns, J. Smart; T. Kelly, J. Leibold, J. Garrels
Second row: T. Gingeras, C. Morita; A. Nickel; R. Blumenthal
Third row: D. Barczik; G. Kist, B. Ward, M. Berejka; J. Bach
Fourth row: T. Broker on Blood Drive Day; Annual staff picnic

First row: P. Reichel, B. Parsons, K. Washington, J. Sabin; J. Terrence, P. Hickey, C. Schneider
Second row: J. Richards; Y. Gluzman, B. Ahrens, H. Reichl, J. Bier, R. Frisque; N. Ford
Third row: J. Wood; R. Salant, S. Jay, J. Drum; F. Otto, C. Mendez; S. Schultz
Fourth row: L. Hyman, E. DeWeerdt, S. Gensel, H. Parsons; D. Owen, A. Kirk, L. Ritcey, M. Cooney

FINANCIAL STATEMENT

BALANCE SHEET
year ended December 31, 1980
with comparative figures for year ended December 31, 1979

ASSETS

	1980	1979
CURRENT FUNDS		
Unrestricted		
Cash	$ 1,343,002	$ 1,041,245
Accounts Receivable	440,985	260,806
Prepaid expenses	82,112	16,339
Inventory of books	90,188	123,202
Due from restricted fund	—	180,485
Due from Banbury Center	128,785	87,939
Total unrestricted	2,085,072	1,710,016
Restricted		
Grants and contracts receivable	2,540,887	2,265,333
Due from unrestricted fund	221,538	—
Total restricted	2,762,425	2,265,333
Total current funds	$ 4,847,497	$ 3,975,349
ENDOWMENT FUNDS		
Robertson Research Fund		
Cash	(24,084)	(233,575)
Marketable securities (quoted market 1980—$10,798,472; 1979—$9,630,113)	9,016,253	8,418,623
Total Robertson Research Fund	8,992,169	8,185,048

LIABILITIES AND FUND BALANCES

	1980	1979
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 327,289	$ 94,971
Mortgage Payable	133,200	133,200
Due to plant fund	808,714	1,001,089
Due to restricted fund	221,538	—
Fund balance	594,331	480,756
Total unrestricted	2,085,072	1,710,016
Restricted		
Due to unrestricted fund	—	180,485
Fund balance	2,762,425	2,084,848
Total restricted	2,762,425	2,265,333
Total current funds	$ 4,847,497	$ 3,975,349
ENDOWMENT FUNDS		

Olney Memorial Fund					
Cash	517	828			
Marketable Securities (quoted market 1979—$19,341; 1980—$20,332)	27,538	26,582			
Total Olney Memorial Fund	28,055	27,410			
Total endowment funds	$ 9,020,224	$ 8,212,458	Fund balance	$ 9,020,224	$ 8,212,458
PLANT FUNDS			**PLANT FUNDS**		
Investments	245,665	230,725			
Due from unrestricted fund	808,714	1,001,089			
Land and improvements	966,110	960,530			
Buildings	6,006,958	5,735,358			
Furniture, fixtures and equipment	1,255,429	1,103,409			
Books and periodicals	365,630	365,630			
Construction in progress	371,787	88,630			
	10,020,293	9,485,371			
Less allowance for depreciation and amortization	2,388,005	1,951,625			
Total plant funds	$7,632,288	$7,533,746	Fund balance	$ 7,632,288	$ 7,533,746
BANBURY CENTER			**BANBURY CENTER**		
Current funds			**Current funds**		
Cash	$ 600	600	Accounts payable	$ 57,733	$ —
Accounts receivable	26,293	95	Due to CSHL unrestricted fund	128,785	$ 87,939
			Fund balance	(159,625)	(87,244)
Total current funds	26,893	695	**Total current funds**	26,893	695
Endowment Funds			**Endowment funds**		
Robertson Maintenance Fund					
Cash	(16,314)	(62,591)			
Marketable securities (quoted market 1980—$1,879,191; 1979—$1,726,973)	1,630,397	1,586,667			
Total endowment funds	1,614,083	1,524,076	**Endowment funds balance**	1,614,083	1,524,076

Plant funds		
Land	772,500	772,500
Buildings	412,672	412,672
Furniture, fixtures and equipment	176,285	164,416
Construction in progress	368,149	49,510
	1,729,606	1,399,098
Less allowance for depreciation	173.596	129,340
Total plant funds	1,556,010	1,269,758
Total Banbury Center	$ 3,196,986	$ 2,794,529
Total—All funds	$24,696.995	$22,516,082

Plant funds		
Plant funds balance	1,556,010	1,269,758
Total Banbury Center	$ 3,196,986	$ 2,794,529
Total—All funds	$24,696,995	$22,516,082

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1980
with comparative figures for year ended December 31, 1979

COLD SPRING HARBOR LABORATORY

	1980	1979
REVENUES		
Grants and contracts	$ 4,066,773	$ 3,269,582
Indirect cost allowances on grants and contracts	1,910,098	1,499,040
Contributions		
Unrestricted	74,334	81,624
Restricted and capital	40,587	774,264
Long Island Biological Association	120,000	6,753
Robertson Research Fund Distribution	375,000	350,000
Summer programs	287,634	288,380
Laboratory rental	20,732	20,732
Marina rental	43,370	39,900
Investment income	181,050	102,999
Publications sales	896,245	837,763
Dining hall	327,328	319,628
Rooms and apartments	215,686	207,061
Other sources	97	20,019
Total revenues	8,558,934	7,817,745
EXPENSES		
Research*	3,782,095	3,002,771
Summer programs*	631,402	633,270
Library	153,052	129,752
Operation and maintenance of plant	1,137,772	1,011,822
General and administrative	910,581	716,487
Depreciation	430,800	405,180
Publications sales*	656,142	510,805
Dining hall*	406,292	324,093
Total expenses	8,108,136	$ 6,734,180

BANBURY CENTER

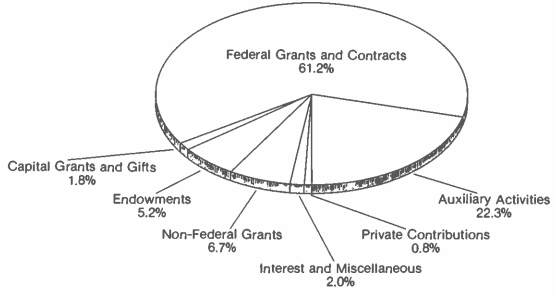
	1980	1979
REVENUES		
Endowment income	$ 80,000	$ 82,604
Grants & contributions	87,800	123,000
Rooms and apartments	23,027	16,890
Publications	176,575	40,768
Transfer from Cold Spring Harbor Laboratory	378,345	40,655
Total revenues	745,747	303,917
EXPENSES		
Conferences	49,182	48,678
Publications	204,210	76,208
Operation and maintenance of plant	99,564	89,777
Program administration	152,708	132,522
Depreciation	43,200	40,656
Capital plant	330,500	44,371
Total expenditures	879,364	432,212
Excess (deficit) of revenues over expenses	$ (133,617)	$ (128,295)

TRANSFERS		
Capital building projects	875,673	430,607
Banbury Center	378,345	40,655
Total transfers	1,254,018	471,262
Total expenses and transfers	9,362,154	7,205,442
Excess (deficit) of revenues over expenses and transfers	$(803,220)**	$ 612,303

*Reported exclusive of an allocation for operation and maintenance of plant, general and administrative, library, and depreciation expenses.
**1980 deficit primarily caused by conversion of current funds and 1979 revenue excess to plant funds for capital building projects.

Note: Copies of our complete, audited financial statements, certified by our independent auditors, Peat, Marwick, Mitchell & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
Year End December 31, 1980



GRANTS

January 1–December 31, 1980

NEW GRANTS

COLD SPRING HARBOR LABORATORY

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
National Institutes of Health	Dr. Bukhari—research	$1,661,439	6/1/80–5/31/83
	Dr. Burridge—research	131,039	4/1/80–3/31/81
	Dr. Feramisco—research	214,758	7/1/80–6/30/83
	Dr. Gingeras/ Dr. Roberts—research	408,085	3/1/80–2/28/83
	Dr. Goldfarb—fellowship	59,244	9/1/80–8/31/83
	Dr. Hicks—advanced bacterial genetics course	97,081	4/1/80–3/31/83
	Dr. Kurtz—research	179,542	4/1/80–3/31/83
	Dr. Mathews—research	266,570	4/1/80–3/31/83
	Dr. Topp—research	303,452	8/1/80–6/30/83
	Dr. So—research	124,370	9/30/80–8/31/82
	Mr. Udry—1980 protein phosphorylation meeting	10,000	
National Science Foundation	Dr. Garrels—research	140,000	9/1/80–8/31/82
	Dr. Hicks—1980 plant workshop	7,373	2/15/80–1/31/81
	Dr. Roberts—research	345,000	8/15/80–7/31/83
	Mr. Udry—1980 symposium support	6,000	5/1/80–4/30/81
	Dr. D. Zipser—research	157,000	5/15/80–4/30/83
Rita Allen Foundation	Dr. Watson—1980 pain workshop	12,500	
American Cancer Society	Dr. Watson—1980 cell culture course	21,120	4/1/80–7/31/81
	Dr. Mathews—research	13,841	1/1/80–3/31/80
Bethesda Research Laboratory	1980 symposium	5,000	
Cetus Corporation	1980 symposium	5,000	
	1980 mammalian vectors meeting	3,000	
Collaborative Research Corp.	1980 mammalian vectors meeting	1,000	
COGENE	Dr. Bukhari—COGENE-India training course	10,000	
Cystic Fibrosis	Dr. Garrels—research	87,230	7/1/80–6/30/82
Department of Energy	Mr. Udry—1980 symposium support	9,000	
	Dr. Hicks—1980 plant workshop	4,000	
E. I. duPont de Nemours & Co.	1981 pain workshop	1,000	
	1980 mammalian vectors meeting	1,500	

NEW GRANTS

COLD SPRING HARBOR LABORATORY

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
Genentech, Inc.	1980 mammalian vectors meeting	3,000	
Grass Foundation	Dr. B. Zipser—neurobiology scholarships	14,000	1980–1981
Lilly Research Laboratories	1980 mammalian vectors meeting	3,000	
Molecular Genetics, Inc.	1980 mammalian vectors meeting	1,500	
Monsanto	1980 mammalian vectors meeting	1,000	
Muscular Dystrophy Assoc.	Dr. Blose—research	19,000	1/1/80–12/31/80
	Dr. Garrels—research	20,000	1/1/80–12/31/80
	Dr. Lin—research	29,750	1/1/81–12/31/81
	Dr. B. Zipser—1980 synapse course	4,000	
	Dr. B. Zipser—1980 neural development course	4,000	
New England Biolabs, Inc.	Dr. Roberts—research	5,000	
	1980 mammalian vectors meeting	3,000	
Upjohn	1980 pain workshop	500	
Volkswagen Foundation	Dr. Watson—neurobiology scholarships	236,088	1980–1982
Whitehall Foundation	Dr. B. Zipser—research	135,000	10/1/80–9/30/83
Helen Hay Whitney Foundation	Dr. Frisque-fellowship	29,000	9/1/80–8/31/82
Damon Runyon-Walter Winchell Cancer Fund	Dr. B. Anderson—fellowship	35,000	10/1/80–9/30/82
	Dr. Goldfarb—fellowship	35,000	10/1/80–9/30/82

BANBURY CENTER

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
National Institutes of Health	purchase of Banbury reports	$82,125	8/80
Alfred P. Sloan Foundation	public information workshops	100,000	1/1/80–12/31/82

CONTINUING GRANTS

COLD SPRING HARBOR LABORATORY

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
National Institutes of Health	Dr. Watson—cancer research center	$13,500,000	1/1/77–12/31/81
	Dr. Watson—general research support	173,612	4/1/76–3/31/81
	Dr. Watson—summer workshops	470,340	4/1/77–3/31/82
	Dr. Watson—symposium support	187,433	4/1/79–3/31/84
	Dr. Watson—neurobiology course support	258,500	6/1/79–3/31/82
	Dr. Watson—herpesvirus meeting	42,280	8/1/79–6/30/81
	Dr. Watson—viruses in naturally occurring cancers meeting	30,000	8/14/79–1/15/80
	Mr. Udry—cancer research facility	1,411,011	9/15/77–indefinite
	Dr. Albrecht-Buehler—research	73,612	9/1/78–8/31/80
	Dr. Blose—research	167,702	12/1/78–11/30/81
	Dr. Bukhari—career development	148,961	5/1/75–4/30/80
	Dr. Bukhari—research	121,351	9/9/77–8/31/80
	Dr. Chaconas—fellowship	19,040	8/1/79–7/31/81
	Dr. Chow—research	238,186	4/1/77–3/31/80
	Dr. Garrels—research	323,073	4/1/79–3/31/82
	Dr. Hicks—research	384,703	7/1/78–6/30/81
	Dr. Klar—research	253,000	7/1/78–6/30/81
	Dr. Sciaky—fellowship	13,800	3/1/79–2/28/80
	Dr. Stringer—fellowship	29,890	2/1/79–1/31/82
	Dr. Topp—research	275,806	1/1/79–12/31/81
	Dr. D. Zipser—fellowship training	852,578	7/1/78–6/30/83
National Science Foundation	Dr. Bukhari—research	330,000	6/15/79–5/30/82
	Dr. Garrels—research and equipment	192,484	7/1/78–6/30/80
	Dr. Hicks—equipment	24,000	8/15/78–1/31/80
	Dr. Roberts—research	210,000	12/15/79–11/30/82
	Dr. Zain—research	65,000	8/15/77–1/31/80
	Dr. B. Zipser—neurobiology course support	60,000	7/1/79–12/31/82
	Dr. B. Zipser—research	120,000	11/1/78–10/31/81
	Dr. D. Zipser—research	136,000	2/15/78–1/31/81
Rita Allen Foundation	Dr. Lewis—research	120,000	8/1/76–7/31/80
American Cancer Society	Dr. Lewis—research	62,500	7/1/79–6/30/80
	Dr. Stringer—fellowship	13,000	1/1/79–1/31/80
American Heart Association	Dr. Blose—resarch	8,570	7/1/79–6/30/80
	Dr. Garrels—research	8,570	7/1/79–6/30/80
Canadian Medical Research Council	Dr. Chaconas—fellowship	24,000	8/1/78–7/31/80

CONTINUING GRANTS

COLD SPRING HARBOR LABORATORY

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
The Camille and Henry Dreyfus Foundation	Dr. Watson—undergraduate research participation	46,000	12/1/78–1/1/81
Institute of Molecular Biology Division of Bethesda Research Laboratory	Dr. Watson—research	7,500	2/1/79–1/31/80
Esther A. and Joseph Klingenstein Fund	Dr. Watson—neurobiology course support	60,000	5/1/79–4/30/82
Leukemia Society of America	Dr. Engler—fellowship	25,000	11/1/78–10/31/80
Muscular Dystrophy Association	Dr. Burridge—research	25,142	7/1/79–6/30/80
	Dr. Feramisco—fellowship	32,000	1/1/79–12/31/80
	Dr. E. Lane—fellowship	25,000	1/1/79–7/1/80
Alfred P. Sloan Foundation	Dr. D. Zipser—computer workshops	31,000	6/1/79–12/31/81
Whitehall Foundation	Dr. Roberts—research	34,500	4/1/79–3/31/80
Helen Hay Whitney Foundation	Dr. Drickamer—fellowship	24,125	9/1/79–5/31/81
Damon Runyon–Walter Winchell Cancer Fund	Dr. Hu—fellowship	26,070	7/1/78–6/30/80
	Dr. Lin—fellowship	28,000	7/1/79–6/30/81
	Dr. Stillman—fellowship	28,000	4/1/79–3/31/81
	Dr. Stow—fellowship	30,000	10/1/78–9/30/80

BANBURY CENTER

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
Esther A. and Joseph Klingenstein Fund	program support	$100,000	3/1/78–2/29/80
Alfred P. Sloan Foundation	program support	100,000	8/1/78–7/31/80

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of the Cold Spring Harbor Laboratory

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Ortho Pharmaceutical Corporation
Smith Kline & French Laboratories

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FINANCIAL SUPPORT OF THE LABORATORY

The Cold Spring Harbor Laboratory is a publicly supported educational institution chartered by the University of the State of New York and may receive contributions which are tax exempt under the provisions of the Internal Revenue Code, particularly Section 501C. In addition, the Laboratory has been formally designated a "public charity" by the Internal Revenue Service. Accordingly, it is an acceptable recipient of funds which would result from the termination of "private" foundations.

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

Methods of Contributing to Cold Spring Harbor Laboratory

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities

You can generally deduct the full amount of the gift on your income tax return, if the appreciation would have been a long-term gain, to the extent of 50% of your adjusted gross income and you need pay no capital gains tax on the stock's appreciation.

We recommend either of the following methods:

(1) Deliver the stock certificates to your broker with instructions to him to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.
(2) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, New York 11724. In a separate envelope send an *executed* stock power.

Bequests

Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

Appreciated real estate or personal property

Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Conversion of private foundation to "public" status on termination

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

For additional information, please contact the Administrative Director, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8300.

THE LONG ISLAND BIOLOGICAL ASSOCIATION

THE LONG ISLAND BIOLOGICAL ASSOCIATION (LIBA) and Its Relation to the Cold Spring Harbor Laboratory

Biological research at Cold Spring Harbor began in 1890 when the Brooklyn Institute of Arts and Sciences was looking for a place to set up a summer laboratory as the headquarters of its Department of Zoology. Representatives of the Institute were invited by Eugene S. Blackford, at that time the First Commissioner of the State of New York, to inspect a site on Bungtown Road across Northern Boulevard (25A) from the Fish Hatchery. The site was found to be ideal, and so the original Laboratory was organized with Mr. Blackford as president of the Board of Trustees. The land was leased from Mr. John D. Jones, whose family since 1819 had operated various industries including shipbuilding, coopering, and textile manufacture (later whaling, also) at the head of Cold Spring Harbor. Bungtown Road, which runs through the Lab property, got its name from the factory that specialized in making bungs—or stoppers—for barrels.

In 1892 the Laboratory's land was leased for a dollar a year from the Wawepex Society, which Mr. Jones had organized as a corporation for holding real estate and for investing funds for the propagation of fishes and for scientific research. In 1904 the Wawepex Society leased additional land to the Carnegie Institution of Washington, which wanted to locate a Department of Experimental Evolution in the Cold Spring Harbor area. Charles B. Davenport, who had been directing the Laboratory since 1896, assumed the additional duties of director of the Carnegie Institution's experimental station. It is interesting to note that Mr. Davenport lived in what came to be known later as the Carnegie Dormitory, the Victorian house on 25A built by John D. Jones before the turn of the century, and recently repainted in its original colors.

The Long Island Biological Association was established in 1924 when the Brooklyn Institute decided to discontinue its research at Cold Spring Harbor and offered its laboratory to two universities. Fortunately, a local group of interested neighbors decided to assume responsibility for the Lab, and thus LIBA came into being. For 38 years LIBA actually operated the Laboratory in conjunction with the Carnegie Institution, but in 1962 it seemed advisable for the Laboratory to be reorganized as an independent unit. Therefore, the property on which it now stands was conveyed to it by LIBA, which, however, still retains reversionary rights. Today LIBA is one of thirteen institutions participating in the support of the Laboratory, each institution being represented on the Laboratory's Board of Trustees.

What has happened, in effect, is that LIBA has become an expanding group of local "Friends of the Laboratory" who help support it through annual contributions. Also, from time to time, the Association undertakes campaigns to finance special important projects for which the Lab cannot obtain funds from the Federal Government or from other sources. For instance, in 1974, LIBA made possible building the James Laboratory Annex and the renovation of Blackford Hall; and in 1976 the re-building of Williams House.

The affairs of LIBA are handled by a board of 28 directors who are elected to office by the membership at an annual meeting. At least twice a year LIBA members are invited to bring their friends to a lecture or an open house at the Lab.

Membership in LIBA requires a minimum annual contribution (tax deductible) of $25 for a husband and wife, $15 for a single adult, $5 for a junior member (under 21). Further information can be obtained from the Long Island Biological Association, Box 100, Cold Spring Harbor, N.Y. 11724, or by telephoning the Laboratory's administrative director, Mr. William R. Udry, at (516) 367-8300.

OFFICERS

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REPORT OF THE CHAIRMAN FOR 1980

LIBA's most important activity in 1980 was completing the fund of $200,000 which its members had undertaken to raise for the purchase of twenty acres of land owned, but no longer needed, by the Carnegie Institution of Washington. The ownership of this land by the Lab is essential for rounding out and preserving its property.

The custom of giving dinner parties for the visiting scientists at the Lab's summer Symposium was successfully continued and greatly appreciated. On June 1st eighteen of our directors and members gave parties for over a hundred guests. This year's hosts and hostesses were:

Mr. & Mrs. Charles O. Ames
Mr. & Mrs. Edmund Bartlett
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Mr. & Mrs. Theodore Wickersham

There were two LIBA meetings open to the whole membership. At the first, the Dorcas Cummings Memorial Lecture, on May 18th, the speaker was Dr. Stephen Jay Gould, professor of Geology and curator of Invertebrate Paleontology at Harvard University. Dr. Gould's lecture was on "The Meaning of the Darwin Revolution;" in it he discussed his fascinating theories about the evolutionary process in Nature. The annual meeting, usually held in December, was postponed until January 18th, 1981 as the speaker we eagerly wanted, Dr. Lewis Thomas, head of Sloan-Kettering Cancer Research Center, was not available at an earlier date. Dr. Thomas spoke brilliantly on "The Value of Basic Scientific Research," stressing its need for wide support and explaining how vital it is that it remain *basic* in spite of the current tendency to commercialize its discoveries. A copy of Dr. Thomas' lecture was distributed to all LIBA members.

On October 26th, LIBA directors and their guests were addressed by Dr. Birgit Zipser, one of the young members of the Lab's scientific staff. Her subject, which intrigued her audience greatly, was "The Application of Hybridoma Technology to a Study of the Molecular Mechanism of Neural Connectivity Essential for the Functioning of the Nervous System." She and her associate, Dr. Ron McKay, have been creating hybridomas which produce monoclonal antibodies that are very specific in binding (or labeling) individual proteins

Stephen Jay Gould and James D. Watson

in nerve cells. A hybridoma is made by fusing a cancer cell (myeloma) with a normal antibody-producing cell. Since the presence or absence of certain proteins indicates what's happening in a nerve cell, by "reading" these labels they can tell, for example, if a particular nerve cell is passing on a message. So their main experiment has been to take a leech, which has relatively few but large nerve cells, and stain the cells with monoclonal antibodies that they had developed for very specific nerve proteins which would be present when the nerve cell was excited, or transmitting. Since they used a fluorescent dye, the cells and their connections lit up most graphically. They are now trying to answer the question: Do these antibodies stain groups of cells that are related functionally when the brain is working? In other words, can they light up the whole circuit of nerves that receive, transmit a stimulus to the brain and back again for a response? A positive answer would, of course, be an incredible tool for studying (and treating) many nervous system problems, including pain itself.

After the lecture a buffet dinner was served to the directors and their guests and to the Lab's scientists in Blackford Hall.

At the annual meeting of members all previous directors were re-elected with the exception of Mr. Richard Olney, 3rd, whose resignation was regretfully accepted because of his moving to Massachusetts. His place was filled by the election of Mrs. Henry Upham Harris, Jr. At the subsequent director's meeting all previous officers were re-elected with the exception of Mr. Walter H. Page, who felt it necessary to devote himself fully to his new responsibilities as chairman of the Laboratory's Board of Trustees. A resolution of regret and appreciation for his long and valuable service to LIBA was adopted. Then Mr. George J. Hossfeld, Jr. was elected to take his place as Vice-Chairman.

I am happy to report that the LIBA membership continues to expand and that the interest of the community in the Lab and its support of its vital research are both increasing.

February 15, 1981 *Edward Pulling*, Chairman
Long Island Biological Association

Lewis Thomas

Birgit Zipser

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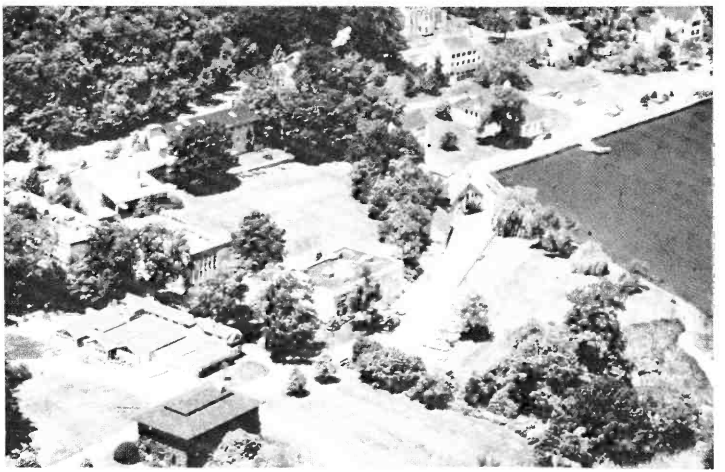

Cold Spring Harbor Laboratory, partial view