

ANNUAL REPORT 1979



COLD SPRING HARBOR LABORATORY

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COLD SPRING HARBOR, NEW YORK

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COLD SPRING HARBOR LABORATORY

COLD SPRING HARBOR, LONG ISLAND, NEW YORK

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DIRECTOR'S REPORT

The stereotype of the eccentric scientist, indifferent to the world about him and motivated only toward the intellectual advancement of his own field of research, is convenient to perpetuate. For the academic, it provides the perfect out from the more than occasional necessity of appearing concerned with the many pressing problems of the imperfect societies in which we all live. A monomania of one form or another is a prerequisite for most intellectual achievements, and trying to do too much all too often is a recipe for future lack of distinction. And even if we are no longer passionate about our intellectual careers, we tend to be reluctant to announce publicly that we are ready to help our neighbors. This would imply that we frequently have ideas that have social consequences. We need not be clever to realize that the appearance of goodness is far from correlated with the actual accomplishment of good works. Far better to maintain the illusion that we always have esoteric ideas in mind.

This way of proceeding is also not without its advantages to our politicians, the ultimate source of almost all money for science. They need to be seen as practical leaders and do not want to be unfairly tagged as unworldly by too much association with minds who cannot be counted on for reliably consistent answers. As the leaders of our countries, they have to devote almost all of their waking moments to today's decisions, leaving to their dreams the long-term future. They know as administrators that the problems of the immediate moment cannot be pushed aside in favor of schemes that perhaps should never have been generated. Most untested new ideas never go anywhere, and old remedies, imperfect as they may be, have that marvelous quality of being apparently predictable. Inherently, none of us want to live with too much uncertainty, yet we as research types seem often to push for new approaches merely because we are bored with the past.

Most new ideas of intellectuals have to be seen for what they are, academic exercises usually put forward without the thought that they might be taken seriously by the outside world. Without the fear of visible failure, the mind meanders faster and further, sometimes toward more practical directions than initially perceived. This is the way it should be. If we believe that our futures

as viable societies demand infusions of the really new, it makes sense, for the most part, to separate the generation of new ideas from the job slots where the consequences of failure are too clearly seen. This is why universities and institutes of pure research must exist as partial oases from the real world. Only in somewhat unhurried atmospheres will we take the time to assimilate the key features of other cultures or to scrutinize carefully the tenets of the moment for their viability over the foreseeable future.

There will thus, of necessity, always be a sense of quiet unease in an academic-type environment. The desire of the students and younger staff to reexamine, if not overturn, the inadequate ideas of the past will often be in conflict with the purposes of the more senior members, who themselves, a generation before, may have come to power through their ability to think differently. A major factor determining the quality of a given institution is thus the ability of its faculty to reward intellectual success even when it leads to the effective academic redundancy of many of its older members.

Happily, the tradition of rewarding intellectual novelty now dominates major universities in every society—that is as long as the ideas promoted are not thought to affect the political (social) stability of the society concerned. Here we should not delude ourselves that these exceptions are all that rare. No one, for example, can imagine a promoter of Marxist economics becoming a professor in today's Chile. And even at Harvard, Marxist economists never seem to make it.

For the most part, however, natural science has been allowed to evolve on its own terms. No society that wishes to advance itself in the modern world can reject scientific progress, and we now find, virtually independent of the political system, that the content of science is left to its practitioners and not imposed from above. There is, for example, no capitalistic form of molecular biology. No matter in which country we look, molecular biologists are apt to have the same common objectives. To be sure, there have been serious efforts to turn the clock back, as witnessed by the debasements of Russian biology when Stalin decided that Mendelian genetics posed a threat to communist doctrine as to why different individuals have different potentials for future

success—or the much less successful attempt by the Nazis to label relativity research as Jewish science—and the rear-guard action by fundamentalist American religious groups to prevent the teaching of Darwin's evolutionary theories.

All in all, however, the more meaningful fact is the paucity of such restrictive political actions during the development of modern science. The efforts of the Catholic Church to muffle Galileo in the 16th century fortunately have not had too many subsequent parallels. Since then, the growing, clear correlation between the existence of a vigorous free science and the generation of technological advances has led more and more nations to organize their support of science in ways that maximize their scientists' control over their own destiny.

Here we cannot overemphasize too much the necessity for the promotion of pure science. Through it are generated the unexpected new facts that later can lead to applied developments like high-speed computers and the drugs that control our fertility. The outcomes of untried scientific experiments are remarkably unpredictable, and we are constantly amazed by how different nature is from what our experts of the past have prophesized. And despite more prognostication that the end of new knowledge is in sight, there is general agreement that there has been no real slowing down of the rate at which important new scientific concepts are established. Most, of course, have no immediate practical consequence. But when one does, say in the development of semiconductors by the solid-state physicists, the payoff may be large indeed.

Although for a short time a growing society may try to live off the new facts produced by others, this approach appears not to be a recipe for long-term worldwide leadership. Witness that the United States only became a major world power when it was the undisputed leader of world science, and that the long-held conception in the United States and Europe that the Japanese get ahead by copying the West is almost certainly false, reflecting more our inability to speak their language than any critical evaluation of the way they educate their students and encourage the development of new ways of thinking.

What almost seems inescapable is that the deeper we understand the ways of nature, the more capable we are of using it for human benefit. Over and over, in retrospect we see that we never had a realistic chance to solve an applied problem until our scientific base was raised. Only, for example, by discovering that there were three immunologically distinct forms of poliovirus could we move beyond the inadequate iron lungs and truly conquer polio.

We must also realize that pure scientists, almost without exception, have no reluctance to exploit their observations for practical use. The fact that most of us do not get involved in practical matters is not a matter of

principle, but usually reflects the perception, sometimes mistaken, that we do not have anything unique to offer the outside world. But when we feel that a new observation has much wider application than a place in a scientific journal, we are unlikely long to ignore the fact that we might enhance our careers, if not our fortunes, by moving our thoughts to the outside world. To show this, I can best draw on my experience in molecular biology, a field that until recently was thought to be one of the more esoteric of the major sciences. Two major examples stand out.

The genetic engineering of microorganisms to produce medically useful hormones and drugs. The now over thirty years of intensive research on DNA, the chemical compound that carries the genetic information of our genes, and on the fundamental genetics of bacteria has led recently to the development of new procedures in which bacteria can be used as effective factories to produce large amounts of human proteins that now are difficult to prepare in medically satisfactory amounts (e.g., the growth hormone and, hopefully soon, the infinitely more illusive antiviral compound interferon). Although vague proposals for genetic engineering have been talked about ever since DNA was pinpointed as the genetic material in 1944, this field became technologically possible only following the unexpected isolation in the early 1960s of a new class of enzymes (the restriction enzymes). These catalysts cut DNA molecules at specific sites and so allow the routine test-tube generation of "recombinant DNA" molecules, say partially of human origin and partially of bacterial origin. As first shown in 1963 by Herbert Boyer and Stanley Cohen, these hybrid molecules can then be put into receptive bacteria, where they multiply as functional minichromosomes and lead to the production of not only proteins of bacterial origin, but also those corresponding to the specific human genes from which they have been constructed. Already three human hormones (insulin, somatostatin, and pituitary growth) have been synthesized by these procedures, and over the next several years we should witness an explosion of such examples, many with great commercial significance. This is not the place, however, to give the technical details of these procedures. Much more interesting are the facts that:

1. There is no way these procedures could have been worked out by applied scientists without prior intense development of pure molecular genetics and bacterial genetics.
2. The first successful recombinant DNA experiments were carried out at two major academic sites (University of California Medical School at San Francisco and Stanford University Medical School) by scientists whose careers until then had been in pure science.
3. The first use of these procedures was to further our knowledge of pure science, and already several major advances in understanding the structure and function of

the genes of higher organisms have occurred that would have been impossible without recombinant DNA.

4. The initial developers of the recombinant DNA methodologies realized from the start that there could be major commercial applications and in due course became consultants and stockholders in several of the new companies that were formed to exploit the technology. They also saw to it that patents were taken out by their respective universities to protect their rights, as well as their universities, in the development.

5. No major pharmaceutical company moved quickly into this work, in part because, on the whole, they had seen no need previously to emphasize applied developments in either bacterial genetics or nucleic acid enzymology. So they didn't possess strong internal lobbies to push the advantages of recombinant DNA. They were also afraid to be labeled as genetic engineers, knowing that the public badly confused work with bacteria with actual experimentation on humans. With the now much diminished public concern about recombinant DNA, however, we may expect the pharmaceutical giants to try to catch up, often by buying up or into some of the newly formed recombinant DNA companies.

Growing industrial exploitation of monoclonal antibodies. Until several years ago, antibodies either for vaccine production or for medical testing or scientific research were made by injecting the appropriate antigens (e.g., diphtheria toxin) into suitable laboratory animals (usually rabbits and guinea pigs, and when large vaccine amounts were needed, sheep and horses). All such resulting antisera are heterogeneous—containing antibodies of varying degrees of strength directed against different chemical groups on the surfaces of the respective antigens. Long desired, but not apparently easy, was a way to make preparations of identically strong antibodies all directed against a single antigenic group. In the 1960s this goal began to look increasingly distant as we began to learn some of the basic facts about the cellular basis of the immunological response. In particular, the immunological response of a single animal usually involves the selective synthesis of large numbers of different lymphocytes, each of which makes its own unique type of antibody. Hence the heterogeneous nature of all available antisera.

It thus became obvious that the only way to produce single-specificity antibodies was somehow to find the conditions under which single-antibody-producing cells could grow in culture—like bacteria. By starting such cultures with single lymphocytes, all the resulting antibodies would be made by genetically identical lymphocytes, and so possess identical antigen-combining sites. All such efforts invariably failed, however, telling us that normal antibody-producing cells lack the ability to multiply indefinitely. This is a great contrast to the behavior of the abnormal antibody-producing cells that generate the cancers we call myelomas. Such cells are

ineffectively immortal and multiply without stop in culture, a condition no doubt related to their cancerous state. Their existence, however, gave to George Kohler and Caesar Milstein, working in Cambridge, England, the idea that if they fused mouse myeloma cells with normal antibody-producing cells from the spleen of a mouse that had been immunized against sheep red blood cells, the resulting hybrid cells might continue to produce the desired antibody against sheep red cells, as well as become immortal. Five years ago, the first such successful experiments were done, giving rise to clones of mouse cells that provide antibodies of desired single specificity (monoclonal antibodies). Since then, monoclonal antibodies have been made against large numbers of different antigens, and this procedure, because it leads to highly reproducible antibodies of high specificity, may soon supplement all current commercial ways of making antibodies. Here it is relevant to emphasize that:

1. This technique could not have been developed without the past two decades of solid advances in understanding the cellular basis of the immune response. Equally necessary was the finding in the mid-1960s by the English cell biologists Harris and Watkins of routine ways for fusing cells.

2. All the key steps were done by pure scientists, this time working in a laboratory for pure molecular biology, supported by the British Medical Research Council.

3. The most important early uses have been in the area of pure immunobiology and cell biology, giving to these fields new armadas of specific antibodies directed against biologically important molecules on cell surfaces.

4. The idea seemed so simple that no thought was given to patent protection until it was too late. Nonetheless, the extraordinary power of the monoclonal antibody procedure has quickly led to multiple commercial uses. As with recombinant DNA, several companies have quickly been formed to exploit it. The fact, however, that most major pharmaceutical houses have major in-house immunological strength has quickly led to their moving to improve their vaccine (medical technology) capabilities through the monoclonal procedures.

Both of the examples cited here are textbook cases of where an advancement in pure science led inescapably to the public good through the production of products not previously effectively available. What is novel here is that molecular biology, prior to these discoveries, was regarded as one of the purest of the sciences. Until a year or so ago, almost none of our students ever thought about jobs in industry, nor were my peers boosting their incomes by being asked for their advice by industry. Now our lives are very different, and many of the peers for whom I have most regard as scientists are shuttling back and forth from their industrial backers. And, of course, they are no longer saying that the doers of

applied sciences are no better than mercenaries for the rich.

I therefore conclude that the main factor that keeps academic thinkers aloof from the real world is not a matter of principle, but the lack of common interest, with most encounters likely to make both sides somewhat edgy. Whereas we as intellectuals have ideas as our chief currency, those in industry or business or agriculture must focus on the economic production of the worldly goods without which our societies would quickly crumble. Our short-term roles are thus so different that very little good is likely to come through too frequent intermixing.

On the other hand, when we do have relevant answers, then we are not the impractical souls of common perception and can make ourselves effectively heard. Our physicists, for example, have never hesitated to enter into inherently political battles about nuclear weapons and how they may be controlled. They know they will rise or fall along with the rest, and their appearance of absentmindedness must necessarily be dropped when they are needed.

At the same time, we must never forget what we can do best. In our searching for ways to understand better the world around us, we are a vital, if not the vital, key for ensuring that the various civilizations of men can long prevail over the inherent chaos of the physical world about us. We thus have as much to lose from false humility as from elitist arrogance. In working toward the advancement of our civilization, we must never forget that ideas are not only beautiful, but necessary.

Highlights of the Year

The Yeast Group confirms its cassette model for the yeast mating-type genes

Almost four years ago, I received a letter from Ira Herskowitz stating that two of his students at the University of Oregon, Jim Hicks and Jeff Strathern, would profit by continuing their collaboration at Cold Spring Harbor and that we should consider the possibility of letting them work year-round in Davenport lab on the genetics of yeast. Its space then was largely unused, except during the teaching of our summer courses on genetics. Exciting to Ira then was the cassette model that Jim, Jeff, and he had devised for mating-type interconversion. This idea opened up a completely new way to think about the control of gene action in yeast, if not in all eukaryotic cells. Purely genetic considerations led initially to the cassette proposal, and it could best be confirmed by recombinant DNA procedures. Why not here at Cold Spring Harbor, provided we continued our habit of giving labs to younger scientists and encouraging them to work at maximal speed?

Happily, Ira's advice was quickly seized upon, and Jeff arrived in the fall of 1977, first working in Ray

Gesteland's lab until he moved to Davenport lab following Jim Hicks's arrival six months later from Jerry Fink's Cornell lab. There Jim had spent two postdoctoral years helping to work out the yeast transformation system. With Amar Klar's arrival from Berkeley, and with Jim Broach also moving from Ray Gesteland's lab in Demerec, Davenport was ready for the big push to clone the mating-type genes to see whether the "silent" loci predicted by the cassette model would be found. After only six months effort, the first mating-type gene was cloned in February, and by the summer's end the correctness of the cassette model was demonstrated at the molecular level, not only from work here, but also through similar experiments done in Seattle by Kim Nasmyth, working in Ben Hall's laboratory.

Important as these results are, they should be seen as opening up a field, not finishing it, and a bundle of new experiments are awaiting to be done to try to work out how the cassettes move from one spot to another. So the fact that the Yeast group has almost to abandon research during the summer course period is increasingly awkward, if not downright depressing. This past fall, we thus began talking about how we might put an addition onto Davenport, and brought in our architects, Moore Grover and Harper, to come up with an aesthetically suitable addition. Now we have plans for an ~3000 sq. ft. addition in the form of a small colonial-type building to the south, connected to Davenport on two levels, with much underground space for biochemical instrumentation. We hope to start construction by early June at the latest, with completion scheduled some ten to eleven months later. The summer of 1981 can thus be happily anticipated, knowing it will not force your yeast geneticists into another enforced absence from real experimentation.

Receipt from NIH of a program-project grant to support our Movable Genes effort

Strongly helpful, if not crucial to the success of our efforts in tumor virology, has been the receipt for the past nine years of a very large program-project grant from NCI which covers the salaries and research monies of key staff members who work on our SV40-adenovirus projects. It gives us an ability to think big, both with regard to the problems we choose, as well as the capacity to recruit highly talented younger scientists to replace staff who have moved on to other institutions. The value of a similar backing for our genetic's program has long been obvious. It would help to formalize a long-term commitment to genetics similar to that which existed here between 1904 and 1963 under the auspices of the Carnegie Institute of Washington. With the creation of our Yeast Group and our already strong programs in bacteriophage μ , directed by Ahmad Bukhari and David Zipser, we again felt strong enough

in genetics to try for program-project support. In May, under the title of "Movable Genes," we submitted a heavy packet of material that proposed long-term support for the salaries and much of the research of our key staff whose research centers on those genetic elements that can move from one chromosomal site to another. Happily, this proposal was well received, and in January 1980 the NIGMS Advisory Council approved our proposal with a high priority. We thus have every confidence that Cold Spring Harbor's long tradition of excellence in fundamental genetics will be maintained.

Isolation of the chicken thymidine kinase gene

Among the most powerful tools now available for studying the genes of higher vertebrates is the transfection technique worked out in 1977 by Michael Wigler and Richard Axel at the College of Physicians and Surgeons. Mike's arrival here in December of 1978 to head our Mammalian Cell Genetics section quickly led to a rash of experiments aimed at producing cloning vehicles through which mammalian and avian genes can be moved from one higher cell to another. Already there has been one major achievement, the use of direct selection procedures to isolate the cellular chicken thymidine kinase gene on a bacterial plasmid, followed by its subsequent reintroduction in a functional form into mouse cells. Manuel Perucho and Doug Hanahan played vital roles in pulling off this feat, which should be reproducible for many vertebrate genes. In particular, we shall seek ways of isolating the "cancer-causing cells" in cells transformed by chemical carcinogens.

Use of recombinant DNA technology to understand the integration of tumor viruses

Perhaps the most serious consequence of the harsh regulations adopted for recombinant DNA was the virtual prohibition of experiments in which the genes of tumor viruses were inserted into *E. coli*. Yet, until this technology was developed, there was, for example, no realistic way to isolate specific regions of transformed mouse cells which contained SV40 sequences. So for more than two years, we felt totally frustrated by regulations that at best could be thought of as well-meaning. The first appearance of flexible rules occurred in England, and to there (ICRF) Joe Sambrook and Mike Botchan went in 1978 to do their first cloning experiments on integrated genomes. By February of 1979 the worst American rules were gone, and since then the cloning of viral genomes has been most actively pursued in James lab. Already we know the exact sequence of one integrated SV40 genome, as well as the cloned sequences of viral chromosomes generated when the SV40 genome is placed in a permissive cell and somehow comes out of the host chromosome. As a result,

Botchan and Sambrook now can argue that excision does not use virus-specific excision enzymes, but involves a process involving replication of the integrated viral DNA followed by an "illegitimate" genetic recombination event that releases the integrated sequences.

Our monoclonal-antibody effort takes off

In the fall of 1978, David Lane, a most-experienced immunologist who trained in London under Av Mitchison, came to James lab hoping to make a set of monoclonal antibodies against the SV40 large T antigen. Using highly purified material made by Bob Tjian, this past year he has not only generated a series of hybridomas of the required specificity, but shown that one of the respective monoclonal antibodies reacts not only with the SV40 T antigen, but also with a normal cellular protein of ~80,000 m.w. whose amount increases following cell transformation induced by a broad variety of agents. The implications of this most unanticipated result are still open, with David now suspecting that the normal protein may have a function like that of the large T, thereby paralleling the relationship between the src-gene product of Rous sarcoma virus and the very similar protein found in smaller amounts in uninfected normal cells.

Given one successful major monoclonal effort, we have extended this technique to other antigens and already have particularly encouraging results, with Jim Lin using striated muscle fibers and myoblast cell membranes to generate monoclonal antibodies of many specificities, some of which we have not seen before. And through immunization of a mouse with the nerve cells of the leech, Ron McKay and Birgit Zipser have a number of monoclonal antibodies that react in quite specific ways to the leech nervous system.

Now we wish to increase still further our range of hybridomas, but we are finding that we are badly short of cell-culture facilities specifically set aside for this purpose. So we have started to plan an addition to James lab, to run to the north, which would be devoted exclusively to the generation of the potentially thousands of different hybridomas that are likely to be generated as we immunize mice with the major classes of cells and viruses. The resulting monoclonal antibodies may prove invaluable as radioactive isotopes for the cell biology research of the next several decades.

Our Symposium marks a milestone in tumor virus research

It was easy to decide to hold this year's Symposium on Viral Oncogenes. The tumor virus field is almost unrecognizable from its state in 1974, when we held our first Symposium on this topic. In this interval, much emphasis has shifted from the viruses themselves to the charac-

terization of those viral genes which directly promote the transformed state. In particular, with the recent isolation of the SV40 (polyoma) T antigens and of the Rous *src*-gene product, the mechanisms of transformation could for the first time be seriously discussed at the biochemical level. Now, for example, many of us know more about protein kinases than we ever thought needed.

The Symposium opened with Howard Temin giving a typically eclectic talk that asked whether much human cancer is, in fact, caused by tumor viruses. Ten years ago, his answer of "no" could have badly disturbed many tumor virologists and, if then commonly accepted, would most likely have kept the tumor virus field from moving so rapidly. Now we see that, even assuming that Howard's views are correct, the massive monies spent on tumor viruses has been most well used, giving us insights into cancer that never would have been generated had, say, the Rous sarcoma virus not been so well studied. Giving the summary this year was Phil Sharp, who did a very neat job comparing the oncogenic products of the DNA and RNA tumor viruses.

As with the past several Symposia, the number of papers presented were virtually more than anyone could absorb. Yet reflecting the high quality of this field almost no one was complaining that we had let in people to speak who had nothing to say. As in most previous Symposia, the audience again exceeded by almost two hundred the number of seats in our lecture hall. Only through the use of our closed-circuit TV, most ably supervised this year by Herb Parsons, could we ever handle such a massive overflow. We clearly need to expand our auditorium, but how to do so is still open. We have considered a balcony, but fear it would be just awful, and so, unlike our mood of a year ago, reluctantly realize that probably only a completely new auditorium will reflect the fact that the science that Cold Spring Harbor has long focused upon is now at the center of biology. It may now be almost impossible to hold an interesting Symposium that does not generate an audience of three-hundred and fifty or so.

This being the case, we must somehow generate funds that most certainly must exceed \$750,000, if not a million dollars. Probably only with such a large sum will we have a building suitable for at least the next twenty-five years (the Busch auditorium was built twenty-eight years ago).

Major changes in our scientific staff

Unlike many Labs, a sizable fraction of our postdocs stay on to become members of our staff, and by the time they leave, we have virtually forgotten them as they were upon arrival. They have become so indispensable to our way of life that we cannot really believe that one day, suddenly, they will be gone, and that we shall feel



Mike Botchan

so vacant. This year's staff loss has been particularly noticeable, with Mike Botchan going to a tenured position at Berkeley, Richard Gelinas to the Fred Hutchinson Cancer Center in Seattle, Ashley Dunn to the European Molecular Biology Institute in Heidelberg, Sayeeda Zain to the University of Rochester Medical School, Jim Broach to join the Department of Microbiology at Stony Brook, and Dietmar Kamp and Regine Kahmann to the Max Planck Institute for Molecular Genetics in Munich.

Leaving us at the end of their postdoctoral positions were Edmund Cheng to join Lan Bo Chen at the Sidney Farber Center, Bill Gordon to go into business in California, Marian Harter to take up a faculty position at the College of Medicine and Dentistry of New Jersey, Kajra Khatoun to return to her teaching position at Karachi University, David Lane to take up his teaching position at Imperial College in London, and Daniela Sciaki to join the Biology Department at Brookhaven National Laboratory.

Newly appointed this January to lead our Protein Structure Group is John Smart, who first came here as a visiting scientist from the Imperial Cancer Research Laboratory. Yakov Gluzman, Jeff Strathern, and Jim Garelis have moved into Staff Investigator slots from their postdoctoral positions, with Jim Hicks in turn moving up to a Senior Staff Investigator position. And Stephen Hughes has joined us as a Senior Staff Investigator in James lab following his postdoctoral period in the Bishop-Varmus group in San Francisco.



Clockwise from top left: Alfred D. Hershey; Alan Garen, Rollin Hotchkiss, Magda Hotchkiss; Hershey Building interior view; exterior view; J.D. Watson, Franklin W. Stahl, James D. Ebert, Alfred D. Hershey, Max Delbrück, Salvador E. Luria



We have also successfully offered Senior Staff Investigator positions, to commence in mid-1980, to John Fiddes, a former student of Fred Sanger at Cambridge and now at UCSF with Howard Goodman, and to Fred Heffron and Magdalene Y. H. So, both products of Stanley Falkow's lab at the University of Washington and now, also, at UCSF. All three have recently been involved in very imaginative gene-cloning experiments, and their arrival here should further strengthen our potentiality for high-powered molecular biology.

Promotion to the Senior Scientist position

Last year we created the position of Senior Scientist whose holders have a continuing rolling commitment of five years of salary support. Promotion to this rank is an important event, since it signifies that we have faith that the individual concerned is capable of a sustained, high-level scientific future. Acting at their April meeting, our Board of Trustees approved the well-deserved advancement to senior Scientist of Tom Broker, Louise Chow, and Terri Grodzicker.

The Robertson Research Fund continues to provide necessary flexibility

Funded in 1973 with a most magnificent capital gift from our neighbor, Mr. Charles S. Robertson, the Robertson Research Fund provides, each year, funds for laboratory renovations, key equipment items, and stipends for selected younger staff and postdoctoral fellows. This year it was especially important in allowing renovation of the ground floor of McClintock lab for our expanding Cell Biology Group, as well as providing funds for much of their needed major equipment and supply monies to tide them over until they obtain expanded grant support. We note here that this year, as in the past, it has been the availability of Robertson Funds that has allowed the creation of an entirely new effort by the Laboratory. Besides ensuring the successful expansion within McClintock of our Cell Biology Group, it has been only through Robertson monies that we were able to ensure that Michael Wigler's Mammalian Cell Group could start up so effectively.

Dedication of the Alfred D. Hershey Building

Of the scientists who have worked at Cold Spring Harbor, none surpasses Al Hershey in the elegance of his experimentation or the crisp clarity of his writing. Already Al's research on bacteriophage has generated many honors including the Nobel Prize, which he shared in 1969 with Max Delbrück and Salvador Luria. There was no doubt that, with time, a building here would bear his name. That occasion came formally to pass on August 23, when the Alfred D. Hershey building

was dedicated before a large number of assembled friends and admirers. The Hershey Building results from a most imaginative rebuilding of the greenhouse and potting sheds put up by the Carnegie Institute around 1910 for plant-breeding research. Long abandoned and seemingly destined for total extinction, they have been most cleverly reprogrammed into offices for scientists working in Demerec laboratory, as well as space for new machine and electronic shops, equipment repair, our safety office, and receiving. The architects Moore Grover and Harper of Essex, Connecticut, are to be congratulated for combining style with simplicity, and Jack Richards is again to be commended for overseeing the not at all trivial construction process.

Among the many guests at the dedication ceremony were Al's long-time close friends, Max Delbrück and Salvador Luria, who joined Jim Ebert of the Carnegie Institute of Washington and Frank Stahl of the University of Oregon to give short speeches in honor of Al, who graciously rose to the occasion by responding that he now knows what Hershey heaven must be like. Following a dinner on the lawn of the newly repainted Carnegie Dorm, a square dance was held, bringing back my fond memories of similar such dances conducted by Carlton McDowell for over thirty years on the same green summer lawn. To mark the occasion, a very handsome booklet summarizing Al's career, together with appropriate pictures from the past and present, was put together by Tom Broker.

The Carnegie Dorm is now again in its original colors

The handsome Victorian house on the corner of Bungtown Road and 25A was built by our founder, Mr. John D. Jones, around 1875-1880 on the site of the ancestral house where he was raised and which burned down in 1862. As far as we can tell, Mr. Jones, the most prominent member of the long-illustrious Cold Spring Harbor Jones family, never lived in his newly built



Carnegie Dormitory

house, living in another family house in Massapequa when he was not in New York as President of the Atlantic Mutual Insurance Company, the largest marine insurers that then existed in the United States. Early in its existence, it was used by Mr. Fred Mather, the first superintendent of the Fish Hatchery that Mr. Jones had established around 1884. When Mr. Jones died in 1893, his nephew, Townsend Jones, took up residence, staying there until 1904 when the Carnegie Institute's Department of Experimental Evolution (later to be known as the Department of Genetics) was established. Then its Director, Charles Davenport, moved in to stay until 1932 when he retired as Director. It then served as a residence for unmarried staff of the Department of Genetics, and in this role became known as the Carnegie Dorm, a designation that still holds today.

With time, its physical state, both outside and inside, gradually declined, with its large size precluding the possibility that any small monetary input would delay the decay. Last summer, knowing that we had to act firmly or totally lose what may well be one of the finest Victorian houses on Long Island, we did extensive carpentry work on its complicated exterior, put on a new roof, and repainted it in its original colors. Helping us here was my wife, Liz, and Frank Matero, an expert in paint analysis, who worked on Theodore Roosevelt's home, Sagamore Hill. At first there were many outcries that any color other than white was inappropriate for our community, but as the painting came to a close, we have found that most neighbors like what we have done, and there is increasing enthusiasm for more such examples of historic preservation. To help cover some of the additional costs involved in the application of the six original colors, a special paint fund was created, to which myself, Edward Pulling, Mary Lindsay, Betty Schneider, and Norris Darrell made donations.

Now we have started work on the interior, putting in a new heating system, which should reduce greatly its now not trivial heating bill. Much new wiring and plumbing are also being installed, with the aim to have it ready for our summer guests. Then in a greatly renewed way, it can resume its necessary dormitory-like role.

A most major gift from the Fleischmann Foundation

When Max Fleischmann set up his very large Foundation in 1952, he set a termination date of 1980. So knowing that almost one-hundred million dollars were to be distributed within just two years, we asked its Trustees whether they would consider a serious proposal from us for capital funds. To our joy they said yes, and we sent in a formal proposal asking for matching funds to complete the financing of our new Animal Facility and of the Sannis Hall residence at the Banbury Conference Center. We also requested more modest funds to make our lecture hall more suitable for closed-circuit TV. Happily our first two requests were

granted in August, providing us with a most-needed \$350,000 for the Animal Facility and \$225,000 for Sannis Hall. This marvelous gift allowed us soon after to sign a construction contract for Sannis Hall, which initially we hoped to have ready this spring. Delays in construction, however, have set back its timetable, with our first use of it now being planned for late summer.

We have also experienced delays in obtaining the final working drawings for the Animal Building. We wanted to break ground early in the spring of this year (1980), but now fear that it may be July 1 before this happens. We are getting increasingly impatient as our tiny mouse house becomes increasingly inadequate with each passing month.

The largest number of visitors so far

This past summer we welcomed over 2600 scientists to our meetings and summer courses. With our nine summer meetings, twelve courses, and four workshops, we were constantly seeing new faces depart before we learned many of their names. The net effect on each of us, however, was very beneficial, giving us many opportunities for establishing new scientific friendships, if not collaborations. There was, in addition, the heavy stimulation provided by the course lectures, for which many of the world's most distinguished biologists came here for a day or so to tell what they are doing to student audiences many of whom have never seen the scientists whose names are attached to the journal articles that fascinate them. Happily, we are increasingly making use of the Banbury Center, where two of our lecture courses and one advanced workshop were held.

Unfortunately, we now have to send to nearby motels many of our meetings' participants, and we were therefore most fortunate in having the opportunity, given to us by the family of Mrs. Nan Wood, to use her most-splendid home, Fort Hill, to house some of our guests. Her father, the noted industrial chemist, William J. Matheson, was President of our Board of Directors from 1904 to 1922, leaving upon his death in 1930 the then-considerable \$20,000 Matheson bequest to the Long Island Biological Association.

The Yeast Course refuses to die

In planning last summer's program, we realized it would be the tenth consecutive year in which we had given our course on Yeast Genetics. Taught most ably by Fred Sherman and Jerry Fink, it has trained a good fraction of our younger generation of yeast molecular biologists, and clearly we had made the right decision to start it up. So why not stop when we were ahead, and we told Fred and Jerry that this would be the last year they would be needed here to teach. By the beginning of the summer, however, we knew we had made the wrong decision, as

there had been a spectacular rise in the number of applicants (more than double that of the previous year), no doubt due to the yeasts increasing potential for recombinant DNA experimentation. So again in 1980, and possibly for many years in the future, the Yeast Course will continue.

Again we have solid support for our Neurobiology Courses

This last year marked the ninth consecutive year that we have mounted a major summer teaching effort in Neurobiology. Sloan Foundation funds covered this program for the first three years, until we obtained an NIH training grant that covered the following three years. We then got caught up in the dilemmas that surrounded the payback provision of the new Training Grant Law, and in 1976 and 1977, only through adroit maneuvering by Bill Udry, and with much help from concerned NIH, NIMH, and NSF administrators, did we get some modest support. Only through monies provided to us by the Banbury Foundation, through the creation of the Marie H. Robertson Fund for Neurobiology, were we able to keep our core curriculum alive.

While increasingly nervous about the attitude of the governmental granting agencies toward our neurobiology summer teaching, we again applied in the fall of 1978 for a long-term commitment, asking NSF for support for our two CNS-related courses in Jones and NIH to support our two Banbury lecture courses, as well as two experimental (the Leech and Experimental Techniques) courses taught in McClintock lab. Happily, almost all the support requested materialized, with NIH early last June granting us all the funds we requested from them. While NSF could grant us monies to support only one of the courses in Jones, a most-appreciated, last-minute grant from the Klingenstein Fund virtually closed the gap, and all six of last summer's courses were adequately funded.

An expanded program of Neurobiology Workshops

Several years ago a sizable Sloan Foundation grant let us commence several summer research workshops to complement our formal teaching courses. These were so successful that we have continued them, seeking out other funds to replace our now-expired Sloan grant. Using funds from the Rita Allen Foundation, we had a most exciting Pain Workshop in Jones, and the Digital Equipment Corporation gave us one of their most versatile MINC computers so that our Synapse Workshop could focus on the use of computers in analyzing neurobiological electrical signals. Our Artificial Intelligence Workshop was again supported by funds from the Cognitive Science Program of the Sloan Foundation, and the *Limulus*-focused workshop was made possible using Marie H. Robertson funds.

A very successful year for publications

This was again a year in which we could be very proud of our publications. At the opening of the year appeared the latest volume in our monograph series, "The Single-Stranded DNA Phages," edited by David Denhardt, David Dressler, and Dan Ray. It came out at twice the initially projected size and so had to be priced at a much higher figure than desired. But this large volume is in no way padded, and it should prove increasingly sought after as the M13-like phages become a common ingredient in DNA sequencing. The "Operon," edited by Jeffrey Miller and Bill Reznikoff, has sold well enough during its first year to cover its initial production costs, and so we plan to bring out a much lower priced paperback edition that should encourage its adoption in molecular genetics courses.

The bringing out of the annual Symposia was again a massive trick, with Volume 43 (DNA Replication and Recombination) running to almost 1400 pages and requiring a two-book set. Through much sustained effort, we had it on hand just as this year's Symposium started. Clearly, we would have liked to get it out quicker, but its production being essentially a linear process, it is subject to so many potential delays that its appearance in less than a year after the meeting is a real achievement for Nancy Ford and her most-dedicated staff. Also most impressive is this year's offering in the Cell Proliferation Series, entitled "Hormones and Cell Culture." Ably edited by Gordon Sato and Russell Ross, its initial sales have been good, and its excellence combined with its great topicality should ensure a wide distribution.

We, also, this year brought out a more polished form of the lab manual for our course on the Transformed Cell, entitled "Experiments with Normal and Transformed Cells." It was assembled by Ruth Crowe, Harvey Ozer, and Dan Ritkin.

The number of books, manuals, and abstracts sold during 1979 was 17,800, to be compared with a 1978 figure of 16,300.

The Banbury Meeting House draws nothing but raves

When we acquired possession of Mr. Charles S. Robertson's estate in the late spring of 1977, we had high hopes for eventually using it in a most-imaginative way. When that was to be was not obvious, however, since we needed both a program to give it its own uniqueness and a Director to carry it out. We also had to somehow convert what was a perfect site for family living into a complex that would successfully hold small conferences in a fashion equal to that done so well in London by the CIBA Foundation. Giving us a great head start was our architects most imaginative transformation of the former Georgian-styled garage into a meeting center with a super central conference room of which I have seen no visual equal. For this we owe Charles

Moore and his colleagues a great debt. Equally important was the recruiting of Victor McElheny to be its first Director, and subsequently his ability to persuade the Sloan Foundation to give us the needed seed monies to begin a high-quality program of meetings on Biological Risk Assessment. This enabled us to go to the NCI with firm plans, and here we must acknowledge the great help that they have given which has allowed us to start up with the highest possible standards.

Already we are almost running at our maximum capacity of six yearly meetings, whose proceedings we transcribe and quickly turn into useful books. That task, combined with the holding of several more informal workshops for journalists and congressional aides, gives to Vic a position where boredom is impossible to imagine.

LIBA never ceases to help us push for the best of science

Existing as we do almost an hour from any major site of biological research, we are seemingly isolated in a world that can know nothing of our virtues and at best regards us with the latent suspicions that exist between any two cultures that speak different languages. The truth is fortunately just the opposite. Through the help provided by our neighbors who comprise the Long Island Biological Association (LIBA), we have firm, warm, local support, which, if widely known, should create envy in virtually every other serious research institution, if not major private university. That we were in a true sense founded by our community, of course, helps, but the credit must go to the fact that we have always tried to work hand-in-hand with our neighbors to solve our common problems and, in doing so, have built up a record of mutual trust that I find truly remarkable. We also have been fortunate that LIBA has always been led by men of such high character, and among these Ed Pulling, in his ability to channel effective support for us, has no peer.

Behind our scientists is a most effective supportive and administrative staff

That we can do so much science so quickly owes much to the fact that as an administrative entity we try to function in a much more personal way than many other institutions where the bureaucracy does not mesh well with those they serve. Supervising that whole show now for over nine years is Bill Udry, whose ability to intelligently adapt to still one more crisis does much to keep us on a steady upward course. Likewise, life without Jack Richards, our super contractor in charge of all of our building here, is just not imaginable. In April of this year, Jack will have been here ten years, joining us just in time to successfully preside over the construction of the James Annex, the real turning point in making it known that the Lab would not decay further, but would

thoroughly transform itself into an institution with aspirations for the very best.

Behind Bill and Jack stand so many competent people that it is unfair to single out a few for special praise. I should like, however, to mention the retirement of Julius Kulmayer, a carpenter of highest quality, who came to us when Jack joined the Lab. Likewise, we are not the same without Bruce DeTroy, who presided so much over our shops for over eight years. And, regretfully, I must note the sudden death of our grounds man, Carmen Dewan, and the premature, sad loss of our most-dedicated artist and photographer, Bob Yaffe. On the more positive side I should mention our new cook, Jim Hope, who has the talent to cheerfully serve five-hundred perfectly cooked, warm lobsters, Stephen Humenick in whom we have a most-innovative mind for our electronic shop, Mike Ockler, who brings high talent as a draftsman and photographer, Herb Parsons, who gives us the audio-visual support we have so long required, and Deborah Lukralle, who keeps our tiny mouse house so effectively run.

A continued necessity for a strong Board of Trustees

Sitting here in our still semi-wild, almost pristine, environment, it is very easy to concentrate inward and not see ourselves as others must. We thus remain fortunate that our Board of Trustees possesses such great strengths in science, academia, law, and business, and is, therefore, able to give us invaluable counsel. Leading them is Harry Eagle, rightfully known as both a scientist of the highest order and one of the best administrators of science that we have had in the United States over the past three decades. He has been aided this year by a particularly strong Executive Committee, which also includes Edward Pulling, Clarence Galston, Bayard Clarkson, Robert Cummings, and Vittorio Defendi. In this capacity they must frequently meet here to give their approval for new actions, and everyone on our staff is greatly indebted for the carefully wise way they commit us to action.

Because of a statutory limit on tenure of service, we lost the services in October of Drs. Herman Eisen and Rollin Hotchkiss, and Dr. Joseph Kates resigned as a consequence of his move from Stony Brook to the Scripps Institute in LaJolla. Arnold Levine, in his move from Princeton to Stony Brook, now becomes our Stony Brook Trustee. Elected to fill the Princeton position is Abraham Worcel, a long-time friend and constant visitor here. Also newly elected is Charles Stevens, chosen by Yale University to represent them following their decision to join our Participating Institutions. Chuck knows us well through his participation in the Synapse Course and gives to our Board of Trustees its first distinguished neurobiologist. We also welcome back Norton Zinder as the Rockefeller Trustee, as well as Walter Page, who has served previously between 1963 and 1969, and 1972 and 1978.

We must soon come to grips with the fact that the forefront of much of our science has commercial implications

Until the past year or so, most of our scientists had never heard of an investment banker, much less met one. Now we know several who say they can take dollars from those fortunate souls who need tax losses and miraculously promise fifteen dollars for every original one they might entrust to our putative genius for manipulating DNA for the benefit of mankind or for making antibodies do tricks that until now only hotshot chemists were capable of.

This most certainly is a new world for us, and joining it has its perils to say the least. At the worst it would so dilute our thinking about difficult science that we would soon not be competitive with the best of our peers. Even if we decided that money was the thing, our amateur status could easily let us fail, leaving us with no true home in either camp. At the best, some foresighted industrial firm would give us piles of money to let us do what we want to do anyway, say, to develop for use in the mouse, if not human cells, the perfect cloning vehicle that has a promoter which can maximally produce any desired gene product. That would let us do wonders scientifically, as well as more than interest those who want to see whether large amounts of authentic interferon would actually work in humans.

So there is no avoiding the fact that some of our most sought after goals may also have a monetary figure attached, and we would be naive not to realize that we may have some bargaining chips. If we were Bell Lab's subsidiary, all the profits would go to ATT, but we would have guaranteed super working conditions, as well as salaries superior to those in academia. But here, as in almost every other research institution or university, we cannot offer the long-term security to do unimpeded research. As a tradeoff to our scientists, they are free to consult with industry, if not to take an equity interest in a new company designed to reap the commercial fruits of molecular biology.

But if our individual staff can join forces with industry, does it make sense for the Lab itself to continue to have a hands-off attitude from any direct involvement in bring-

ing our ideas to the market world by not acquiring some equity in any company that wishes to use our ideas. The conventional response, that such a course is bound to divert us as a lab from our real purpose of high-level biology, may very well be right. Here we need badly the wisdom of our Trustees to whom we have already referred this problem.

Financially we end the year in strong shape

Thanks in part to our large Fleischmann gift, our year end finances are stronger than at any time since I have been Director. We have been running at more than full utilization of our research and meeting facilities, and none of our service and maintenance staff are underemployed. Our Lab's productivity, seen from the economist's viewpoint, has been on an upward course, which we hope to maintain by investing almost all the free cash we generate into new laboratories and equipment that will let us do even better science. We do this believing that the safest way to protect our future is to keep at the forefront of science, as opposed to the piling up of a large reserve of cash to draw on in case our nation's support of science badly stalls.

Here we are optimistic and assume that our country's fortune will never fall so low that it loses the will to keep itself scientifically strong. We are, of course, aware that we are passing through a most hazardous economic period for our nation, with no evidence that our current government or plausible successor will do more than parrot the slogan of the balanced budget to remedy the fact that we are too much a nation of consumers and that we must work more effectively and receive less in the short term if we are to enter the twenty-first century as a commanding power. Now our Lab should act, as we hope our nation will, by looking to see where the future is, not where the past was, and to maintain our long tradition of staying ahead of the conventional wisdom that worked for our predecessors, but is inadequate for our successors. If we continue to be so regarded by those who know us well, we shall continue to have a meaningful future.

March 6, 1980

J. D. Watson



YEAR-ROUND RESEARCH

TUMOR VIRUSES DIVISION

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

MOVABLE GENETIC ELEMENTS DIVISION

Molecular Genetics
Insertion Elements and Plasmids
DNA-Protein Interactions
Yeast Genetics
Mammalian Cell Genetics

CELL BIOLOGY DIVISION

Cell Motility
Cell Biochemistry
Quest 2-D Gel Laboratory

NEUROBIOLOGY DIVISION

Neurobiology Laboratory
Neurobiology Workshops:
Invertebrate Neurobiology Workshop
Exploratory Synapse Workshop
Computer Modeling
of Specific Neural Networks
Pain Workshop

First row: A. Dunn; E. Cheng; L. Lipsich; R. Harshey

Second row: M. DuBow, A. Bukhari, C. Chaconas, D. Evans, N. Sarvetnick; D. Lane, K. Willison; L. Jordan, K. Burrige

Third row: R. LaPorta; R. Frisque, B. Ahrens; C. Fraser, M. Wigler

Fourth row: M. Mathews, B. Stillman; F. Asselbergs; P. Bullock; S.-L. Hu; W. Keller

Fifth row: N. Stow; R. Gelinas; J. Strathern, J. Hicks; L. Chow

TUMOR VIRUSES

From the research reports that follow, you can see that 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.



James Laboratory

MOLECULAR BIOLOGY OF TUMOR VIRUSES

J. SAMBROOK, B. Ahrens, P. Barkley, A. Barros, M. Botchan, J. Dorso, A. Dunn, R. Frisque, L. Garbarini, Y. Gluzman, R. Greene, T. Grodzicker, W. Hoefler, D. Holtzman, S.-L. Hu, R. Kelch, D. Klessig, D. Lane, R. McGuirk, R. McKay, R. Pashley, C. Paul, M. Quinlan, S. Smith, D. Solnick, C. Stephens, N. Stow, J. Stringer, W. Topp, S. Weirich, I. Wendel, J. Wiggins, K. Willison

During the past year, the Tumor Virus Group 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 groups: (1) the organization of viral DNA sequences in malignantly transformed cells, (2) the genetics of the transforming viruses SV40 and adenovirus 2, (3) the properties of proteins coded by viral genes which cause transformation, (4) the mechanism of RNA splicing, (5) the properties of malignantly transformed cells grown in culture, and (6) the interaction of SV40 with early mouse embryos.

The organization and expression of viral DNA sequences integrated in the genome of adenovirus-transformed rat cells

Adenovirus-transformed cells

J. Sambrook

All cells transformed by adenovirus 2 (Ad2) carry viral DNA sequences, which, being covalently joined to those of the host, are passed on to the cells' descendants like any other part of the cellular genome. Although a good deal is known about the number of integrated viral segments present in different cell lines and their general organization, nothing is known about the mechanism by which integration occurs, the nature of the cellular attachment sites, the exact locations of the integration sites in the viral genome, or the mechanism by which viral DNA sequences are expressed. Direct access to the three latter problems has recently become possible with the development of techniques to propagate segments of eukaryotic DNA in prokaryotes. During the past year we have used these techniques to clone from lines of adenovirus-transformed cells the integrated viral DNA and its flanking host sequences. We have used these clones to understand in detail the organization of the integrated viral segments and to determine the sequences that connect the viral DNA to the cellular genome.

We have studied the viral sequences in two lines (F4 and F17) of rat embryo brain cells transformed by Ad2. Both are part of an extensive collection isolated after infection of brain cells of Sprague-Dawley embryonic rats with viral particles. Like all other cells transformed by adenovirus 2, F4 and F17 contain only a fraction of the viral genome. Cells of the F17 line carry 3.5 copies of the left-hand 14% of the viral DNA. This sequence is common to all cells transformed as a consequence of

infection with adenovirus-2 particles: often (as is the case with F17 cells) it is the only viral sequence that is present. Cells of the F4 line appear to be considerably more complicated: they carry about 16 copies of the segment of the viral genome that extends from the left-hand terminus to about map position 63, as well as a small stretch of sequences from the extreme right-hand end of the viral DNA (Sambrook et al., *Cold Spring Harbor Symp. Quant. Biol.* 39:615 [1975]). Thus, F4 cells contain a more extended set of sequences than is necessary to establish or maintain transformation, and those sequences are present in considerably higher copy number than usual. Nevertheless, the DNA of F4 cells after cleavage with *EcoRI* yields only two fragments that contain Ad2 DNA sequences. These bands, whose approximate molecular weights are 23,000 and 10,500, do not comigrate with any known *EcoRI* fragment of Ad2 DNA. Thus, F4 cells, which carry a large number of copies of viral sequences, are measured by renaturation kinetics and display a simple distribution of viral DNA sequences among the variously sized *EcoRI* fragments of cellular DNA. The best explanation of this paradox is that all 16 viral insertions are identical, so that, high copy number notwithstanding, the cell-virus junctions are unique. This hypothesis is open to test. Accordingly, we set about cloning into bacteriophage λ the two *off-sized EcoRI* fragments that contain adenoviral sequences, using standard methods. The starting material was either F4 cellular DNA, digested to completion with *EcoRI* but not further fractionated, or similar preparations of DNA that had been enriched for adenoviral sequences by sedimentation through sucrose density gradients. However, despite screening a total of recombinant phages somewhat in excess of 10×10^6 , we were unable to identify any that contained adenoviral DNA sequences. The reason for this failure is unknown. Forced to approach the problem another way, we took advantage of an unpublished method developed by Hanahan to generate and screen by hybridization large numbers of recombinants synthesized *in vitro* by ligation of DNA to the plasmid pBR322. Fearing that insertion of the entire integrated viral set would prove too great a burden for the plasmid to carry efficiently, and hoping to gain preliminary information about the organization of the viral sequences, we used a combination of two restriction enzymes. F4 DNA was cleaved sequentially with *EcoRI* and *HindIII* and cloned in pBR322. Eighty-six recombinants were obtained that contained

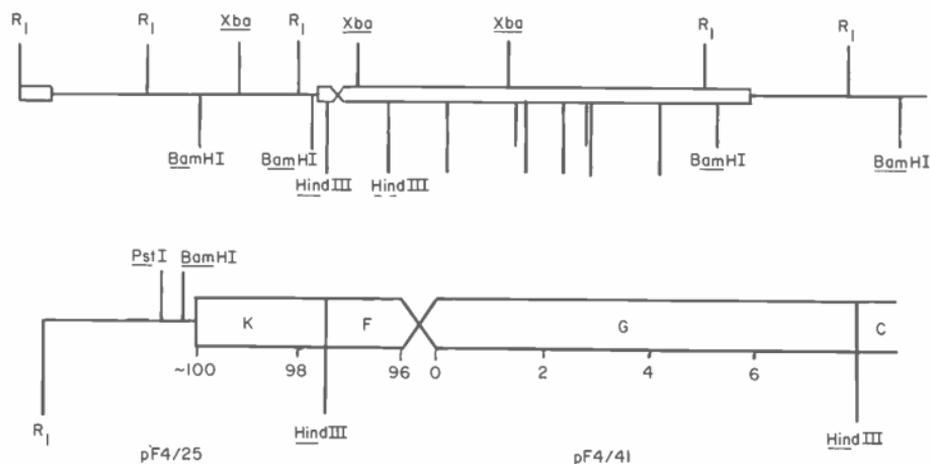


Figure 1
 The structure of adenovirus 2 DNA sequences integrated in F4 cells. The viral DNA sequences are represented by the blocked areas; the flanking cellular sequences are shown as thin lines. The upper diagram shows the structure of a tandem array of viral and host sequences; the lower diagram shows in more detail the structure of the junction between cellular and viral sequences and of the inversion which connects the right and left ends of the adenovirus genome.

adenoviral sequences. Their structures have been analyzed in detail by restriction endonuclease digestion, hybridization, and DNA sequencing. From the results of these experiments, it is possible to deduce an unambiguous structure for the viral sequences integrated in F4 cells (see Fig. 1). Starting from the left-hand end there occurs, in order, 1 kb of cellular sequence; a tract of viral sequences approximately 1400 nucleotides in length, beginning 2 nucleotides from the right-hand end of the viral genome and terminating soon after the *Bg*II site at map position 95.8; and, finally, a tract of viral sequences approximately 21,000 nucleotides in length, beginning near the left-hand end of the viral DNA and terminating at map position 63.

As far as we can presently tell, the structure of each of the 16 copies of the adenoviral sequences present in F4 cells is identical. Furthermore, each appears to be inserted into an identical sequence of cellular DNA. It seems most likely to us that this situation arose as a consequence of an amplification event that took place after integration had been established. Experiments to test this idea are in progress.

By reassocation kinetics, cells of the F17 line have been shown to contain about 3.5 copies of the leftmost 14% of the Ad2 genome. When the products of digestion of F17 DNA by endonuclease *Hind*III are assayed for their content of viral sequences, two bands are detected. Because the segment of viral DNA present in these cells contains only one site for endonuclease *Hind*III, at map position 8.0, the results strongly indicate that there is a single site at which viral se-

quences are integrated. Consistent with this conclusion is the observation that after cleavage with *Eco*R1, F17 DNA yields only one band that hybridizes to Ad2 DNA. This fragment was cloned in bacteriophage λ gtWES \cdot AB by standard techniques. Its structure has been partially elucidated by restriction endonuclease cleavage and hybridization. As far as we can tell, the cloned fragment contains a sequence of adenoviral DNA that is colinear with the viral genome from a point close to the left-hand end (between nucleotide 1 and nucleotide 188) to a point just after the *Hind*III site at map position 17. Little more information is available, except that the host sequences flanking the viral insert in F17 cells are different in their pattern of restriction from those flanking the viral sequences in F4 cells. This observation is consistent with the conclusion that adenoviral sequences integrate in different locations in the genomes of different lines of transformed cells.

SV40-transformed cells

M. Botchan
 J. Stringer
 J. Sambrook

Stable transformation of cells by SV40 is accompanied by integration of viral DNA into the cellular genome. This endows the cell with a heritable source of virally encoded tumor (T) antigen proteins that are involved in the maintenance of the transformed state. The mechanism by which integration occurs is not known; however, at the level of analysis resolved by restriction

endonuclease digestion of total transformed-cell DNA, it appears that integration is not site-specific with respect to either cellular or viral chromosomes. During the past year, we have been investigating whether this apparent lack of specificity extends to the level of nucleotide sequence or whether integrative recombination is mediated by DNA sequence homology. To this end, we have cloned in various lambdaoid phages DNA fragments that contain the integrated SV40 DNA along with its flanking cellular DNA sequences. We have obtained cloned fragments from the genomes of two transformed rat cell lines, 14B and SVRE 17, both of which contain a single integrated SV40 tract, and from closed circular molecules produced by excision of the integrated SV40 DNA following fusion of 14B cells with monkey cells. We have analyzed these clones by restriction endonuclease digestion, heteroduplex formation, and DNA sequence determination.

The structure of the integrated DNA in 14B cells is somewhat complex. SV40 DNA is attached to cellular DNA at nucleotide 850; it then proceeds as a continuous tract colinear with the SV40 genome through the origin of replication and the early region to nucleotide 2244. At this point there are 40 nucleotides that are not recognizable as SV40 DNA; these are followed by a resumption of SV40 sequence beginning at nucleotide 857 and continuing to nucleotide 1656 where cellular DNA resumes. This second stretch of SV40 DNA is inverted with respect to the first SV40 DNA sequence. The cause of this inverted arrangement of SV40 sequences is not known. It may be the result of either two neighboring integration events or a rearrangement of the viral genome during integration.

The role of sequence homology in the integration event that gave rise to 14B cells will remain unresolved until the segment of cellular DNA that took part in the recombination event is sequenced. We have cloned a 10-kbp fragment from untransformed rat cells that is colinear with the cellular DNA on one side of the SV40 DNA integrated in 14B cells. Interestingly, the DNA sequences on the other side of the SV40 DNA are not present in the 10-kbp fragment, suggesting that some rearrangement, deletion, or insertion of cellular sequences occurred during integration.

The structure of the integrated SV40 DNA in SVRE 17 cells is simpler than in 14B cells. Viral DNA is present as a single tract colinear with the SV40 map from 0.76 map units through the origin of replication and early region around to a position somewhere between 0.98 and 0.95 map units. Sequence analysis of the recombinant joint around 0.76 map units shows it to be at nucleotide 518. This is clearly different from any of the cell-virus junctions in 14B. Although the other cell-virus joint in SVRE 17 has not yet been completely sequenced, it is already clear that it is not the same attachment site at nucleotide 1656 that is

found in 14B cells. The cellular sequences adjacent to integrated SV40 in SVRE 17 are also different from any of the flanking cell sequences in 14B. Furthermore, the cellular DNA tracts in the cloned fragments from cell line 14B and SVRE 17 do not cross-hybridize, indicating a lack of sequence homology within the cellular DNA sequences flanking the integrated SV40 genomes.

Excision of SV40 DNA

J. Sambrook

J. Stringer

M. Botchan

The excision products found in 14B cells following fusion with permissive monkey cells are heterogeneous in size and are composed of both viral and cellular DNAs. Eight excision products formed after fusion of 14B cells with monkey cells have been cloned. They range in size from 3 kbp to 10 kbp and are all structurally related. All can be aligned with the same cell-virus joint at nucleotide 850. The DNA sequence at this joint has been determined in one of the cloned excision products and it is the same as that found in the DNA cloned directly from the integrated 14B genome. The cloned excision products contain various amounts of viral and cellular DNAs, but all the viral DNA tracts are colinear with one another and with the viral DNA cloned directly from the 14B genome. The simplest explanation of these structures is that they were produced by recombination between cellular DNA sequences flanking the SV40 insertion or between integrated SV40 DNA and flanking cell sequences. We have recently cloned from the genome of 14B cells another DNA fragment that overlaps the first clone from 14B, described above. This clone will be useful for further analysis of the cell sequences in the rescued viral products.

The cloned excision products and the cloned integrated DNA fragments obtained from 14B cells constitute a set of parental sequences and recombinant progeny sequences. Analysis of the DNA sequences before and after excision will establish what role DNA homology plays in the excision process.

We can conclude from our analysis of cloned DNA fragments that the low level of specificity for integration holds true at the level of DNA sequence. We will soon know whether DNA sequence homology mediates integration and excision of SV40 DNA.

Transcription of integrated viral sequences in transformed cells

S.-L. Hu

Much work has been focused on the expression of viral sequences in transformed cells. Viral transcripts from two single-copy lines of rat cells transformed by

SV40 (SVRE 14B and SVRE 17) and one line transformed by Ad2 (F4) have been studied according to the method of Berk and Sharp (*Cell* 14:695 [1978]). In general, cytoplasmic poly(A)-containing viral transcripts from these transformants have the same topology and splicing patterns as those found early in viral lytic infection. The relative abundance of individual species, however, may vary. In one of the lines investigated, F4, two additional viral transcripts unique to the transformed cells have been observed. These transcripts contain sequences from both the left end and the right end of the adenovirus-2 genome. They appear to originate from the early-region-4 promoter and extend into the early region 1A, transversing the junction connecting the right and left end sequences. These transcripts are terminated and spliced in the same manner as that of normal transcripts from early region 1A. Whether they code for any protein that is responsible for any of the transformed properties of F4 is currently under investigation.

In all three lines of transformed cells investigated, no transcripts from viral late promoters can be detected. Whether this indicates a failure in the initiation of these transcripts or some defects in their processing is still not clear.

Another interesting observation concerns the existence of hybrid RNA molecules containing both viral and cellular sequences in SVRE 14B. This transcript appears to originate from the proximal cellular sequences, extend through the viral early region, and terminate at the normal site for early SV40 mRNA. This transcript is polyadenylated, but not spliced, and is present only in the nucleus of the transformed cell. Whether this hybrid transcript can serve as a precursor to the spliced viral mRNA in the cytoplasm is not clear at present. If such should be the case, the 5' end of this hybrid transcript must be processed so that it will coincide with the 5' end of normal viral early mRNA, since the structure of viral transcripts present in the cytoplasm of SVRE 14B is indistinguishable from that of normal early mRNAs. The availability of clones containing the integrated viral genome together with its flanking cellular sequences should allow us to study the structure and the biosynthesis of these hybrid transcripts in greater detail.

In summary, it appears that all transcriptional regulatory elements (promoters, splicing signals, terminator sites, etc.) normally functional in early viral infection are also operative in transformed cells. However, because of the rearrangement of the viral genome in the integrated state, some viral transcripts produced in certain lines of transformed cells may not be identical to those observed in lytic infection. Finally, in the nucleus of some transformed cells, we have observed chimeric transcripts containing both viral and cellular sequences.

The genetics of the transforming viruses SV40 and adenovirus 2

Origin-defective mutants of SV40

Y. Gluzman
R. Frisque
J. Sambrook

Replication of SV40 DNA begins at a unique site on the viral chromosome and proceeds bidirectionally around the circular molecule. To understand the structure of the viral origin of replication and its role in the expression of viral genes and in the mechanism of transformation, we isolated a new set of nonconditionally lethal origin-defective mutants. This class of mutants cannot be complemented by any helper virus in their conventional eukaryotic hosts. Therefore, the isolation of these mutants was achieved by utilizing SV40 DNA cloned in a plasmid vector and propagated in *Escherichia coli*.

The mutations at the origin of viral DNA replication were introduced at the *Bgl*I site (which is located at the origin). Cloned DNA was cut with *Bgl*I, and mutations were introduced by treatment of the resulting linear DNA with either single-strand-specific nuclease S1 or DNA polymerase I. Mutated *Bgl*I-resistant DNAs were recloned and used for biological studies.

All *Bgl*I-resistant SV40 DNAs were found to be origin-defective mutants; however, the majority of them were capable of synthesizing biologically active T antigen in transfected mammalian cells. Thus, despite the very close physical proximity of the putative early promoter and the origin of DNA replication, these two sites are functionally separated. Origin-defective mutants were able to transform rat cells with efficiencies similar to that of wild-type SV40 DNA. Thus, a functional origin of DNA replication is not necessary for the maintenance of transformation. Because transformation was initiated by DNA transfection using the calcium phosphate precipitation method, we can state that the origin of SV40 DNA replication does not affect the efficiency of transformation in this situation; however, whether the viral origin is required for efficient transformation after infection of cells by virions is still unresolved and is still under investigation.

The origin-defective mutants were also used to transform permissive monkey cells. The mutant DNA expresses functional T-antigen; however, the lack of a viral origin allows the permissive cells to survive infection with mutant DNA and become transformed. Because these cells were not selected to survive either virion infection or uncontrolled viral DNA replication, they possess a combination of very useful properties: they are permissive to infection with SV40 and express functional T antigen. We have shown that these two

properties allow replication of SV40 *tsA* virus at the nonpermissive temperature. These cells can thus be used as a host for SV40 vectors, which contain a viral origin of DNA replication and an intact late region, but have the early region replaced by different heterologous DNAs. This system has the advantage of allowing the propagation of pure populations (without helper viruses) of eukaryotic transducing viruses.

Cotransformation of adenovirus-2 DNA and the herpes simplex virus 1 thymidine kinase gene in human cells

T. Grodzicker
D. Klessig

We are deriving human cell lines that can express different adenoviral genes by cotransformation of Ad2 restriction enzyme fragments with the herpes virus thymidine kinase (*tk*) gene. We hope to use these lines as host cells for various types of viral mutants, as well as for studying the control and expression of viral genes in the *tk*⁺ cells.

In collaboration with Michael Wigler, we have cotransformed a *tk*⁻ human cell line (obtained from Carlo Croce, Wistar Institute) with large Ad2 fragments (30% or 60% of the genome) and a cloned *Bam* fragment carrying the herpes virus thymidine kinase gene. *tk*⁺ cells were isolated after selection and maintenance in HAT medium. The transformed lines were initially screened to see if they are able to complement the growth of Ad5 Δ 312, a deletion mutant lacking the sequences between ~1% and 4.5% of the viral genome (Jones and Shenk, *Cell* 17:683[1979]). Several lines were obtained that complement Ad5 Δ 312, some giving extremely high, and others intermediate, levels of complementation. Some cell lines are also able to complement the growth of Ad5 Δ 313, a mutant lacking sequences between positions ~4% and 10% of the viral genome.

Several of the cotransformants that complement the mutants are quite stable, whereas others are fairly unstable even when grown in the continuous presence of HAT medium. Thus, some *tk*⁻ subclones complement Ad5 Δ 312 as well as the parental line, others give reduced levels of complementation, and some fail to support the growth of the mutant viruses. Analysis of the adenoviral sequences in the transformed cells by restriction enzyme analysis and Southern hybridization showed that the amounts of viral DNA are the same in all *tk*⁺ subclones regardless of their ability to complement the mutants. Thus, *tk*⁺ subclones do not lose viral DNA, but its expression is altered or reduced. This situation is also found in SV40-*tk* mouse-cell cotransformants (D. Hanahan, M. Botchan, D. Lane, L. Lipsich, M. Wigler, unpubl.).

All of the lines are unstable to some extent in the

absence of HAT, and therefore *tk*⁻ revertants can easily be obtained. In this case, all of the *tk*⁻ revertants have lost all of the thymidine kinase and the adenoviral DNA they had contained (see Mammalian Cell Genetics Section for further discussion of this phenomenon).

The amount and arrangement of viral sequences in the transformed cell has been analyzed by blot hybridization. Most of the cell lines contain a single insertion of the herpes virus *tk* fragment and a single insertion of adenoviral DNA. The adenoviral DNA is composed of a segment from the left end of the genome and extends for varying lengths in different lines. However, one line (B1) contains about eight different insertions, two of which are amplified. All of the insertions in B1 are missing a small segment from the extreme left-hand end of the genome, suggesting that the final arrangement of viral DNA may have arisen from recombination and amplification of an initial set of viral sequences. Interestingly, this line complements the growth of viral mutants the least, even though it contains the most viral DNA, suggesting that the viral sequences are poorly expressed. The viral RNA in these lines is presently being analyzed. Preliminary results indicate that the two lines (A2 and A5) that complement very efficiently and contain a single copy of the left end of the adenoviral genome synthesize the early-region-1A and early-region-1B mRNAs. In contrast, the B1 cell line produces the normal early-region-1B mRNAs but not the early-region-1A mRNAs. In addition, a prominent, unspliced RNA that is encoded by the *Hind*III C fragment (coordinates 7.5–17) and may extend into the *Hind*III G fragment (coordinates 0–7.5) is found in the cytoplasm of B1 cells. The loss of the left-end viral DNA sequences at least to coordinate 0.8, together with the absence of normal early-region-1A mRNAs, suggests that the promoter for this gene family may have been lost or altered during the initial recombination event that led to the formation of the B1 cell line.

New *tk*⁻ lines are being constructed with different viral fragments, as well as with plasmids containing the thymidine kinase gene and various adenoviral genes on the same molecule.

Suppression of Ad2⁺ ND1 nonsense mutants

T. Grodzicker

In collaboration with Jim Lewis, we have been studying the use of antibiotics that cause misreading of nonsense codons and suppression in mammalian cells. We have isolated both amber and ochre mutations in the SV40 segment of the hybrid virus Ad2⁺ ND1 and have shown that they can be suppressed by yeast suppressor tRNAs when mRNA from the mutants is translated *in vitro*, or *in vivo* when yeast

suppressor tRNA is microinjected into mutant-infected nonpermissive cells (Grodzicker et al., *J. Virol.* 19:559 [1976]; Gesteland et al., *DNAs* 74:4567 [1977]; A. Graessmann, R. Gesteland, and T. Grodzicker [unpubl.]).

Paromomycin is an aminoglycoside antibiotic that has been shown to cause misreading of poly(U) in several *in vitro* translation systems derived from a variety of eukaryotic cells (see, for example, Wilhelm et al., *Biochemistry* 17:1143 [1978]) as well as phenotypic suppression of many yeast nonsense mutants (Singh et al., *Nature* 277:146 [1979]; Palmer et al., *Nature* 277:148 [1979]). We have translated mRNA derived from two amber Ad2*ND1 mutants in a reticulocyte lysate translation system and have shown directly that paromomycin causes suppression of the nonsense mutation. In the presence of 4–30 $\mu\text{g/ml}$ of paromomycin, mutant mRNA is translated to yield the wild-type 30K protein in addition to the 19K amber fragment normally produced by the mutants. At 60 $\mu\text{g/ml}$ of the drug, protein synthesis is quite severely inhibited. We plan to screen other putative adenoviral nonsense mutants to see if they can be suppressed by paromomycin. Preliminary evidence suggests that the drug does not readily enter cells, even when the cells are treated with a variety of reagents that facilitate the uptake of various macromolecules. We intend to test a series of related antibiotics to see if they can be taken up more readily by mammalian cells.

The role of adenoviral DNA-binding protein in late gene expression

D. Klessig
T. Grodzicker

Five host-range mutants (Ad2hr400, hr401, hr402, hr403, and Ad5hr404) of human adenovirus serotype 2 and 5 (Ad2 and Ad5) that overcome the block to growth of wild-type adenovirus in monkey cells have been isolated. They form plaques and multiply efficiently in both monkey and human cells. The alteration in each of these mutants allows the full expression of all viral late genes, in marked contrast to the depressed synthesis of many late proteins in monkey cells infected with the parental Ad2 or Ad5. The altered gene encodes a diffusible product, since the mutation acts *in trans* to enhance the synthesis of wild-type Ad3 late proteins during coinfections of monkey cells with Ad2hr400 and Ad3. Restriction enzyme analysis of the genomes of all host-range mutants show that none of them contain major alterations. In addition, earlier work (Klessig and Hassell, *J. Virol.* 28:945 [1978]) indicated that Ad2hr400 does not contain SV40 sequences, which in some adenovirus-SV40 hybrids allows efficient multiplication in monkey cells. The mutation responsible for the ex-

tended host range has been physically mapped by marker-rescue experiments using isolated restriction enzyme fragments of the mutants to transfer the new phenotype to wild-type adenovirus. The alteration in each of the five mutants is located in a region (coordinates 62–70.7; coordinates 62–68 for Ad5hr404) which encodes predominantly the 72K DNA-binding protein (DBP). More detailed mapping using Ad2 hr400 fragments places the mutation (coordinates 62.9–65.6) entirely within the DBP gene.

Viral DNA replication that requires DBP occurs normally in monkey cells infected with wild-type Ad2 or Ad5. Thus, DBP must have a second function that is impaired in these abortive infections. The action of DBP is probably mediated through its interaction with host cell components. Thus, in infected human cells, DBP interacts properly with all cellular proteins needed for its functions in both early and late stages of lytic infection. In monkey cells, however, DBP can function with host proteins needed for viral DNA replication, whereas it fails to interact correctly with other host factors important for late gene expression. It seems probable that the host-range mutations alter DBP so that it can interact correctly with monkey as well as human cell components.

Mutants in early region 1A of adenovirus 2

D. Solnick

Genetic experiments have been designed to further our understanding of the adenoviruses of transforming genes and their role in viral gene expression. We have been isolating and characterizing early-region-1A mutants generated by one of several site-directed mutagenesis techniques. To date, eight point mutants and six deletion mutants have been isolated. The point mutants were obtained by nitrous acid mutagenesis of the small *Xba*I restriction fragment (map position 0.0–3.8) from a phenotypically wild-type Ad5 variant containing a single *Xba*I restriction site located at 3.8 map units. The mutagenized fragment was ligated to the large *Xba*I fragment containing the remainder of the viral genome, and the mixture was then transfected onto human 293 cells. These Ad5-transformed permissive cells support the growth of viral mutants with lesions at the left-hand end of the viral genome. The resulting plaques were then checked for enhanced plaquing efficiency on 293 cells relative to that on HeLa cells. The deletion mutants were generated by a modification of a technique developed by Tom Shenk (*Cell* 13:181 [1978]), which selects for spontaneous mutants lacking a particular restriction site. In this case, the deletions remove the *Sma*I site located at 2.9 map units.

One of the point mutants has been partially characterized. Interestingly, this mutant synthesized one, and only one, of the two principal region-1A cytoplasmic

mRNAs, as determined according to the method of Berk and Sharp (*Cell* 12:721 [1977]). In the wild-type infection, these two mRNAs have each lost one intervening sequence and are exactly or very nearly coterminal. The right-hand boundaries of the intervening sequences are coincident, whereas the left-hand boundaries are different (Berk and Sharp, *Cell* 14:695 [1978]; Chow et al., *J. Mol. Biol.* 139:265 [1979]; Perricaudet et al., *Nature* 281:694 [1979]). It is suspected, therefore, that these two mRNAs are derived either in sequence or in parallel from the unspliced nuclear transcript detected by Berk et al. (*Cell* 17:935 [1979]). The mutant, then, may contain an altered splicing signal required for one of the processing pathways of the region-1A transcripts.

A second project involves the construction of an adenoviral variant containing a second major late promoter. Such a variant could be useful as a vehicle for the expression of closed eukaryotic genes. We are initially trying to obtain a virus that overproduces the region-1A proteins. Both early region 1A and the major late promoter have been separately cloned in *E. coli* using the pBR322 vector. The appropriately oriented ligation product from these two clones was then recloned in pBR322. This final clone is now being inserted into a nonessential region of the adenoviral genome with the hope that the late promoter will produce a functional transcript of region 1A.

Cloning of adenovirus-2 early cDNAs

N. Stow

The four major early regions of Ad2 encode a large number of overlapping mRNAs that exhibit different patterns of splicing and this considerably complicates the analysis of their fine structures and biological functions. One possible way of circumventing these problems is to purify and amplify the mRNA sequences in the form of cloned cDNA copies.

Early in infection, Ad2-specific sequences constitute only a small proportion of the total poly(A)-containing RNA, and much time has been spent in finding an efficient method of cloning cDNA copies of these low-abundance species while maintaining their structural integrity. Initial experiments with the more abundant late Ad2 mRNAs indicated that maximum cloning efficiencies are obtained by synthesizing double-stranded cDNA copies (as opposed to DNA-RNA hybrids) and inserting these by G/C tailing into the *Pst*I site of the plasmid vector pBR322. Efficiencies of 10^1 – 10^3 colonies/ μ g double-stranded cDNA were obtained by transforming *E. coli* χ 1776, using a procedure developed by D. Hanahan.

This result suggested that it might be possible to obtain cloned copies of early messages by employing total poly(A)-containing RNA from infected cells as template for the synthesis of double-stranded DNA.

This cDNA would then be used in a "shot-gun" transformation of χ 1776, and colonies containing virus-specific sequences could be identified by hybridization. Starting with double-stranded cDNA, which had been shown to be reasonably intact by blotting, 47 clones containing Ad2 DNA sequences were obtained by using the approach outlined above. Hybridization experiments indicated that sequences from early regions 1A, 1B, 2, 3, and 4 were represented, and several clones are now being subjected to a detailed structural analysis.

Restriction enzyme analysis and DNA sequencing (in collaboration with Eric Lifsen and Daniela Sciaky) are being used to correlate the structures of these clones with previously described message species and to identify protein coding regions, splice sites, and polyadenylation signals.

Those clones containing the complete coding sequences for individual Ad2 early proteins will be used in systems that allow their expression as an aid to understanding the biological roles of these gene products.

The transforming proteins of SV40

Studies of SV40 tumor antigens

D. Lane

Three separate hybridoma cell lines have been derived that secrete monoclonal antibody to the large T antigen of SV40. The lines were derived from a fusion between spleen cells from a mouse immunized against the purified Ad2*D2 hybrid virus T-antigen-related protein isolated from infected HeLa cells. All three lines have been through three separate rounds of cloning in soft agarose and now appear to be stable secretors. Clone 1 and clone 2 are being grown in pristane-primed mice, and the ascitic fluid from these hybridoma-bearing animals contains large amounts of the monoclonal antibody. All three lines grow well in mass culture and the media contains high concentrations (1–10 μ g/ml) of the antibody.

The binding site of clone 1 on the T-antigen protein has been shown to be retained even after the antigen is treated with boiling SDS and 0.1 M DTT. Furthermore, the binding site has been localized on the T protein by the finding that clone 1 does not bind the 30K T-antigen-related polypeptide encoded by the Ad2*ND1 virus but does bind the 56K and 42K polypeptides made by the Ad2*ND2 virus.

Clones 2 and 3 recognize binding sites on SV40 T antigen that are not present on the T-antigen-related polypeptides made by Ad2*ND1 and Ad2*ND2. In both cases, the binding sites are sensitive to the denaturing effects of SDS. A large number of uses for these monoclonal reagents are envisioned and their further characterization is now in progress.

The host protein that binds to large T antigen has

been characterized in a variety of different transformed cells, in collaboration with William Topp. The protein has been isolated preparatively from an SV40-transformed rat cell line and used to immunize rabbits. The relationship between the presence of the protein and its association with large T antigen with the transformed state of the cell has been studied in both tsA-transformed and flat revertant cell lines. In the flat revertant cell lines examined so far, no loss of the host protein has been discovered and it is still complexed to large T antigen. Thus, the presence of the protein in a complex with large T antigen does not automatically mean that the cell is transformed. Results with the tsA-mutant-transformed cells are more complex, but preliminary data suggest that the ability of the host protein to complex with large T antigen is thermosensitive in these lines. It has also been noted that the temperature sensitivity of the transformed state in different tsA-transformed cells is correlated with the half-life of large T antigen in the cell.

An immunoassay for the interaction of SV40 T antigen and DNA

R. McKay

Standard immunoassay procedures using monoclonal antibodies have been adapted with the aim of monitoring the binding of SV40 T antigen to DNA. Two advantages of these procedures are foreseen: (1) The DNA binding properties of the protein can be assessed at very early stages of purification. (2) The assay procedure can be used to obtain kinetic information on protein-DNA interactions. An absolute binding constant derived from a Scatchard plot depends on the fulfillment of several criteria, namely, that (1) equilibrium is reached, (2) the reaction is fully reversible, (3) bound and free forms can be measured without perturbing the equilibrium, (4) the protein is univalent, (5) binding sites are independent, and (6) one of the reactants is homogeneous. Some of these criteria are known to be fulfilled by this assay, others may not be. In particular, the independence of binding the three tandem binding sites at the replication origin (Tjian, *Cell* 13:165 [1978]) may only be achieved using DNA sequences that have been experimentally altered.

At the present time we have shown that there is a protein complex in Ad2^{*}D2-infected HeLa cells that binds to the DNA restriction fragment containing the SV40 origin up to 1000 times "better" than it does to other DNA fragments. One assumes that this binding activity is due to the active form of the T-antigen-related protein found in these cells. Recovery of the protein from the DNA fragments is required to prove this assumption. This "high binding" complex also binds preferentially to another site on SV40 DNA.

The mechanism of splicing

Incomplete splicing of fiber mRNA in monkey cells

D. Klessig

Monkey cells are nonpermissive hosts for human adenoviruses due to a block in the synthesis of certain late viral proteins. Analysis of the RNA produced in abortively infected monkey cells indicated that the depressed synthesis of many of the late proteins can be ascribed to the reduced concentration of their corresponding mRNAs (Klessig and Anderson, *J. Virol.* 16:1605 [1975]). An exception is the capsid polypeptide, fiber. Fiber RNA concentration is reduced 5- to 20-fold in monkey cells, whereas fiber protein synthesis is depressed 100- to 1000-fold. In collaboration with Louise Chow, experiments designed to detect defects in the cytoplasmic fiber RNA that would account for its poor translatability have been conducted and show that the RNA had a normal, capped tripartite leader at its 5' terminus. However, a large proportion of these molecules contained long sequences between the tripartite leader and the main body that failed to be spliced out of the initial transcript during maturation of the fiber mRNA. Similarly, region-3 RNAs at late times also contained the same long upstream sequences in abortive infections. In contrast, few cytoplasmic fiber RNAs or region-3 RNAs from a productive infection with an Ad2 mutant (Ad2hr400) which grows lytically in monkey cells retained such long upstream sequences. The other late RNAs from abortively or productively infected monkey cells had splicing patterns identical to those seen in permissive human cells. These findings, together with the observation that the host-range mutation in Ad2hr400 is located in the DBP, suggest a role for this protein in RNA processing in addition to its function in viral DNA replication.

Attempts to achieve splicing in vitro

A. Dunn

We have continued our analysis of the transcription of viral genes in human cells infected with the non-defective hybrid virus Ad2^{*}ND1dp2. Ad2^{*}ND1dp2 contains two independent insertions of SV40 sequences that have replaced a 6% fragment of the Ad2 chromosome which is nonessential for lytic growth. One of the SV40 insertions is located about 300 bp upstream from the beginning of the major late gene which encodes the structural protein fiber. A consequence of this arrangement is the synthesis, during the late phase of productive infection, of viral mRNAs containing covalently linked Ad2 and SV40 se-

quences. Previous work in our laboratory has shown that the cytoplasm of infected cells contains large quantities of two distinct and separable populations of mRNAs that are associated with the SV40 sequences proximal to the 3' end of the fiber sequences. These mRNAs, which are under the control of the major late adenoviral promoter, carry the traditional tripartite leaders, but differ in the presence or absence of an extra leader (the "y" leader) component located 2000 nucleotides downstream from the gene.

We have considered the possibility that the hybrid mRNA carrying the γ leader is the immediate precursor to the mature mRNA containing only the tripartite leader. These molecules might be a suitable and convenient choice as substrate for use in identifying and isolating the enzymatic machinery responsible for removal of intervening sequences in precursor RNAs in eukaryotes. This choice of substrate offers several advantages including (1) the ability to isolate purified ^{32}P -labeled precursor RNA in large quantities and (2) the precursor and mature mRNA have unique and diagnostic fingerprints which we have observed experimentally and which are predicted from the nucleotide sequence analysis of a cloned copy of the closely related Ad2 fiber mRNA, carried out in our laboratory in the last year.

Initial experiments, carried out in collaboration with Walter Keller at the University of Heidelberg, have resulted in the demonstration of an activity, present in both infected and uninfected cells, that reduces the molecular weight of the supposed precursor to that of the mature mRNA. This activity, which is dependent on divalent cations, appears unstable under a variety of storage conditions, and we have experienced great difficulty in the day-to-day reproducibility of our assay. Nevertheless, we have shown that these semi-purified fractions include a double-strand-specific RNase which, on theoretical grounds at least, we believe to be a likely component of the splicing apparatus. Interestingly, those same fractions do not appear to contain a T4-like ATP-dependent ligase. Perhaps the covalent linkage between conserved sequences of mRNA is mediated by the joining of 5'-OH and 3'-PO₄ groups, as has been convincingly shown for the processing of tRNA in yeast.

We have also considered the possibility that RNA is only spliced when it is in its natural environment as part of a ribonucleoprotein complex. We are presently isolating RNA complexes from the nuclei of infected cells to determine whether they include all of the processing machinery.

Finally, we are synthesizing viral RNA in the isolated nuclei of virus-infected cells. The majority of this RNA is unspliced and is initiated at the major late Ad2 promoter. Preliminary experiments suggest that this same result can be obtained in lysed nuclei, thus pro-

viding a soluble system in which to demonstrate *in vitro* splicing.

Transformation of cells in culture

W. Topp

R. Frisque

Y. Gluzman

We have been involved in the study of how DNA viruses transform cells in tissue culture and the relationship of this process to tumorigenicity. (1) We have characterized cells transformed by the human papovavirus JC. Although JC virus (JCV) is tumorigenic in hamsters and owl monkeys, there have been no reports until now of it transforming cells *in vitro*. An analysis of the phenotype of JCV hamster brain transformants suggests that they are quite similar to cells transformed by SV40, showing good colonial growth suspended in methylcellulose. A number of the JCV transformants are tumorigenic. However, JC transformants are morphologically flatter and, in general, retain the cytoplasmic actin networks characteristic of normal cells. (2) The transforming ability of SV40 mutants defective in the viral large (*tsA*) or small (*d154/59*) T proteins has also been investigated and the phenotypes of transformed cells compared with those of wild-type transformants. The large T protein appears to be involved in the maintenance of transformation in both rat and hamster cells. However, hamster embryo fibroblasts transformed by *d154/59* mutants were phenotypically indistinguishable from their wild-type counterparts, indicating that the small T protein is not required. This is in contrast to our previous results with rat cells in which small T protein was clearly required for full transformation. (3) Replication-defective mutants of SV40 have recently been isolated in our laboratory. SV40 DNA was cloned in a plasmid vector, mutagenized at the origin of DNA replication, and analyzed (see above). Many of these mutants have very small deletions or substitutions, induce T antigen in permissive and nonpermissive cells, and complement *tsA* virus in lytic infection. We find that these mutants give efficiencies of transformation identical to those of wild-type SV40 when rat cells are infected by coprecipitation with calcium phosphate. This result argues strongly against the involvement of viral DNA replication in the initiation of transformation by this route. (4) We are beginning studies related to adenovirus transformation. Because of the confusing situation in Ad2 of overlapping genes and numerous gene products, it has been difficult to precisely define which DNA sequences and polypeptides are involved in transformation. We are attempting to introduce small mutations at specific sites (using cleavage with restriction endonucleases) in cloned

subgenomic species of Ad2 DNA containing the transforming region. The transforming efficiencies of these molecules relative to that of the parent will be used to map functions important for transformation. Initial experiments indicate that cloned adenoviral DNA fragments are able to transform rat embryo fibroblasts.

Interaction of SV40 DNA and virus with early mouse embryos

K. Willison

Preimplantation mouse embryos have been infected with SV40 either by incubation of morulae with virus or by microinjection of purified DNA into the blastocyst. These infected embryos were implanted into pseudopregnant foster mothers and live mice were obtained. DNA was extracted from the tissues of these mice and from the liver of the foster mothers. The DNAs were then analyzed for the presence of SV40 sequences by Southern blotting.

Five litters were obtained after DNA injection. All the members of two litters contained SV40 DNA sequences in a form that comigrated with circular SV40 DNA extracted from lytically infected CV-1 cells. The liver DNA of the foster mothers that carried these two litters also contained free (nonintegrated) SV40 DNA sequences. None of the members of the other three litters that were analyzed contained free SV40 DNA. One male mouse (1 of 12 analyzed), however, did contain SV40 sequences in high-molecular-weight DNA that was extracted from somatic and germ line tissue. The arrangement of the SV40 DNA has been examined using five different restriction enzymes and the data suggest that it is integrated. To prove that this is the case, experiments are in progress to clone the integrated sequences using the Charon 4A phage vector system.

Four litters were obtained after viral infection (the infections were carried out by Francoise Kelly, Institut Pasteur, Paris; DNA samples were analyzed at Cold Spring Harbor Laboratory). DNA was extracted from the livers and brains of some members of each litter (nine animals analyzed). In no case could any free SV40 DNA be detected by Southern blotting. However, two mice from different litters appeared to contain integrated SV40 DNA sequences. A detailed restriction enzyme map is being constructed using Southern blotting.

The aim of this work has been to find a method for introducing DNA sequences into adult mice, and so far the results are encouraging. The efficiency of integration is high (3 of 23 mice examined) and this encourages us to explore the possibility that SV40 could serve as a useful vector for introducing genes into living animals.

The presence of free SV40 DNA in two litters and the foster mothers of these same litters is most readily

explained as the result of a persistent viral infection. No proof of virus production has been obtained as yet nor have the cell type(s) infected been identified. This observation warrants further attention in light of the fact that all mouse cells in tissue culture are nonpermissive for SV40 production. Perhaps some cells in the developing mouse embryo, when infected early enough, can support virus multiplication and hence start an infection in the mother.

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NUCLEIC ACID CHEMISTRY

R.J. ROBERTS, J. Brooks, P. Bullock, R.E. Gelinas, T.R. Gingeras, M. Kelly, M. Moschitta, P.A. Myers, D. Sciaky, M. Wallace, G. Wong, C. Yen, B.S. Zain

Following the discovery of RNA splicing in 1977, the efforts of this laboratory have been directed toward the determination of sequences within the adenovirus-2 (Ad2) genome that are involved in splicing. Initially, our interest centered on those three regions of the Ad2 genome (coordinates 16.6, 19.6, and 26.6) that encode the three leader segments of the major late rightward transcription unit. However, it soon became apparent that many other regions of the Ad2 genome give rise to mRNAs that are processed by splicing. Clearly, these regions also needed to be analyzed at the primary sequence level before a complete description of Ad2 splicing would be possible. Consequently, during the summer of 1978, it was decided to concentrate the efforts of our laboratory on a project aimed at determining the complete primary sequence of the Ad2 genome. This sequence would not only provide a basis for an understanding of RNA splicing, but also could ultimately be linked to the plethora of biological information being gathered by other groups at Cold Spring Harbor Laboratory.

Ad2 sequencing

The basic strategy with which we have tackled this project was outlined in last year's Annual Report and has not changed significantly during the course of this year. The large size of the Ad2 genome, which we now believe to be in excess of 36,000 bp, has meant that the work this year has been involved primarily with data gathering. The present status of the information available is shown in Table 1.

In brief, essentially complete sequence information is available from at least one strand of the genome for the region from 0% to 37.3% of the genome and from 89.7% to 100% of the genome. These sequences are presently being confirmed by obtaining sequence data

from the complementary strand. This process is both straightforward and efficient using the chain-termination method because the map positions of appropriate primers can be predicted from the first strand sequence. The remaining regions of the genome still require more data gathering before the first strand sequence can be completed. The sequence from coordinate 73.6 to coordinate 75.9 is a part of the EcoRI F fragment whose sequence has already been reported (Galibert et al., *Gene* 6:1 [1979]).

More than 200,000 nucleotides of sequence have been derived so far and have yielded more than 30,000 nucleotides of unique sequence. The manipulation and analysis of this amount of data have required the use of computer methods and these are described below. Although this year has mainly been devoted to data collection, some analysis of these data has already been possible. In particular, the three regions of the Ad2 genome around coordinates 16.6, 19.6, and 26.6, which encode the major leader components of late mRNAs, have been sequenced and the positions of splicing within these sequences have been determined. In the case of fiber mRNA, some mRNA species have an extra leader component and the sequences surrounding its coding position have also been deduced. It has been possible to locate and sequence the region of the Ad2 genome encoding the main body of fiber mRNA and to then locate the position at which this main body becomes spliced to either of the two leader conformations. By comparing the genome sequence within this main body of fiber with N-terminal protein sequence data (C.W. Anderson and J.F. Lewis, pers. comm.), we have located the start of the fiber-protein coding sequence.

Like most other eukaryotic mRNAs, we have found that the sequences at the splice points of Ad2 mRNAs contain the dinucleotides GU at the 5' end of interven-

Table 1

Region of Ad2 genome (coordinates)		l-strand sequence (%)	r-strand sequence (%)
HindIII G	(0 - 7.9)	99.9	>50
HindIII C	(7.9-17.0)	99.9	>80
HindIII B	(17.0-31.5)	95.0	10
HindIII I	(31.5-37.3)	99.0	0
HindIII J	(37.3-41.0)	80.0	0
HindIII D	(41.0-50.1)	40.0	0
HindIII A	(50.1-73.6)	10.0	>70
EcoRI D	(75.9-84.0)	0	>90
EcoRI E	(84.0-89.7)	0	>50
EcoRI C	(89.7-100)	>50.0	99

ing sequences and AG at the 3' end of intervening sequences. There are no other obvious sequence homologies around these splice points and, indeed, the sequences surrounding these late Ad2 splice points are considerably different from the "preferred" sequence suggested by Chambon and his coworkers (*Proc. Natl. Acad. Sci.* 75:4853 [1978]). We have also examined the possible RNA secondary structures that might be adopted by the RNA in the region of these splice points. Although some interesting features have emerged, there is as yet no conserved feature that might suggest a molecular basis for the specificity of splicing.

We have also examined the possibility, first suggested by L. Phillipson (unpubl.), that VA RNA might act as a template for the splicing process. We can find no obvious homology between VA RNA and the splice points, which would suggest a simple template function. Furthermore, again using computer predictions of secondary structure, we find that VA RNA could adopt a particularly stable secondary structure with rather few single-strand regions present and available for hybridization.

One early mRNA splice point is now known. N. Stow (Tumor Virus Section) has partial sequence data from a cloned cDNA copy of an early mRNA and, by comparison with the genomic sequence present in *EcoRI* fragment C, the splice point has been deduced. This mRNA is from early region 4 and contains leader sequences encoded around coordinate 99 that have become joined to sequences beginning at coordinate 96. Although the sequences around the splice point contain the usual GT, AG dinucleotides at their ends, they bear little resemblance to any of the other junction sequences present in Ad2. The sequences present in the leader around coordinate 99 do not contain an AUG initiation codon and the first such codon appears within the first 50 nucleotides beyond the splice point at coordinate 96.

Computer programs

From the inception of this project, it was apparent that the amount of data involved was too great to allow its ready manipulations without automated methods. Because such computer methods were not available, we undertook to develop our own software specifically designed to handle large amounts of sequence data. We were fortunate to obtain the help of a professional programmer, Mr. John Milazzo, who is a full-time employee of the Computing Center at Stony Brook. Much of his spare time over the last year has been devoted to collaborating with us in the development of computer strategies for the assembly and analysis of DNA sequences. At the present time, sequences are transcribed manually from the original autoradiograms and then entered manually into the computer.

Two copies are made within the computer, one entering a master archive and the other entering a working file. As data accumulate within the working file, the various strings of nucleotides can be compared and searched for identical or complementary stretches. Once such stretches have been identified, they can be used to join together the original strings of nucleotides and thus the original, short strings of nucleotides gradually become fused into a long, continuous string that ultimately will be the sequence of the entire Ad2 genome. A variety of subroutines are available to manipulate the various strings of nucleotides including, for example, programs to locate restriction enzyme sites.

Aside from the ease of manipulating large amounts of sequence data by computer methods, the most significant advantage of this approach is the fidelity with which one can make copies of any given piece of sequence information. Even the most careful individual makes errors when manually copying a sequence from one place to another. From our own experience we have found that, because of the manual steps involved in transferring information from the original autoradiograms into the computer, errors can accumulate at a rate of about 1 per 200 nucleotides. We are presently exploring a method that we hope will eliminate this source of error. This new method makes use of a device called a "digitizing tablet" upon which is placed the autoradiogram. The digitizing tablet is connected electronically to a pen, such that when the pen touches it, the x-y coordinates of the point of contact are recorded. By then using the pen to touch the individual bands within a sequencing gel, their x-y coordinates are recorded and can be translated into a sequence by an appropriate program. Such a program is being developed at the present time. In addition, a number of modifications to the existing programs for the assembly of DNA sequences will also be made in the coming year so as to reduce the amount of manual intervention necessary to convert primary data into a complete sequence. The programs that we have written so far are of general application and have been made available to other members of the scientific community.

Restriction endonucleases

The collection of restriction enzymes continues to grow and more than 200 such enzymes are now known and more than 70 different specificities are represented. Among the new specificities, the recognition sequences of *DdeI* (C↓T_NAG) and *XhoII* (Pu↓GATCPy) have recently been characterized in this laboratory. In addition, we have shown that *BacI* and *EccI*, first isolated by H. Mayer, are isoschizomers of *SacII* (CCGCGG) and *MsiI* is an isoschizomer of *XhoI* (CTCGAG). We have also shown that three other en-

zymes, *Sbol* and *Sbri*, isolated by H. Takanami, and *Shyl*, isolated by F. Walter, are isoschizomers of *XhoI*. Finally, we have shown that *XnII* is an isoschizomer of *PvuI* (CGATCG).

Using recombinant DNA techniques, we have attempted to transfer the genes for a number of restriction endonucleases into *Escherichia coli*. Initially, we hoped to transfer and select these genes directly; however, our experience would suggest that, in general, this will not be possible. Presumably, this is because of the acquisition of a powerful specific endonuclease, which, even when accompanied by its cognate methylase, still results in the destruction of the host cell DNA. To try to overcome this difficulty, we have now concentrated our attention upon the prior cloning of the methylase genes. Consequently, we have carried out a number of experiments to characterize the patterns of methylation that serve to protect DNA against the effects of certain restriction endonucleases. Among enzymes that recognize sequences containing the tetranucleotide GATC, we find that methylation of the A residue within the sequence protects DNA against the effects of *MboI* (GATC), *BclI* (TGATCA), and *CpeI* (TGATCA). On the other hand, methylation of the C residue within this sequence protects DNA against the action of *BamI* (GGATCC), *BglII* (AGATCT), *PvuI* (CGATCG), *XhoII* (PuGATCPy), and *Sau3A* (GATC). Thus, by cloning the genes for the *Sau3A* methylase and the *dam* methylase of *E. coli* (which methylates GATC), we should obtain hosts in which it will be possible to clone all of these restriction endonucleases. Recently, we have successfully isolated clones containing the *dam* methylase of *E. coli*.

During the course of these studies of methylase specificity, we have encountered an unusual situation. It has previously been shown that the restriction endonuclease, *HpaII*, recognizes the sequence CCGG but cannot cut at that sequence if the internal C residue is methylated. However, if the external C residue is methylated, or if neither C residue is methylated, the enzyme will cleave that sequence. Another restriction endonuclease, *MspI*, also recognizes this same sequence. In contrast to *HpaII*, this enzyme will cleave the sequence when the internal C residue is methylated, and so it was assumed that protection would be afforded by methylation of the external residue. This turns out not to be the case. When DNA from the *Moraxella* species, which is the source of *MspI*, is prepared, it is found to be resistant to the action of both *MspI* and *HpaII*. Because *HpaII* can cleave DNA in which the external cytosine is methylated, we are faced with the conclusion that the modification responsible for protecting *MspI* DNA against the action of *MspI* must involve either the methylation of both C

residues or some other kind of modification. In general, relatively little is known about the types of modification that serve to protect the host DNAs against the effect of their own restriction endonucleases, but in those cases that have been studied, it has always been found to involve methylation. It could be that *MspI* will prove to be the first example of an organism using a different kind of modification.

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The Electron Microscopy Section has continued to study the transcription and splicing of adenoviral RNAs and the replication and transposition of bacteriophage Mu DNA. In doing so, we have combined electron microscopic analysis with the techniques of DNA cloning, restriction enzyme mapping, and polynucleotide sequencing. In addition, we have collaborated with others to align the physical maps of the chromosomes with the genetic and functional maps.

Adenoviral RNA synthesis

Adenoviruses cause throat infections or conjunctivitis in people and can grow lytically in human cell culture. Although they are unable to complete the infectious cycle in rat cells, the several classes of human adenoviruses elicit, to varying degrees, transformation of these cells and tumorigenesis in rodents. Hence, they are bona fide tumor viruses and have been studied intensely in their own right and as model systems for eukaryotic gene expression:

The human adenovirus-2 (Ad2) chromosome consists of 36,500 bp of DNA. After infection of permissive cells, the viral genome is transcribed into different sets of RNA molecules at early (1–8 hr), intermediate (8–16 hr), and late (16–30 hr) times after infection. For the past several years, we have been mapping the chromosomal locations of the viral transcripts and, together with others at Cold Spring Harbor and elsewhere, we have found that almost all cytoplasmic RNAs are derived from discontinuous segments of the genome. This spliced structure is generated from continuous primary transcripts of DNA by the deletion of intervening sequences and the religation of the conserved segments that constitute functional RNAs.

The map coordinates, splicing patterns, and relative abundances of the early, intermediate, and late RNAs were described in previous Annual Reports. One of the more interesting RNAs at intermediate times is an *r*-strand transcript with main body sequences from coordinates 30.5–39.0, which encodes a 52K protein (J. Miller, R. Ricciardi, B. Roberts, B. Paterson, and M. Mathews, pers. comm.; Lewis et al., *Cold Spring Harbor Symp. Quant. Biol.* 44:[1980]). This RNA has the tripartite leader that is found on all the other products of the *r*-strand transcription unit or, more frequently, a four-part leader (Chow et al., 1979, 1980). This extra segment, which we call the “i” leader, is derived from genome coordinates 22.0–23.2, between the normal second and third leaders (Fig. 1). The *i* leader can also be found in all the other *r*-strand messages, especially at intermediate times after infection.

In the presence of Ara C (an inhibitor of DNA replication), the production of the 52K transcript is dramatically elevated, at the expense of the dozen other possible products we have previously characterized (Chow and Broker, *Cell* 15:497 [1978]). Furthermore, about 85% of the 52K RNA and of the other *r*-strand RNAs have the four-part leader. Molecules with this extra leader might be RNA splicing intermediates. To test this possibility, Kilpatrick has cloned short segments of the Ad2 chromosome that contain various combinations of the coding regions for the late RNA leaders. These will be hybridized to radioactively labeled RNA synthesized *in vivo* to follow their kinetics of appearance, interconversion, and turnover in an attempt to define the precursor-product relationships.

Using cell-free translation of RNA, Jim Lewis and coworkers identified a 13.6K protein that is made at intermediate times and is encoded near map coordinate 20. Extensive electron microscopic examinations of the RNA by Louise Chow revealed that the only likely segment that could be correlated with the 13.6K protein was the *i*-leader segment. More recently, one of Kilpatrick's cloned Ad2 segments containing the coding sequences for the *i* leader (but no other leaders) was used successfully by Lewis to select the 13.6K RNA. This strongly supports the notion that the leader is a message—a novel situation—and provokes speculation about the role of the 13.6K protein in the early-to-late transition in gene expression.

Ad2 RNA synthesis in monkey cells

Klessig and Anderson (*J. Virol.* 16:1650 [1975]) and Farber and Baum (*J. Virol.* 27:136 [1978]) have shown that the block to the growth of human Ad2 in monkey CV-1 cells can be attributed, in part, to a reduced concentration of certain late mRNAs in the cells. The most severe reduction is in the mRNA for the fiber protein; it seems to be anomalously affected because the fiber protein level is even more reduced than that of the RNA. The nature of this defect was examined by electron microscopy of fiber (and other late) RNA in collaboration with D. Klessig (Klessig and Chow, 1980). A major manifestation of the defect is evident at the level of RNA splicing. About half of the fiber transcripts are incompletely spliced and have long RNA sequences remaining between the tripartite leader and the main body of the fiber message (Fig. 2). Because only the 5'-proximal message in polycistronic eukaryotic RNA is translated (Kozak, *Cell* 15:1109 [1978]), such long fiber RNA would not be expected to produce fiber protein. A mutant of AD2

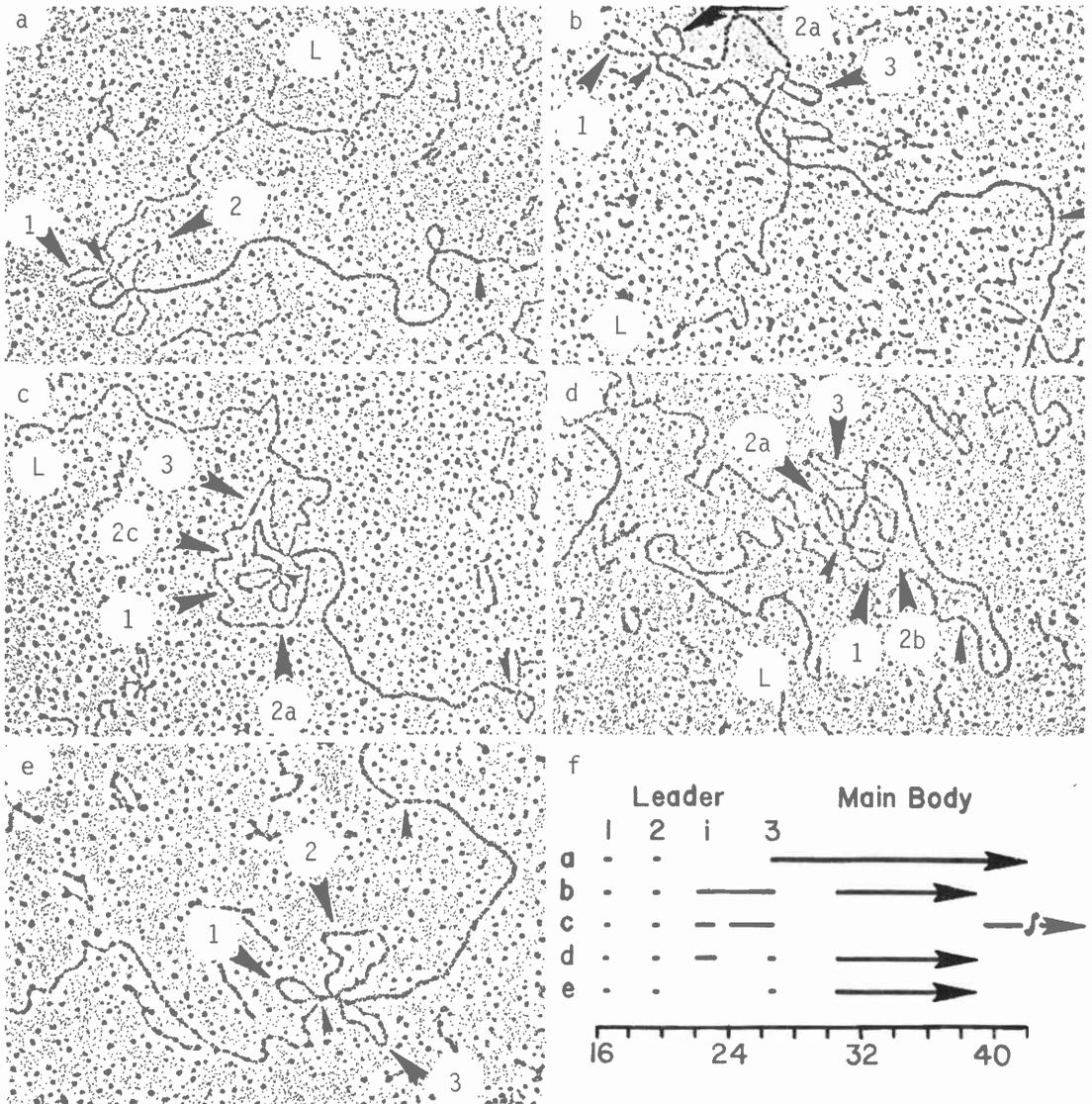


Figure 1
 Heteroduplexes of single-stranded Ad2 DNA with late cytoplasmic RNAs having abnormal 5' leader segments. (a) A rare cytoplasmic RNA with normal first and second leader segments. The sequence between the third leader and the main body of the message has not been removed; thus, there are only two leader loops, 1 and 2. (b) An RNA (coordinates 30.5–39.0) for the 52K protein which has normal first and second leaders and a long third leader from coordinate 22.0 to coordinate 26.8, forming leader loops 1, 2a, and 3. (c) An RNA with a normal first and second leader, an *i* leader, and a longer-than-normal last leader (coordinates 24.0–26.8). The leader loops are labeled 1, 2a, 2c, and 3. The 5' end of the main body of the RNA is located at coordinate 39.0 (d) An RNA for the 52K protein with a four-part leader 1, 2, *i*, and 3, generating leader loops 1, 2a, 2b, and 3. (e) An RNA for the 52K protein which has a normal tripartite leader constraining the DNA into leader loops 1, 2, 3. (f) Graphic representations of a through e.

(HR400) that grows in monkey cells (Klessig, *J. Virol.* 21:1243 [1977]) shows almost normal production of mature fiber RNA both in amount and in structure. The HR400 mutation maps in or near the gene for the DNA-binding protein (Klessig and Grodzicker, *Cell* 17:957 [1979]), implicating it in the RNA-splicing process. The tenfold reduction in the amount of fiber

message could be due to any of several defects: (1) premature termination of transcription, (2) failure to polyadenylate fiber transcript, or (3) instability of incompletely spliced fiber message. In last year's Annual Report, we presented some evidence suggesting that the tripartite leader is transferred sequentially down the primary transcript from the 5' end of one gene to

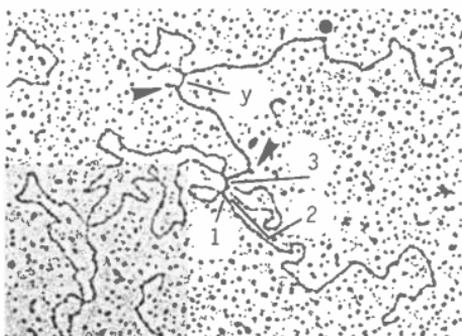


Figure 2
Heteroduplex of Ad2 DNA with incompletely spliced fiber RNA isolated from monkey CV-1 cells. The coordinates of the RNA are 16.5-16.6/19.5-19.7/26.5-26.8/72.5-73.5/73.9-77.6/78.6-79.1/86.2-91.3. In the heteroduplex, the seven conserved segments are separated by six deletion loops corresponding to the intervening sequences. Leader segments 1, 2, 3, and y are labeled, two small deletions loops are indicated by arrowheads, and the 3' end of the RNA is indicated by a dot. Mature fiber RNA contains only four segments, the first three leaders and the main body from coordinates 86.2-91.3.

the next. It appears that splicing to the leader to the main body in monkey cells is very slow or is blocked in the vicinity of the 100K-33K-pVIII gene family. This is compatible with our splicing model.

RNA synthesis by class-B adenoviruses

Last year our laboratory showed that the late transcripts of class-B adenoviral serotypes Ad3 and Ad7 have virtually the same coordinates as those mapped for Ad2, a class-C adenovirus (Kilpatrick et al., 1979; Chow and Broker, *Cell* 15:497 [1978]). The leader sequences of RNAs within the same class (Ad1, Ad2, Ad5, Ad6 or Ad3, Ad7) will anneal with DNA from

another member of the same class; however, they will not anneal with DNA from another class, indicating sequence divergence even though the locations of the coding regions are highly conserved.

To examine these leader divergences in greater detail, Engler and Kilpatrick have cloned DNA restriction fragments of Ad3 and Ad7 into the pBR322 plasmid vector. Engler has further characterized them by re-cloning and by detailed restriction endonuclease mapping. DNA sequence analysis of Ad3 and Ad7 clones containing the first leader coding sequences has been carried out. Examination of the sequence indicates an unexpectedly close homology with the Ad2 sequence of the same region (Fig. 3). Green et al. (*Virology* 93:481 [1979]) have reported that Ad3 and Ad7 share only about 15% homology with Ad2, based on retention on hydroxyapatite after solution hybridization. However, Engler finds that over 80% of the nucleotides are identical. There are very few deletions or insertions and most changes are transitions or transversion. By analogy to the Ad2 sequence, the Ad3 and Ad7 DNA sequences coding for the first leader (41 nucleotides) have been recognized and there are six differences between Ad2 and Ad7 or Ad3. The splice junctions are identical, but differences are observed as close as the third and fourth nucleotides into the intervening sequence. The TAT(A)_n sequence indicative of eukaryotic promoters (D. Hogness, pers. comm.) is identical for the Ad2, Ad7, and Ad3 r-strand transcription units.

An unexpected dividend of the few observed differences between the viruses comes from examination of the potential secondary structures in the sequence. Ziff and Evans (*Cell* 15:1463 [1978]) noted, in the single-strand DNA, a potential stem-loop structure in the vicinity of the promoter and the 5' end of the late Ad2 RNA. This cannot exist in Ad7 or Ad3 due to 8-bp changes (Fig. 4). But a different stem-loop structure can be drawn precisely between the promoter and the 5' end of the RNA (Fig. 4a). In fact, an analogous structure also exists in the Ad2 DNA sequence (Fig.



Figure 3
Comparison of the Ad7 and Ad2 DNA sequences in the vicinity of the major late promoter and the first leader segment (coordinates 16.5-16.6). The Ad7 sequence was determined by Jeff Engler and the Ad2 sequence by Ziff and Baker (*Cold Spring Harbor Symp. Quant. Biol.* 44 [1980]). Sequence differences are indicated by the shaded areas and reveal less than 20% divergence of the leader as well as of the flanking regions.

Table 2*General patterns of adenoviral RNA splicing*

1. Almost all mature mRNAs are derived from two or more discontinuous segments of the genome.
2. Splices may remove a few nucleotides (e.g., about 100 in early-region-1A and -1B RNAs) to many (21,700 between the third consanguinous leader segment and the fiber message).
3. The first two nucleotides of a deleted intervening sequence are GU and the last two are AC, as in virtually all eukaryotic RNA splicing (based on sequencing studies in many laboratories).
4. Splices are faithful to the nucleotide; they can correctly join coding regions. (Early regions 1A, 1B.)
5. Splices can also be upstream of coding regions (between leader segments) or downstream of the translated portion of the message. (Early region 1B.)
6. Conserved segments are always in the same order as in the primary transcript of the genome, suggesting a directional, intrastrand event.
7. Most, if not all, splicing occurs in the nucleus.
8. The capped 5' end and the polyadenylated 3' end of each primary transcript are conserved through all splicing events, and all deletions are therefore internal.
9. The presence of the 5' cap nearby (within 50–100 bases) seems to potentiate certain splice sites; its absence seems to disqualify sites that can otherwise be used. (The early and the late leaders of region-2 transcripts are usually not present in the same mRNA.)
10. There are a number of early and late RNA families with common 3' ends. The sites of polyadenylation determine the sets of product messages derived from the precursor transcript such that the 5' end of the primary transcript is spliced to one of several sites relatively near the poly(A). (Early region 3, region-4–region-2 hybrid RNAs, and late *r*-strand transcripts; see Figs. 5 and 6.)
11. A site that is a normal downstream junction site can be bypassed if poly(A) is not nearby. (Region-4–region-2 hybrid RNAs.)
12. Segments that are conserved when present in one primary transcript may be spliced out when flanked by different 5' and 3' ends. (Early-region-3 fiber, early-region-4–region-2, and late *r*-strand mRNAs from different 3' families.)
13. Deletions are not necessarily processive in the 5'→3' direction; certain downstream splices can occur prior to other upstream splices. (Early region 4, late *r*-strand leader segments, and fiber transcripts.)
14. Some multiple deletions may occur in alternative orders. (Early region 4, late *r*-strand leader segments, fiber transcripts, and other late *r*-strand products.)
15. Splicing of certain segments does not necessarily occur in a single complete step, but can proceed by a series of partial deletions. (Early region 4, late *r*-strand leader segments, fiber transcripts, and other late *r*-strand products.)
16. Late tripartite leaders appear to undergo a gene-to-gene transfer during coupling to distal coding regions in polycistronic transcripts. The leaders are (apparently transiently) coupled to the 5' ends of upstream genes. (*r*-strand transcripts.)
17. Leader sequences and main body sequences may be spliced somewhat independently; they can be at different stages of maturation. (*r*-strand transcripts.)
18. Splice signals in separate transcription units can faithfully recombine if transcription readthrough puts them in the same RNA molecule. (Region-4–region-2, region-1A–region-1B, and late *r*-strand-unit–early-region-3.)
19. Many splice junction sequences can have alternative 5' or 3' splice partners. (All early regions, and major late *r*-strand transcripts; see Figs. 5 and 6.)
20. When a message switches from an early to a late promoter, splicing within the main body can continue in the same pattern despite different leaders. (Early regions 2 and 3.)
21. Splices that occur efficiently in one host may be inefficient in another host. (Region 3 and fiber: HeLa vs monkey cells.)
22. The rate or efficiency of splicing can depend on cellular growth conditions whether cells are grown in monolayers or spinner cultures.
23. Cycloheximide, an inhibitor of protein synthesis, depresses the appearance of the more extensively deleted members within a family of transcripts. (Early regions 1A, 1B, and 4.)
24. Adenoviral RNA splicing is catalyzed by host proteins, but some viral proteins play some role in the process at intermediate to late times after infection.
25. The designations "intron" and "exon" are inappropriate for adenoviral transcripts since most segments can serve both roles, depending on the message.

References to nucleotide sequence analyses: van Ormondt et al., *Gene* 4:309 (1978); Maat and van Ormondt, *Gene* 6:75 (1979); Maat et al., *Gene* 1980; Akusjarvi and Pettersson *Cell* 16:841 (1979); Zain et al., *J. Mol. Biol.* 135:413 (1979); Perricaudet et al., *Nature* 281:694 (1979); Perricaudet et al. *Proc. Natl. Acad. Sci.* (1980).

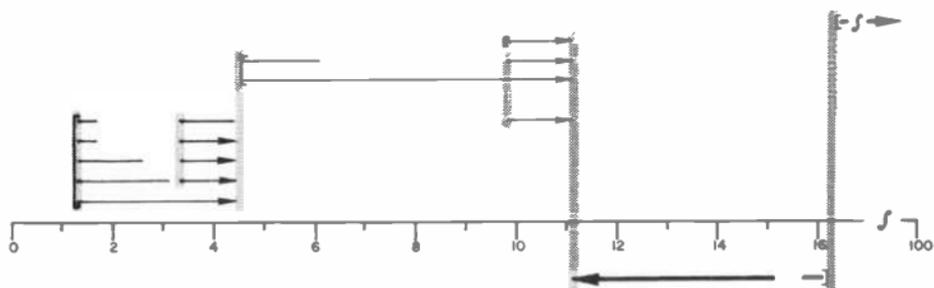


Figure 5: Coincident RNA processing sites in the *Ad2* chromosome between map coordinates 0.0 and 17.0. Arrows indicate the direction of transcription along the r strand or l strand of the DNA. The conserved segments constituting early RNAs (Chow et al., 1979) are depicted by thin arrows and those in late RNAs (Chow and Broker, Cell 15: 497 [1978]) by thick arrows. Gaps in arrows represent intervening sequences removed from the mRNAs by splicing. The short deletion in the region-1B RNA was detected by Berk and Sharp (Cell 14: 695 [1978]) using a nuclease-digestion assay. Short DNA or RNA regions that serve multiple roles in the synthesis and splicing of different RNAs (derived from either the same strand or opposite strands) are indicated by the stippled areas.

tions in various RNAs transcribed from the same strand or from opposite strands were discussed by Broker and Chow (1979) and are emphasized in Figures 5 and 6. It is remarkable that RNA synthesis and processing signals are so closely clustered in the short segments

between protein-coding regions. The promoters and 5'-leader segments of the late RNAs for the 72K single-stranded DNA-binding protein overlap protein-coding regions on the opposite strand, the first examples of such an overlap.

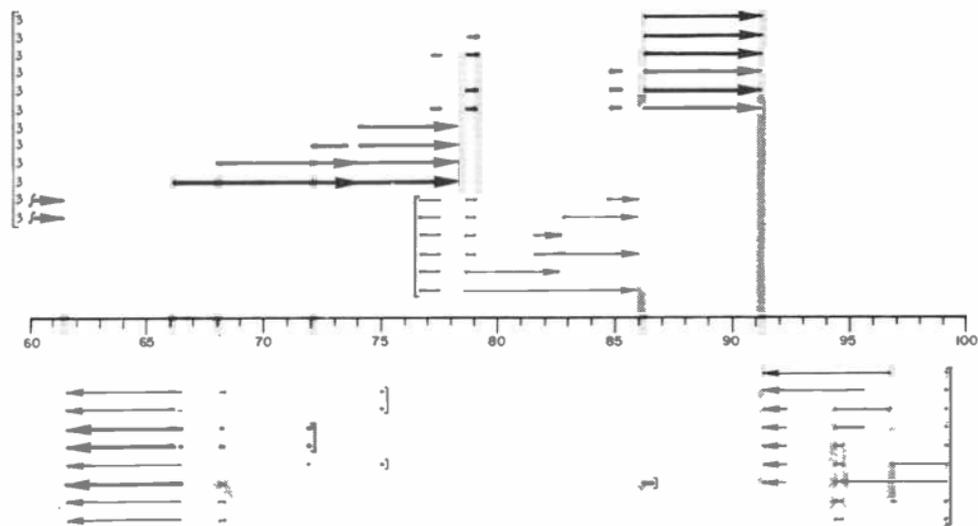


Figure 6 Coincident RNA processing sites in the *Ad2* chromosome between map coordinates 60.0 and 100.0. The conventions described for Fig. 5 are maintained. All late r-strand RNAs have a tripartite leader (3) derived from genomic coordinates 16.5–16.6/19.5–19.7/26.5–26.8. The fiber RNAs have multiple forms that include the consanguinuous tripartite leader and all combinations of ancillary leaders x, y, and z, which coincide with segments in early-region-3 RNAs. The region-3 RNAs originate from a promoter at coordinate 76.6 at early times and can be transcribed from the major late promoter (coordinate 16.5) at late times. The single-stranded DNA-binding protein RNAs are transcribed from different promoters, two active primarily at early-early times and two at intermediate to late times. Each DNA-binding-protein RNA can be observed in an alternative form with a small splice at coordinate 66.3.

Cloning and mapping of specific bacteriophage Mu DNA restriction fragments

Partial correlations of genetic and physical maps of bacteriophage Mu are obtained by determining the Mu DNA segment present in λ pMu transducing phages (Magazin et al., *Virology* 77:677 [1977]) or by defining the genetic markers in certain Mu DNA restriction fragments cloned into bacteriophage λ or plasmids (Moore et al., in *DNA Insertion Elements, Plasmids, and Episomes*, p. 567, Cold Spring Harbor Laboratory [1977]). To extend such correlations, Jeff Engler has cloned EcoRI-BamHI restriction fragments of Mu DNA. These clones have been characterized by electron microscopic heteroduplex methods and by restriction endonuclease digestion and gel electrophoretic assays, as well as by genetic complementation tests. In collaboration with R. Forgie and M. Howe, additional genetic markers were tested and the two BamHI cleavage sites in Mu DNA have been placed within cistrons E and F.

The relationships between bacteriophage Mu and D108

Phage D108 is a Mu-like phage, but it is heteroimmune. It is closely related to Mu in both DNA sequence and genetic properties (Hull et al., *J. Virol.* 27:513 [1978]). By electron microscopic heteroduplex analysis, we have detected two regions of the genomes which are nonhomologous, in addition to three others previously described (Fig. 7). One of the newly found nonhomologies is a 200-nucleotide-pair substitution located in the invertible G segment, which has been postulated to encode the tail fiber of the phage. This sequence divergence might explain the differences in the host ranges of Mu and D108 (Howe, *Virology* 55:103 [1973]; Hull et al., *J. Virol.* 27:513 [1978]). The other identified difference between the two genomes is an insertion of 0.5 kb in the β segment of D108 relative to that of Mu. The genetic consequence of this insertion is unknown.

Upon coinfection of sensitive cells with Mu and

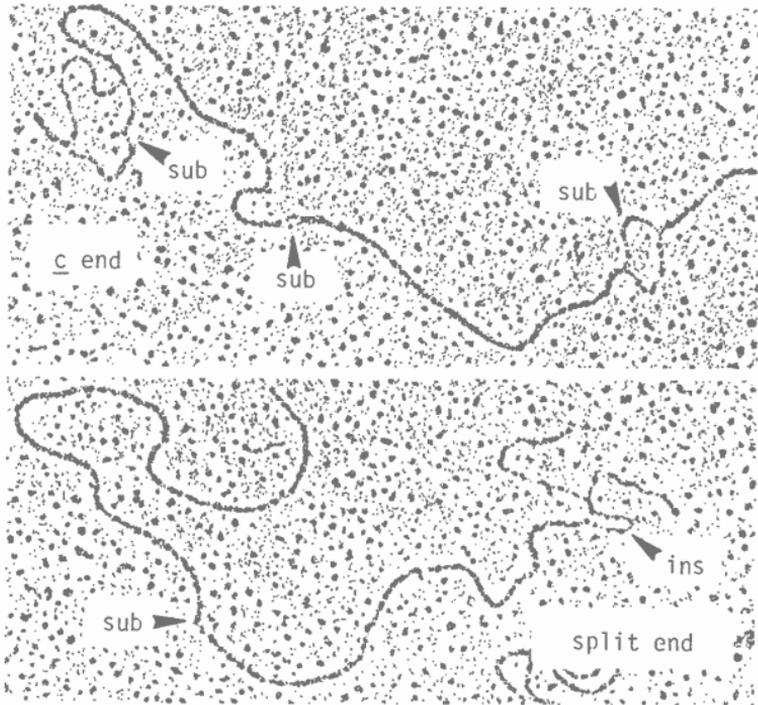


Figure 7

Heteroduplexes of DNAs from phages Mu and D108. (Top) Three substitution loops near the left end of the genomes at coordinates 0.3–4.1%, 13.1–13.5%, and 20.5–22.3%; (bottom) a substitution loop within the invertible G segment in the (+) orientation at coordinates 89.6–90.1% and an insertion of 500 nucleotides in D108 (relative to Mu) in the beta-region near the right (split) end at 99.7%.

D108, only 0.5–4% of the progeny are Mu. This is similar to the exclusion phenomenon observed with the related T3 and T7 phages or the T-even phages. However, when Mu-infected cells are superinfected with D108 after a 5-minute delay, mixed bursts are obtained in which 80–95% of the progeny are Mu. The molecular basis for these observations is under investigation.

We have also found that the replication-transposition system of D108 complements that of Mu poorly. Cells harboring a mini-Mu genome (consisting of the two terminal segments of Mu) replicate and transpose only rarely when the host cells are superinfected by D108. This mini-Mu has been shown to replicate and transpose efficiently in the presence of a Mu helper (D. Kamp, R. Kahmann, and L. Chow, unpubl.).

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PROTEIN SYNTHESIS SECTION

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In the past year, studies in the Protein Synthesis Laboratory have concentrated on the proteins of the adenoviral particle and of cells productively infected by the virus or transformed by it. Several areas have received special attention, including (1) the proteins encoded by the left end of the viral genome, (2) the interactions that contribute to the regulation of viral gene expression, and (3) the properties of small virus-coded RNA species. Identification of viral proteins and correlation of the proteins with their cognate mRNAs and genes is now well advanced, particularly in the case of the structural components of the viral particle produced late in infection. Extensive progress has also been made in recognizing the viral products synthesized in the early phase of the infectious cycle, before viral DNA replication has occurred, but it is clear that there is still some way to go before the catalog is complete. Transformed cells need express only one or two of the viral genes, although four early genes have been found to exert at least some influence over the transformation process. It is in this aspect of viral gene expression that some of the most profound unanswered questions persist.

Our analyses are based on the translation of mRNA from infected or transformed cells in vitro, using a cell-free system from rabbit reticulocytes rendered dependent on exogenous mRNA by treatment with micrococcal nuclease. The mRNA is preselected by molecular hybridization and/or by size fractionation to provide information on its locus of origin and to

permit correlation with RNA species characterized by other means. The proteins generated in vitro are identified by SDS gel electrophoresis and compared with their counterparts made in vivo by such methods as peptide mapping, two-dimensional electrophoresis, and immunoprecipitation.

Discovery of "new" viral proteins

Several additional virus-coded polypeptides have come to light in the last year using these techniques. It is convenient to introduce the salient facts here because these products will figure in the subsequent discussion.

E1A—28K: The smallest of the E1A proteins, with an apparent molecular weight of 28,000, is translated from the smallest mRNA from this region. It comes to account for a substantial share of the early-region-1A (E1A) protein synthesized at late times in infection (see Figs. 1 and 2).

E1B—58K: Under most conditions this is a minor in vitro product, first seen by Halbert et al. (*J. Virol.* 31:621 [1979]), although in vivo it appears to be a major component of T antigen. We are presently investigating its relationship to other viral proteins.

E1B—18K and 22K: These two polypeptides are made in vitro in small amounts relative to the 15K protein from the same region. Their mRNAs are selected by hybridization to a DNA fragment from the right end of the E1B region, but nothing is known

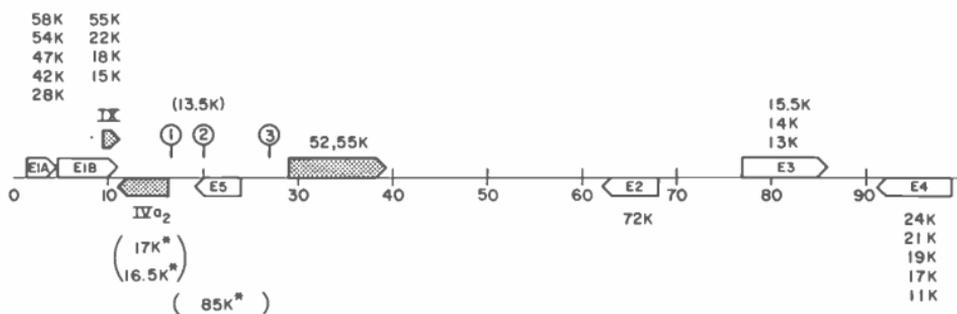


Figure 1

Adenoviral proteins synthesized in the absence of viral DNA replication. The conventional early gene blocks, E1–E4, and the newly positioned E5 block which contains a conventional early gene, are shown as unshaded arrows. Stippled arrows represent genes falling into the "intermediate" category. The direction of each arrow gives the polarity of transcription in each gene block. Polypeptides translated in vitro from RNA deriving from each gene block are listed above or below their point of origin. Parentheses and asterisks signify that precise map locations and polarity assignments, respectively, have not yet been made in some instances. The circled numbers 1–3 mark the loci of the elements of the tripartite leader associated with the 52K, 55K mRNA and late mRNAs.

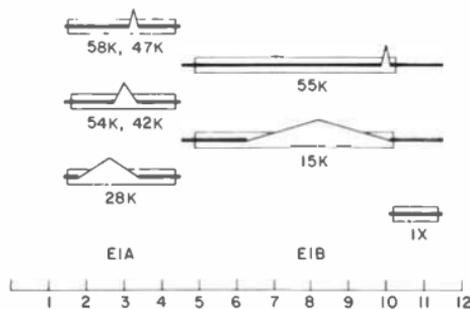


Figure 2
Coding assignments for early-region-1 mRNAs. The thick lines represent the map locations of the sequences and splices contained in the E1A and E1B mRNAs (drawn from several sources). The proteins encoded by each RNA species are indicated, and the boxed areas show the likely extent of the coding regions.

about the size of the mRNA or the precise location of its coding region.

16.5K and 17K: The RNAs encoding these polypeptides map within the region that codes for the IVa2 protein at 11–17 map units. The proteins have been detected as *in vitro* translation products of RNA from high-multiplicity infections made in the presence of cytosine arabinoside (Ara C, an agent that blocks DNA replication and prevents the appearance of the conventional late mRNAs though not of IVa2 mRNA), but not in preparations made in the presence of cycloheximide (which blocks protein synthesis as well as DNA replication and late RNA production). Neither the precise location of the coding regions for these proteins nor their relationship to each other and to component IVa2 is known at present.

13.5K: This polypeptide is made from an *r*-strand transcript selected by a DNA fragment located between 17 and 22 map units. It may represent the leftmost protein encoded by the major late transcription block running from map position 16.4 toward the right end of the genome. The mRNA is selected by DNA containing the tripartite leader sequence that is present on most late mRNAs, but we do not yet know whether the 13.5K protein RNA is initiated at the promoter used by the major late block. It is found in largest amounts in preparations isolated at late times after infection and can be seen in many early RNA preparations, including ones made from cells almost totally blocked for protein synthesis.

85K: The DNA fragments located between 17–22 and 22–26.5 map units select RNA that is translated into a polypeptide with a molecular weight of 85,000, coincident with the late structural protein III (penton base). Although the mRNA for the latter could hybridize to the same fragments by virtue of its tripartite

leader sequences, the 85K polypeptide is detected in the translation products of early RNA, which fails to evidence any of the other late proteins. Accordingly, we consider the 85K early protein a likely candidate for the gene product of the newly mapped early region 5, defined by mutants of the ts36 group (Galos et al., *Cell* 17:945 [1978]).

52K, 55K: This pair of closely related polypeptides constitutes the leftmost product of the late gene block as conventionally defined. The 52K, 55K-protein mRNAs possess the tripartite leaders and map between 29 and 39 map units, with coding sequences between about 30 and 34 map units. However, the mRNA encoding these proteins is unconventional in that it is readily detectable in preparations made in the absence of viral DNA replication, whereas the other mRNAs of the major late transcription unit remain cryptic.

Early region 1A

Studies in other laboratories have shown that the products of this region constitute the core of the transforming potential of the virus (van der Eb et al., *J. Supramol. Struct.* [Suppl. 3]: 128 [1979]) and also (perhaps by related activities) act as positive effectors to promote the expression of other early viral genes (Berk et al., *Cell* 17:935 [1979]; Jones and Shenk, *Proc. Natl. Acad. Sci.* 76:3665 [1979]). By cell-free translation of RNA isolated from adenovirus-2 (Ad2)-infected cells, we have identified five polypeptides encoded in this region, with apparent molecular weights of 58,000, 54,000, 47,000, 42,000, and 28,000. In the closely related Ad5 strain, the corresponding polypeptides migrate with apparent molecular weights of approximately 2000 greater, but for simplicity we will use the Ad2 designations throughout this report. Translation of early RNA, made at 6 hours postinfection in the presence of cycloheximide or at 16 hours in the presence of Ara C, yields predominantly the four largest E1A proteins and relatively little of the 28K polypeptide. In contrast, late RNA made at 20 or 29 hours postinfection codes for reduced amounts of the four large proteins, whereas the synthesis of the 28K polypeptides rises to levels comparable with the other proteins from this block.

Electrophoresis through agarose gels in the presence of methylmercury hydroxide separates three size classes of E1A mRNAs. The largest species, centered at 1.05 kb, directs the synthesis of the 58K and 47K polypeptides; the second largest species, 0.9 kb, codes for the 54K and 42K polypeptides; and the smallest RNA species, 0.6 kb, encodes the 28K polypeptide. The observation that the four large proteins are translated from only two size classes of RNA is open to several interpretations. One possibility is that there are indeed only two species of mRNAs, and

the pairs of polypeptides that derive from each one are generated by anomalies in translation or by posttranslational modification. Alternatively, both mRNA size classes might contain two comigrating species each coding for a unique polypeptide. We have not yet arrived at a definitive resolution of this question, but experiments with E1A mutants (described below) confirm the pairwise behavior of the proteins and locate the source of the duality between 3.7 and 4.5 map units. This issue aside, the results from our translation experiments correlate readily with data from this and other laboratories on the structures and kinetics of appearance of E1A mRNAs, permitting assignment of proteins to individual RNA species (see Fig. 2).

Mutant E1A proteins

Two sets of mutants of adenovirus that are defective for growth in HeLa cells contain lesions in the E1A region and give rise to aberrant proteins. RNA from cells infected with the host-range mutant *hr1* (Harrison et al., *Virology* 77:319 [1977]) fails to produce the 58K and 42K polypeptides from region E1A, but directs the synthesis of the 54K and 42K pair together with a rather diffuse group of polypeptides with apparent molecular weights of 26,000–28,000. The RNA species encoding these shorter products correspond in size to the mRNA that in wild-type infections codes for the 58K and 42K proteins. Therefore, we conclude that the *hr1* mutation has generated a nonsense codon in the region between 2.8 and 3.2 map units that is specific for the largest species of E1A mRNA, resulting in the truncation of its normal products (58K and 42K) to 26K–28K.

Another group of host-range mutants contains viruses that carry deletions of the E1A region (Jones and Shenk, *Cell* 17:683 [1979]). In the most severe case (*dI312*) no viral messenger activity can be detected by cell-free translation. This virus is missing almost all of the E1A region and, by physical criteria, all viral mRNAs. The mutants *dI311* and *dI313* contain smaller deletions of the E1A region and produce mRNAs that encode altered proteins. Two polypeptides are made *in vitro* in each case, of sizes 38K and 34K (*dI311*) and 40K and 36K (*dI313*), in place of the four normal proteins of 42K–58K. Fractionation of the RNAs by gel electrophoresis suggests that the polypeptides from each mutant are made on separate mRNAs, corresponding to the species that in wild-type infections give rise to the four largest E1A proteins. Both of these mutants lack E1A sequences to the right of map position 3.7 and their mRNAs produce two proteins instead of two pairs of proteins. This suggests that the feature that causes each RNA to make a pair of proteins maps between 3.7 and 4.5 map units. In *dI313*, the deletion fuses part of the E1A and E1B regions, with the result that the E1A mRNAs, and probably the pro-

teins for which they code, contain some sequences from the E1B region. Additional minor polypeptides with molecular weights of 50,000–55,000 are seen in some preparations of E1A mRNA from *dI311* and *dI313* infections, but the origin of these polypeptides, which differ in the two viruses, is not understood.

Pleiotropic effects of E1A products

The E1A proteins have been clearly implicated in cell transformation, and in the last year it was reported that they also exert a controlling influence over the expression of other viral early genes. We have studied this point using the *in vitro* translation technique to estimate the amounts of viral mRNA present in HeLa cells infected with mutant viruses or with wild-type virus in the presence of inhibitors of protein synthesis.

In agreement with the results of others, we find that the *dI312* lesion produces the most severe obstruction to viral gene expression, and we have not detected the synthesis of any viral products whatsoever. The *hr1* mutation, which foreshortens one of the two pairs of E1A proteins, leads to a reduction in the amounts of mRNAs from the other early regions. In our hands the effect is quantitatively variable but always less dramatic than reported in the literature, the RNAs from regions E2 and particularly E4 being affected to a greater extent than those produced by the E1B and E3 regions. Therefore, it seems reasonable to conclude that the E1A region products include positive effectors required for the expression of the other early regions E1B, E2, E3, and E4. One tempting hypothesis that can be framed around the data is the speculation that the E1A 58K and 47K proteins are primarily concerned with the *l*-strand mRNAs of regions E2 and E4, whereas the responsibility of the E1A 54K and 42K products lies with the E1B and E3 *r*-strand products.

As far as this pleiotropic effect is concerned, the functionally significant parts of the proteins would seem to be encoded to the left of map position 3.7 on the genome, because only minor reductions of early mRNA concentrations are observed in cells infected with the *dI311* and *dI313* mutants whose deletions are to the right of this point. On the other hand, both of these mutants are defective in viral DNA synthesis, so the region lying between 3.7 and 4.5 map units presumably encodes a portion of the E1A proteins involved directly in replication.

The proposition that the E1A proteins are "pre-early" or "immediate early" viral products required to actuate other early genes has been probed by experiments using drugs to inhibit protein synthesis. Cycloheximide, the drug conventionally used for this purpose, gives rise to a pattern of gene expression not differing greatly from that observed in the presence of Ara C. However, as shown previously, it does markedly affect quantitative representation of the various

early mRNAs, the cellular concentration of many, but not all, early viral mRNAs being augmented—perhaps by interference with synthesis of a negative effector or via stabilization of mRNA against degradation. However, cycloheximide is only 95–97% effective in blocking protein synthesis *in vivo* and it could be that the residual activity suffices to produce adequate amounts of an immediate early protein. We have therefore turned to anisomycin, a more potent inhibitor of protein synthesis, to investigate this question. At 10 μM , anisomycin gives effects similar to those observed with cycloheximide, but, at 100 μM , protein synthesis is reduced to less than 1% of the control level and the pattern of early mRNA expression is grossly altered. The concentrations of most early RNAs, including those from region E1A and especially those from regions E2 and E4, are severely depressed, but the amounts of the mRNAs for the 13.5K and 52K, 55K polypeptides are unaffected. On the basis of these experiments, these proteins would be good candidates for immediate early status.

The “early-late switch”

Although the existence of a network of controls operating during the early phase has only recently been recognized, the conventional distinction between early and late stages of infection is well established. The transition between these phases does not occur in the absence of viral DNA replication, i.e., in the presence of inhibitors of early protein synthesis or of DNA synthesis, in infections with defective viruses or of nonpermissive cells, or simply chronologically prior to replication in the normal infection cycle. However, the nature of the link between replication and the transcription of late genes is obscure. We have employed a superinfection assay to examine the expression of viral genes introduced into cells already at a late stage of a prior infection and the effects thereon of metabolic inhibitors. This enables us to discriminate between two classes of hypothetical linking mechanisms: those that propose that the nature of the DNA template is the determining factor and others that emphasize concurrent alterations in the transcriptional apparatus of the cell.

In these experiments, we use a pair of closely related viruses that cross-complement for all known adenoviral functions, chosen such that the products of the initial infection can be distinguished from those of the superinfecting virus. The first virus is an Ad5 mutant, ϵ 125, conditionally defective in an early function, and the superinfection is carried out with a variant of Ad2, Ad2*ND1dp2, carrying two insertions of SV40 sequences, one of which is under early control and the other, under late control. These markers, and other electrophoretic differences between the products of the two viruses, allow the expression of early,

“intermediate” (see below), and late genes to be monitored.

Upon superinfection of cells in the late phase of a primary infection, both early and late products of the superinfecting genome can be detected. However, blockade of DNA replication from the time of the superinfection prevents expression of the late genes of the superinfecting genome, although active transcription of the late genes of the first agent continues in the same cells. Control experiments exclude several less-interesting possible explanations of this finding, in terms of gene dosage or serotype-specific effects, for example, and we conclude that the mere presence in the cell of the machinery for late transcription does not secure the expression of late genes from unreplicated viral DNA. Other data argue against the transient involvement of unstable factors acting on the first genome to activate its transcriptional capacity. It seems, therefore, that only genomes which themselves have undergone replication are available for late gene expression, although ongoing replication is not obligatory. The nature of the replication-induced modification is presently under investigation, and current efforts are directed toward the isolation and definition of the late template.

“Intermediate” phase of gene expression

These results notwithstanding, the demarcation between early and late genes has become less clear-cut in recent months. Last year we demonstrated that virion component IX, which is made in large amounts at late times, is also produced in the absence of viral DNA synthesis. Recently, we have extended this observation to include polypeptide IVa2, another constituent of the viral particle, whose mRNA has now been detected in cells that are blocked for DNA replication with Ara C and contain no other “true” late RNAs. The mRNAs for polypeptides IX and IVa2 are much less evident in preparations from cells held in the early phase of infection by cycloheximide. These observations suggest the existence of an “intermediate” category of products expressed strongly at late times, modestly in the absence of viral DNA replication, and only weakly in the absence of protein synthesis.

It is perhaps not surprising that the genes for polypeptides IX and IVa2 should be under separate control from the majority of late genes, since they are located outside the major late transcription unit and have their own transcriptional promoters. However, it was more surprising to discover that mRNA for the 52K, 55K polypeptides, which are encoded between 30 and 34 map units, is present in early RNA preparations that lack the other late species. The 52K, 55K-protein mRNA, like other RNAs of the major or late block, carries the tripartite leader sequence, and there-

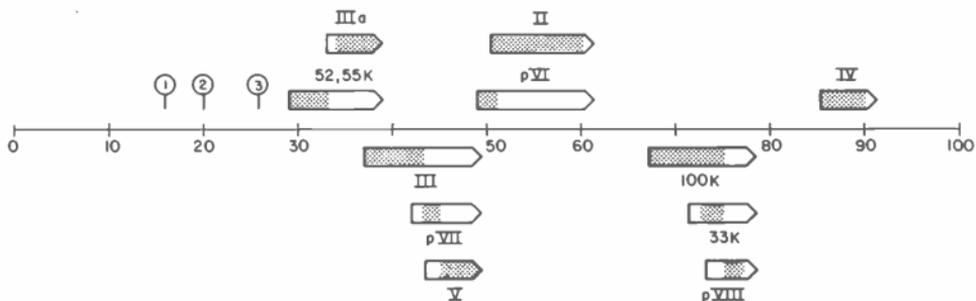


Figure 3
Identification and mapping of late genes. The arrows indicate the map locations and direction of transcription of the 11 mRNAs, grouped in five families, comprising the major late block. The stippled portions mark the positions of the protein-coding sequences. All these RNA species carry the same 5'-terminal tripartite leader sequence whose points of origin are shown by the coded numerals.

fore presumably is also initiated at map position 16.4. Consequently, it becomes necessary to postulate modulation of transcription at the level of processing, as well as at initiation, to explain how the 52K, 55K-protein mRNA can be formed when other late mRNAs are lacking.

The late gene block

In collaboration with Bruce Paterson (National Institutes of Health) and Bryan Roberts (Harvard Medical School) and his coworkers, we have completed a fine-structure analysis of the major late gene block using cell-free translation in combination with three other techniques: (1) gel electrophoresis of mRNA in the presence of the denaturant methylmercury hydroxide to provide size estimates of the messages; (2) hybridization selection to locate the genomic origins of the mRNAs; and (3) hybrid-arrested translation to map the protein-coding sequences. The diagram in Figure 3 summarizes the main conclusions. In accord with results from several sources, the late mRNAs are arranged in five families of one to three mRNAs each, all sharing the common tripartite 5'-leader sequence. The RNAs of each family are also coterminal at their 3' ends and their coding sequences are invariably 5' proximal. Our analysis revealed the existence of one new gene, encoding the 52K, 55K polypeptide pair, at the left end of the block, and we also resolved certain anomalies in previous maps by establishing the order of polypeptides III-pVII-V within the second family of mRNAs. In addition, the data show that those mRNAs containing extensive untranslated regions are prone to lose these superfluous sequences and hence migrate in the gel as a polydisperse streak. The mechanism responsible for this phenomenon is being studied.

Protein structure studies

Partial protein sequence data, obtained in collaboration with Carl Anderson (Brookhaven National Labo-

ratory), have allowed us to locate precisely the N termini of four proteins with respect to the DNA sequence. Two of these are prominent late proteins, hexon (II) and fiber (IV); the others are the E1B 15K protein and polypeptide IX, which are also relatively easily obtained in a highly radioactive form. This advantage is not shared by many early proteins that, in addition, often exist in a number of related forms.

Region E1A codes for at least six polypeptides, with molecular weights of 38,000–50,000, extractable from infected cells. Three of these focus at an isoelectric point of about 6.0 and exhibit molecular weights of 50,000 (A), 46,000 (B), and 42,000 (C), whereas the remaining three E1A proteins focus at pH 5.9 and exhibit molecular weights of 46,000 (D), 42,000 (E), and 38,000 (F). In an attempt to further define the relationships among these proteins, we have compared the tryptic peptides of each of the species labeled *in vivo* with [³H]proline. That all six species are closely related is demonstrated by the detection of at least six proline-containing peptides common to all of the E1A proteins.

The structures of the mRNAs from this region are consistent with the hypothesis that some of the peptides that occur in the larger E1A proteins might be absent from some of the smaller species. We have observed a proline-containing peptide that occurs in the E1A proteins, A, B, D, and E, but is absent from the smaller proteins C and F. This peptide is therefore a potential candidate for a peptide derived from sequences that are "spliced out" of the mRNA(s) coding for the smaller E1A proteins C and F. These data also suggest that protein C is more closely related to protein F than to protein E and, therefore, that the three E1A proteins focusing at pH 6.0 might show a diagonal, rather than a horizontal, molecular-weight relationship to the three species focusing at pH 5.9. Such a uniform shift of proteins A, B, and C to a more acidic isoelectric point with a concomitant decrease in

molecular weight might be accomplished by a post-translational modification such as phosphorylation or proteolytic removal of a basic portion of the molecules. At least one distinct proline-containing peptide occurs in proteins A, B, and C, but not in proteins D, E, and F, and may represent a peptide that has been modified or deleted posttranslationally.

Experiments are also in progress to define the relationship of these six E1A proteins isolated from infected cells to the E1A 42K, 48K, 54K, and 58K species synthesized *in vitro*.

Several other viral proteins have also been subjected to peptide analysis. The data show that three 21K proteins and one 19K protein, all resolved by two-dimensional gel electrophoresis of infected cell extracts, are closely related to one another and to 21K, 19K, and 17K polypeptides made *in vitro* from early-region-E4 mRNA. The 11K polypeptide from the same region gives rise to different peptides and seems unrelated. Some of these peptides have been characterized further by sequence analysis in order to correlate them with the DNA sequence and ultimately to define the complete sequence of amino acids in the original proteins.

Small virus-coded RNAs

The two small noncoding VA RNAs still pose several conundrums, and we have continued our investigations of their structure and function. Together with U. Pettersson (University of Uppsala) and his coworkers in Sweden, we have completed the DNA sequence analysis of the VA RNA gene region and have identified the sequence of VA-RNA_{II} by combining this information with RNA sequence data. Computer-assisted model building predicts virtually identical hairpinlike structures of considerable stability for both VA RNAs. However, neither the structural sequences themselves nor the sequences lying to their 5' side (potential promoter sites) show much homology. We have also begun to explore the range of variation permissible in the sequence of VA-RNA_I. Within group-C adenoviruses, few base changes distinguish the different serotypes: the VA-RNA_I of Ad2 is identical to that of Ad6 and differs by 2 nucleotides from that of Ad5 and probably by only 1 nucleotide from that of Ad1. One mutant, generated in collaboration with Terri Grodzicker, contains a four-base insertion that, like the natural variations, is located near the center of the molecule. The mutation has little impact on its probable secondary structure and is not associated with any detectable physiological perturbation in the infectious cycle.

A clue to their role in adenoviral infections may emerge from experiments on binding of VA RNA to mRNA. Under suitable conditions, we have been able

to form complexes by hybridizing labeled VA-RNA_I to mRNA and to separate the complexes from unreacted VA RNA by chromatography on oligo(dT)-cellulose. RNA isolated from infected cells in the late phases is more effective in binding VA RNA than is uninfected cellular RNA. VA-RNA_{II} also appears to form similar complexes, but these have not been extensively studied. Further analysis of the complex is being carried out with the aid of a plasmid containing a DNA copy of fiber mRNA and of cloned fragments of the viral genome, with a view to characterization of the sequences in the VA RNAs and mRNAs that are involved in the binding.

Sequence analysis of the mRNA coding for virion component IX, carried out with Ulf Pettersson (University of Uppsala) and coworkers, and Daniel Klessig, is now essentially complete. The RNA comprises 485 nucleotides, excluding the poly(A) tail and inverted 5'-terminal base, of which 420 nucleotides code for amino acids. Identification of the 5'-terminal sequence confirmed our expectation that this mRNA (like the VA RNAs) is not spliced, and in this respect it seems to be unique among adenoviral mRNAs. From this observation, we deduce that whatever the role of splicing is, it is not essential for messenger production.

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MOVABLE GENETIC ELEMENTS

It has become evident in the last few years that spatial arrangements of genetic material may undergo rapid changes and that different DNA segments may fuse or dissociate by mechanisms occurring commonly in nature. These mechanisms can now be given a general term, "specialized recombination". Movable genetic elements, specific DNA sequences that undergo rapid rearrangements, are the primary exponents of specialized recombination. The realization that movable genetic elements occur commonly in nature is changing the way we think about the evolution, organization, storage, reassortment and expression of genetic material.

Studies on movable genetic elements at Cold Spring Harbor are a continuation of a long tradition of genetics. Barbara McClintock did her famous work on controlling elements in maize in the early fifties at Cold Spring Harbor. She proposed that the variegated phenotype of kernels of corn (and that of corn plants themselves) is a result of interaction of movable genetic elements with different genes and that this interaction occurs by transposition of the controlling elements to, and excision from, the affected loci. She further proposed that the process of transposition itself could be controlled by a component of the transposable system. Later, the work on bacteriophage lambda at Cold Spring Harbor helped to understand how one DNA molecule is inserted into another.

The present era of movable genetic elements can be traced to the discovery of bacteriophage Mu and bacterial transposable elements. The idea that Mu is a prototype of transposable elements was mainly developed from studies at Cold Spring Harbor. Transposition of Mu DNA and flip-flop of the G segment of Mu are now well recognized processes of genetic rearrangements.

Recently, two groups of scientists studying genetic rearrangements have joined the Cold Spring Harbor Laboratory. One group studies the molecular genetics of yeast, with a focus on transposition of mating type genes in *Saccharomyces cerevisiae*. The other group is studying the molecular genetics of mammalian cells; in particular, the integration of exogenously added genes in the mammalian cell genome. Thus, the Division of Movable Genetic Elements at Cold Spring Harbor encompasses studies on prokaryotes, lower eukaryotes and higher eukaryotes.

These are exciting times in genetics. New technologies are making it easy to analyze the structure and functioning of genes. The impact of studies on basic genetic mechanisms, on biology, medicine, and agriculture will thus continue to increase.

MOLECULAR GENETICS

D. ZIPSER, E. Cheng, D. Kwoh, T. Kwoh

For the past two decades, a central theme of molecular biology has been the study of gene expression. There is no single mechanism for modulating the expression of a gene. Control can occur at many levels ranging from amplification of the number of DNA copies through the synthesis, transport, or degradation of mRNA to the transport, modification, and ultimately to the degradation of proteins. Over the past year our laboratory has been engaged in a variety of projects which were motivated by a desire to understand various aspects of the control of gene expression. An example is our work on bacteriophage Mu-1. This bacteriophage integrates at random into its host chromosome and causes mutations. It has a mechanism of DNA replication with features significantly different from other known bacteriophages and also contains a variety of interesting control systems. One, which has attracted our attention most strongly, is the G-loop inversion system. The G loop is a 3000-bp segment of DNA in the Mu genome, which can exist in either of two possible orientations. Mu possesses a function called *gin*, which is absolutely essential for changing the orientation of the G loop. It has now been established that in one orientation the G loop allows Mu to infect certain strains of *Escherichia coli*, whereas in the other orientation efficient infection of other strains occurs but the ability of Mu to grow on *E. coli* is virtually eliminated. We are ultimately interested in developing an in vitro system that will enable us to study the mechanism by which the G loop is inverted. To do this, we must obtain significant quantities of the *gin* protein. This is necessary in order to identify the protein and to have it in high enough concentrations so that the inversion reaction can be run at reasonable efficiency in vitro. Our approach to identifying *gin* and obtaining it in large quantities has been to clone this gene on a amplifiable plasmid. This approach had previously enabled us to identify the repressor protein of Mu and to map the Mu operator. The cloning approach we took made it worthwhile to clone the entire Mu genome in small pieces. We used random shearing and terminal transferase to construct our clones. During this year we have isolated a large number of plasmids, each containing a section of the Mu genome of a few thousand base pairs. In addition to sections containing the *gin* function, we have all the essential genes of Mu cloned in this fashion. These clones have been analyzed genetically and physically using restriction enzymes to determine which sections of Mu DNA they contain, and they have been analyzed by complementation to determine which gene products they produce. We now have clones that produce all the essential functions of Mu, together with the *gin* and

mom functions. We are focusing our attention on clones that contain the proteins of the G-loop region and the G loop itself, together with *gin*. Other people have begun to use our clones to study a variety of functions associated with Mu. To identify the *gin* protein, we have transferred the plasmid, which has been shown by physiological analysis to make *gin* in large quantities, to minicells. In these cells it is possible to identify the products made by plasmids, since the minicell mother cells bud off small enucleate cells that contain no host DNA but do contain plasmid and express only those proteins coded by the plasmid. This is a well-worked-out technology that we have used in identifying the Mu repressor. With it, this year, we have been able tentatively to identify the *gin* protein, together with several other proteins from the G loop in both of its orientations. If our preliminary identification of *gin* is correct, and this can only be proven by further genetic analysis, then we are fairly lucky since it is produced in large quantities and has a molecular weight of about 15,000, which makes it quite easy to separate from other proteins. We already have mutations induced in the *gin* function on the cloned plasmid that should enable us to unambiguously confirm that the protein we believe to be *gin* is in fact, that function. This will lay the foundation for our further work on the in vitro system.

As mentioned above, one of the important features in controlling the expression of genes is to be able to destroy the products of those genes when they are no longer needed. This is often done by protein degradation. This year we have continued our study of the *lon* protein degradation system of *E. coli*. This system is quite specific and has the interesting feature that protein degradation is in some way energy-dependent. One of our important findings this year is that the *lon* system works on the soluble form of proteins and probably not on the insoluble form, which sometimes occurs with aberrant proteins. We were able to determine this by cloning a mutant of β -galactosidase, X90, which produces a large fragment that is rapidly degraded by the *lon* system under normal circumstances. However, when this mutant was cloned on a multicopy plasmid, the fragment was synthesized in huge quantities, on the order of 50% of the cell protein. Somewhat to our surprise, the fragment that was normally rapidly degraded had become completely stable. We were able to analyze the reason for this and it became clear that stability was associated with precipitation of the protein; at these huge concentrations apparently protein-protein interactions occurred, leading to insolubility. We found the X90 fragment present in a low-speed centrifuge pellet in

strains producing it at extremely high levels, whereas it was always present in the soluble fraction under normal conditions. A variety of restriction enzyme analyses and suppressibility tests indicated that we were in fact dealing with genuine X90. From this we concluded that the highly multimeric form of this protein which led to insolubility, also made it resistant to degradation by the *lon* proteases.

E. coli has a lot of proteolytic enzymes, most of which are not energy-dependent. The presence of these enzymes has greatly complicated the study of the energy-dependent enzyme, and this year we have managed to identify and purify to homogeneity one of the important energy-independent proteases of *E. coli*, which we have called protease III. We have also been able to isolate mutations in the protease-III gene and map them. These protease-III⁻ mutants lack one of the major energy-independent proteases of *E. coli* and are excellent vehicles for clarifying the study of the more complex energy-dependent protein-degradation systems, since they eliminate one of the spurious sources of substrate degradation.

Prior to this year, the Molecular Genetic Section had concentrated almost entirely on prokaryotic systems. This year we began a collaboration with M. Wigler, who is now next door to us at the animal cell facility. Dr. Wigler brought from Columbia a marvelous new system for the transformation of animal cells. This new technique allows virtually any exogenous DNA to be introduced into cultured mouse cells. This means that the expression of DNA in eukaryotic cells can be studied in great detail using genetic-engineering techniques on cloned DNA outside the host and then introducing the DNA into mouse cells. The collaborative projects we have initiated this year involve such techniques as introducing *lac* operon genes from bacteria into animal cells to determine what features of DNA structure are required for their expression there. We have started a project to introduce dihydrofolate reductase genes from bacteria into animal cells to construct a positively selectable universal vector that would be able to have a basis for selection in trans-

formation of both bacteria and cultured animal cells. In addition, we have started to transfer many of the thymidine kinase genes, which have been cloned from animal cells into bacteria, to find out what modifications of them are necessary for their expression in bacteria. In this way we also hope to develop vectors that are selectable in bacteria and animal cells both of which have selective techniques for thymidine kinase. An additional collaborative project involves a detailed genetic analysis of the thymidine kinase gene of herpesvirus to map the sites required for its transformation, transcription, and control within animal cells. This project is based on our ability to create a large library of deletions in cloned thymidine kinase genes using currently available genetic-engineering techniques.

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INSERTION ELEMENTS AND PLASMIDS

A.I. BUKHARI, G. Chaconas, M. Dubow, D. Evans, R.M. Harshey, K. Messina, N. Sarvetnik

The transposable genetic elements in prokaryotes fall in two main groups. One group causes duplications of 9 host base pairs at sites of insertion, whereas the other group generates 5-bp duplications. The 5-bp group includes Tn3, bacteriophage Mu, IS2, and the $\gamma\delta$ sequence. A remarkable recent event in molecular genetics is the demonstration that the phenomenon of 5-bp duplication transcends the prokaryote-eukaryote boundary. G. Fink and coworkers at Cornell University have found that a transposable element in yeast causes a 5-bp duplication; G. Rubin at Sydney Farber Institute in Boston has reached a similar conclusion for an element in *Drosophila*. Thus, it would seem that the process of bacteriophage Mu DNA transposition has even wider applicability than was thought previously. During the past year, our efforts to dissect the mechanism of Mu transposition were focused on examining the behavior of small plasmids containing either whole Mu or internally deleted Mu's (called mini-Mu's).

Construction of mini-Mu plasmids

All of the plasmids used for studies on Mu replication and transposition were derived from a 9.09 kb plasmid pSC101. We removed from a pSC101 plasmid containing the whole Mu genome the central 27-kb *Pst*I fragment of Mu DNA. This prototype mini-Mu contains 1.6 kb from the left end of Mu and about 7.5 kb from the right end, including the invertible G segment. Within this mini-Mu, we cloned various selectable markers such as the entire *lac* operon of *Escherichia coli*, a gene for ampicillin resistance (encoding β -lactamase), and a gene for kanamycin resistance. The prototype mini-Mu was further manipulated by first recombining the gene for *amp^r* at the right ex-

trinity of the G segment. (This was done by a genetic cross involving the Mu *amp* previously isolated by D. Leach and N. Symonds, at the University of Sussex.) Since the *amp^r* gene contains a *Pst*I cleavage site, we were able to cut out the G segment, removing all but the 1.6-kb segment of Mu DNA at the right end. In addition, we cloned the *lac* operon and the *amp^r* gene within this 3.2-kb mini-Mu. Many of the important plasmids constructed by us during the past year are shown in Table 1.

These plasmids provide us with valuable tools with which to dissect the process of Mu transposition and replication. We can study the various events during the life cycle of Mu by monitoring the markers we have inserted within the ends of Mu.

Replication and transposition of mini-Mu sequences

By genetic, biochemical, and electron microscopic methods, we have shown that the mini-Mu sequences carried in our plasmids undergo Mu-mediated transposition, replication, and packaging when provided with Mu helper functions in *trans*. When a phage lysate is prepared from a strain containing mini-Mu and a helper Mu phage, 5–20% of the phage particles carry mini-Mu sequences, indicating that mini-Mu sequences increase in number during Mu growth. Figure 1 shows a packaged mini-Mu that was heteroduplexed with wild-type Mu DNA.

The replication of mini-Mu is shown directly by assaying the activity of β -lactamase for mini-Mu*amp* and the activity of β -galactosidase for mini-Mu*lac*. When these mini-Mu's are provided with normal Mu functions, the levels of the enzymes begin to increase. This increase is in good agreement with the previously reported kinetics of wild-type Mu DNA replication.

Table 1
Plasmids Containing Mu DNA

Plasmid	Number	Mu DNA	Mu G region	Selectable marker	Transposition
pSC101::Mu	pMC321	entire Mu	+	—	+
pSC101::Mukan	pMC398	entire Mu	+	kanamycin ^r	+
Mini-Mu	pGC121	c+S	+	—	+
Mini-Mukan	pGC102	c+S	+	kanamycin ^r	+
Mini-Mu <i>amp</i>	pMD861	c+S	+	ampicillin ^r	+
Mini-Mu <i>lac</i>	pCL198	c+S	+	Lac ⁺ phenotype	+
Right end	pCL151	S only	+	—	—
Left end	pGC302	c only	—	—	—
Mini-Mu	pCL222	c+S	—	—	+
Mini-Mu <i>lac</i>	pGC401	c+S	—	Lac ⁺ phenotype	+
Mini-Mu <i>amp</i>	pGC501	c+S	—	ampicillin ^r	+

All mini-Mu's contain the immunity gene *c* and only the beginning of the *A* gene. The left and right ends of Mu are denoted by *c* and *S*, irrespective of the extent of DNA present at the ends.

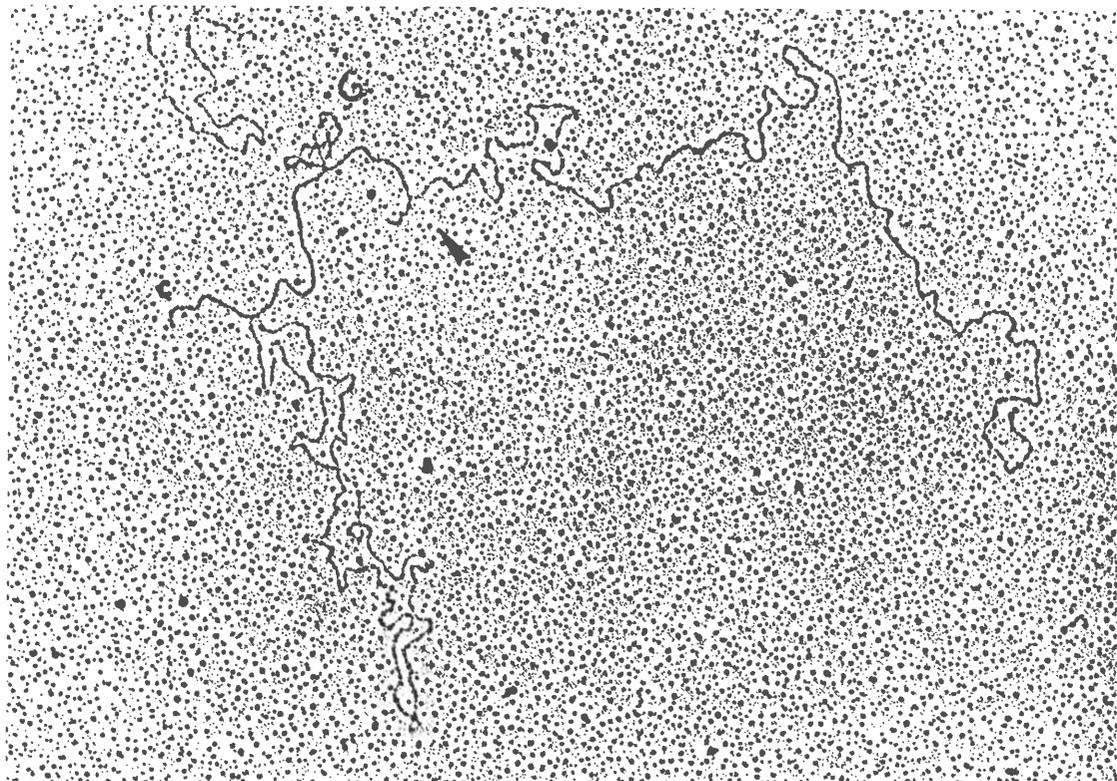


Figure 1
A packaged mini-Mu sequence. Mini-Mu DNA in a transducing lysate is heteroduplexed with wild-type Mu DNA. A large loop near the left end (c end) represents the deletion in mini-Mu. An underwound G segment can be seen. The arrow indicates a normal split end and a very long split end, indicating the packaging of extra-long host DNA attached to the mini-Mu sequence.

The replication of mini-Mu requires both functional *A* and *B* genes of Mu.

High-efficiency transposition of mini-Mu sequences has been shown by a genetic assay that involves integration of the mini-Mu sequences into a conjugally transferable plasmid such as *F'prolac*. Following induction of a strain containing a Mu prophage and mini-Muamp, mini-Mulac, or mini-Mukan the mini-Mu sequences appear in about one in ten transferred episomes. Many of these mini-Mu sequences still have the plasmid attached to them; the integrated DNA has the structure mini-Mu–plasmid–mini-Mu. This type of structure has been seen with all transposable elements and is referred to as a cointegrate.

Behavior of Mu- and mini-Mu-containing plasmids during transposition

A singularly important advantage of using small plasmids with Mu insertions is that the plasmid molecules can be separated from the chromosomal DNA and their different forms can be differentiated by agarose gel electrophoresis. In a series of experiments,

we extracted the DNA from strains containing the Mu or mini-Mu plasmid and subjected it to electrophoresis in low-percentage-agarose gels followed by transfer of the DNA to nitrocellulose paper. Separate hybridizations with ³²P-labeled pSC101 and Mu DNA revealed the position of the plasmids and the replication of Mu DNA. We found that the plasmids are not cleaved to generate either a linear form or smaller products; instead, they migrate with *E. coli* DNA. This interaction of the plasmids with the host chromosome is of two types: (1) formation of cointegrates of the structure Mu-plasmid-Mu and (2) association of circular plasmids with the chromosomal DNA.

Analysis of cointegrates

That some of the plasmid DNA migrating with the host DNA after induction represents cointegrates has been shown by restriction, blotting, and hybridization of DNA. When the DNA was digested with *Kpn*I, which cleaves only once within Mu or mini-Mu sequences, linear plasmids of the original size were released.

When the DNA was cut with *Xho*I, which cuts the plasmid DNA once but does not cut the Mu sequences, a smear of plasmid DNA was generated. This would be expected if Mu-plasmid-Mu sequences had integrated at different sites in the host DNA.

As mentioned above, we have studied the cointegrates genetically by inserting them in conjugally transferable plasmids. As much as 90% of the integrated Mu sequence can be in the form of cointegrates. The cointegrates are resolved in *rec*⁺ cells, generating the original Mu plasmid and a copy of Mu (or mini-Mu) on the conjugal plasmid. This resolution is much reduced in *recA*⁻ cells. It has yet to be determined whether there is a Mu function that will resolve cointegrates slowly in a *recA*⁻ background. We have also studied the cointegrates by transduction experiments. When Mu lysates are used for transduction of plasmid

markers, we can recover the original plasmid in the recipient cells. This can only happen if cointegrates are packaged in the phage particles and are then resolved in the recipient cells. The packaging of cointegrates is being examined carefully.

Electron microscopy of plasmid-host association

Electron microscopy of mini-Mu plasmids after Mu induction showed that many circular plasmids were in contact with the host DNA. About 20% of the observable plasmids were seen associating with the host chromosome in this manner. This association requires functioning of the A and B genes of Mu. Figure 2 shows examples of such association. These structures apparently represent either intermediates in the transposition process or the end products of transposition.

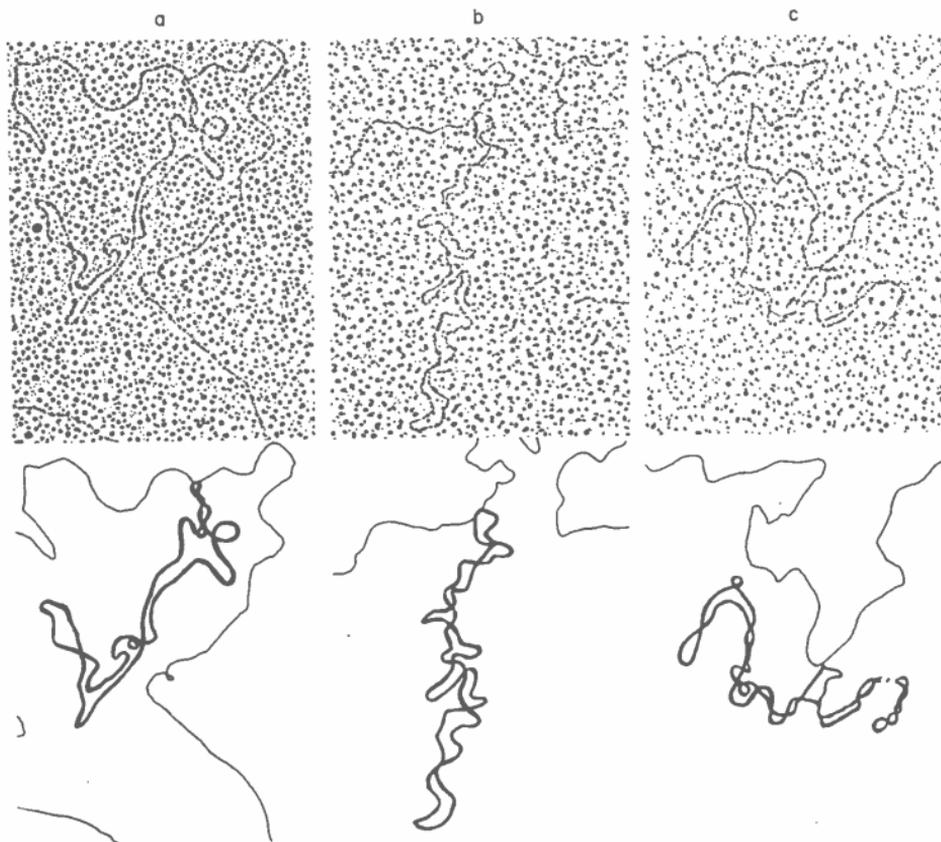


Figure 2
Mini-Mu plasmids in contact with the chromosomal DNA during transposition.

Analysis of the nature of the contact between the plasmids and the host DNA promises to provide important clues to the mechanism of transposition.

The Mu transposition problem in 1980

The results discussed above, i.e., plasmids that contain Mu sequences remain intact during Mu replication and associate with the host chromosome in their entirety, lend further support to the replication-integration hypothesis that we proposed a few years ago. It was proposed in this hypothesis that the predominant event during Mu transposition is replication of Mu DNA in situ to generate integrative intermediates. The results further strengthen the assumption that the ends of Mu DNA are the primary determinants of replication and transposition. The A-gene and B-gene proteins of Mu evidently act upon these ends to trigger the twin processes of transposition and replication.

Cointegrate formation appears to be a prominent feature of the process of Mu transposition and, indeed, of all other prokaryotic transposable elements. Occurrence of cointegrates in response to Mu induction has been seen in at least two other laboratories working on Mu. It has yet to be determined whether the cointegrates are obligatory intermediates in transposition.

Flip-flop control of gene expression in Mu

One experiment on the expression of genes within the 3-kb invertible G segment of Mu is noteworthy. In this experiment, we inserted the *lacZ* gene without its promoter into the G segment as follows. We cut λ trplac phage DNA (λ trplacW205, provided by W. Reznikof, in which the *lacZ* gene is fused to *trp* and is under *trp* promoter control) with *Hind*III and *Kpn*I. The *Hind*III cut is in the *trpB* gene and the *Hind*III-*Kpn*I fragment contains a part of *trpB*, *trpA*, *lacZ* and a part of the *lacY* gene. The *Kpn*I cut is very close to the end of *lac* DNA. To put this fragment into a *Kpn*I site within the G segment, we used a *Hind*III-*Kpn*I linker of about 500 bp derived from the adenovirus-2 DNA, since this purified fragment was readily available at Cold Spring

Harbor. The cloning was done in mini-Mu pSC101.

We have put this plasmid in the *trpA*⁻, Δ *pro**lac* strain and have shown that the cells switch back and forth from the Lac⁻Trp⁻ phenotype to the Lac⁺Trp⁺ phenotype. If we use ampicillin selection for enriching Trp⁻ cells, we get colonies that are Trp⁻Lac⁻. Using the Lac⁻Trp⁻ cells, on the other hand, we can directly select Trp⁺. By screening for colonies that have become fixed in one G orientation, we can isolate *gin*⁻ mutants. A by-product of this work is the potential for the study of Mu promoters, using *lac* and *trp* as indicators of promoter activities. We can also test whether the G segment can be transposed by examining for *lac* transposition.

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DNA-PROTEIN INTERACTIONS

D. KAMP, R. KAHMANN

For the past year, we have extended our studies on site-specific recombination with the main focus on the integration/transposition process of bacteriophage Mu DNA and the inversion system of the G segment of Mu DNA.

Integrative recombination of bacteriophage Mu

The heterogeneous host DNA sequences attached to the ends of bacteriophage Mu DNA have been demonstrated directly by the dideoxy chain-terminating sequencing method of Sanger et al. (*Proc. Natl. Acad. Sci.* 74:5463[1977]) These experiments have confirmed our previously determined position of the crossover sites and, furthermore, have shown that the host DNA sequences immediately adjacent to the attachment sites are not randomly distributed but reproducibly contain a certain set of predominant nucleotides (Fig. 1), which we shall call the host attachment site. In view of the very low insertion specificity of bacteriophage Mu, this result was quite unexpected. It seems to indicate that Mu insertion does not occur at randomly distributed sites, as was thought previously, or alternatively it could mean that the Mu packaging machinery preferentially encapsidates Mu prophages inserted next to a specific host DNA sequence. A comparison of the preferential sequences at the two ends revealed that these sequences do not reflect a 5-bp duplication of host DNA (5-bp duplications of host DNA have been found to flank the two prophage junctures analyzed). We have inferred from this result that in mature Mu DNA, not all molecules contain the duplicated host sequences. An integration model for Mu was developed that accommodates these findings.

We have extended our investigation of the Mu transposition process. The multicopy plasmid pTM2, which contains only the extreme ends of a Mu prophage (called TnM1) and which does not express any Mu functions, was exposed to a Mu helper phage *in vivo*, and the phage progeny derived from such an infection was analyzed biologically and physically. About 10–20% of the phage progeny contain the entire pTM2 plasmid. After digestion with restriction enzymes, all pTM2 plasmid fragments appear in their relative intensities, indicating that the integration of the pTM2 plasmid did not occur via a Campbell-type mechanism. We have also eliminated the possibility that multimeric forms of pTM2 are packaged or that dimeric forms of pTM2 are the required substrate for transposition. The structure of those molecules containing the entire TnM1 sequence was shown by restriction enzyme analysis to be a cointegrate TnM1-pBR322-TnM1-host DNA. The presence in these molecules of a direct repeat of the TnM1 sequences was substantiated by the finding that a bacteriophage Mu lysate propagated on a strain containing pTM2 is capable of transducing pTM2 with very high fre-

quency (2×10^{-1}) into a rec⁺ host. Under recA conditions, the transduction frequency is greatly reduced, indicating that recombination between the direct repeats of TnM1 sequences within the cointegrate structure is essential for the resaturation of the pTM2 plasmid.

This very sensitive transduction assay was also employed to study the biological activity (e.g., transposition of the entire plasmid and formation of cointegrate structures) of recombinant plasmids containing either only Mu attL (pLM445) or Mu attR (pRM445), respectively. The transduction frequencies observed were 7×10^{-7} for pLM445 and 1×10^{-6} for pRM445. These values are very low relative to pTM2 transduction frequencies, but are significantly higher than those of a pBR322 control (6×10^{-9}). Quite surprising is that pRM445 is transduced at all since the transduction assay requires not only transposition to the host chromosome but also packaging; the latter is believed to be initiated exclusively at the left end of the Mu DNA molecule. Using this transduction assay, we have further shown that D108, a Mu-related phage, can be used as a helper for the transposition and cointegrate formation of pTM2. However, the frequency is about 100-fold reduced compared to a Mu helper phage. We are currently sequencing the D108 attachment sites to determine whether this reduced complementation can be attributed to sequence differences of the Mu and D108 attachment sites.

Inversion of the G segment of bacteriophage Mu DNA

In continuation of our studies on the invertible G segment of Mu, the puzzle of the noninfectious G(-) phage particles has been resolved. The original observation was that a phage mutant Mu 445-5 G(-) is noninfectious for *Escherichia coli* K12. However, when Mu 445-5 G(-) phage is grown on an *E. coli* K12 strain containing the recombinant plasmid pGM1, which expresses the Gin function, a few plaque-forming phages are released. The same result is obtained when the pGM1-containing host for propagation of the G(-) phage has a mutation conferring resistance to Mu infection. The plaque-forming phages released were propagated and it was shown by restriction enzyme analysis that they were Mu 445-5 G(+). This indicated that whatever prevented the Mu 445-5 G(-) phage from entering a cell could be overcome in a few rare cases and could become visible by inverting the G segment of the entering G(-) phage back to the G(+) orientation.

These results tempted our search for a bacterial host fully sensitive to Mu 445-5 G(-) infection. In the beginning, bacteria were isolated from soil samples and tested for their sensitivity to G(+) and G(-) phages, respectively. The results were, however, ambiguous; we observed killing of the host at high phage

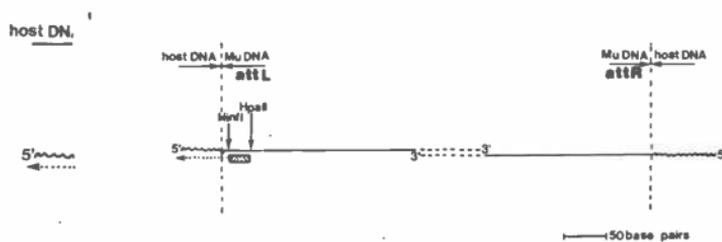
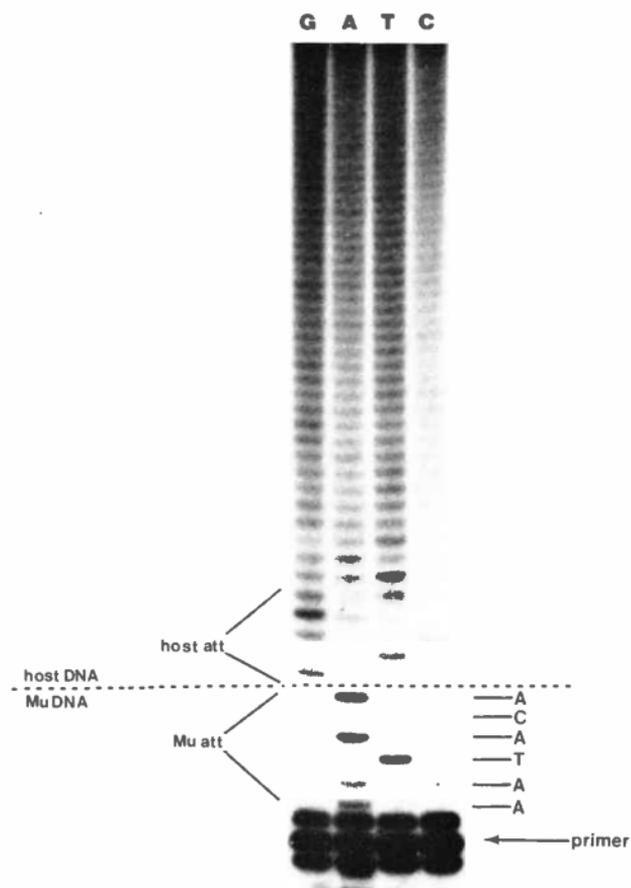


Figure 1
 Autoradiograph of sequence gel from the left Mu-host DNA juncture (- - -). The lower diagram shows schematically the relative position of hybridization of the restriction fragment used as primer (□) and the direction of synthesis by polymerase (· · ·). (~~~~) Heterogeneous host DNA.

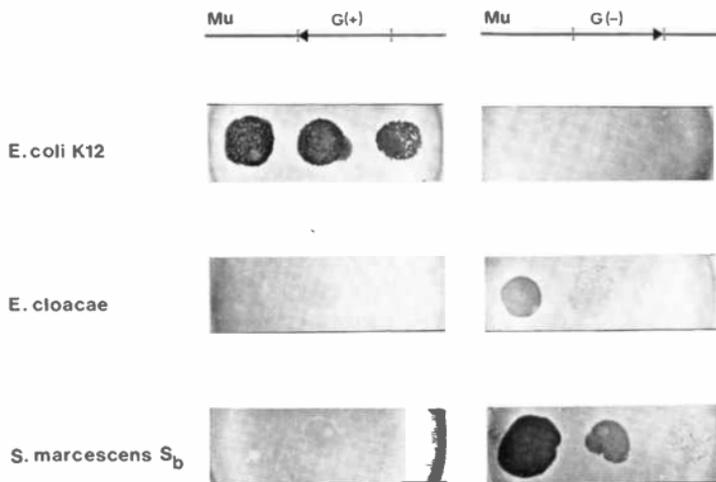


Figure 2

Host range of bacteriophage Mu. Bacteriophage Mu 445-5 G(+) and Mu 445-5 G(-) lysates propagated on E. coli K12 were spotted in serial dilutions on plates seeded with E. coli K12, Enterobacter cloacae, and Serratia marcescens, respectively. Phage growth results in clearing of the lawn at high phage concentrations and in the appearance of single plaques at low phage concentrations.

concentrations, but were unable to detect single plaques. We therefore resorted to at least partially characterized strains. Of those tested, *Serratia marcescens* and *Enterobacter cloacae* were sensitive to infection by Mu 445-5 G(-), but insensitive to infection by Mu 445-5 G(+) phage (Fig. 2). Mu 445-5 G(-) phage can lysogenize both strains, and phage lysates derived from *Enterobacter* or *Serratia* have a plating efficiency of 1 on their respective hosts, but they plate on an *E. coli* K12 r^-m^- host with an efficiency of 10^{-7} . This indicates that the Mu 445-5 G(-) phage has maintained its G(-) phenotype through propagation on *Serratia* and *Enterobacter*. Since the Mu 445-5 phage has a *mom^-* phenotype, the plating efficiencies of Mu *mom^+* and Mu *mom^-* phage lysates were determined in control experiments. Both values are identical, eliminating the possibility that the Mu-encoded DNA-modification function *Mom* is involved in any of the effects observed. We can thus conclude that through inversion of the G segment of bacteriophage Mu an alteration of the phage host-range specificity is achieved. This raises the interesting possibility that *E. coli* K12 may not be the natural host for Mu and that, instead, soil bacteria such as *Serratia* could potentially be carriers of Mu or Mu-related prophages that so far have gone undetected.

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YEAST GENETICS

J.B. HICKS, J. BROACH, A. KLAR, J. STRATHERN, S. Ismail, C. McGill, J. McIndoo, C. Michels, J. Wood

The Yeast Genetics Group in Davenport laboratory was formed in the spring of 1978, with the specific objective of characterizing the genetic switch that controls mating type in yeast cells and, in addition, to establish an ongoing research effort at Cold Spring Harbor Laboratory in the rapidly expanding area of yeast molecular biology. Previous studies by ourselves and others have shown that this genetic process may involve an extremely specific rearrangement of the cell's DNA and that it has implications for the study of growth and development in higher animals and plants. We therefore brought our independent research projects together to attack this problem.

Traditionally, Davenport laboratory has been the site of summer courses and workshops and has a rich heritage of research in molecular genetics. The summer courses continue, but now, for the first time, Davenport is being used full time all year round. The dual use of the building created some problems. We had no sooner arrived at the laboratory in April 1978 when we had to pack up and move out to make room for the summer courses. In September, we moved back into Davenport laboratory to begin our first full year of research, and it was a very successful year for all of us. Thanks to the Robertson Fund, we were able to equip and supply the laboratory until Federal grant money became available. Research grants were awarded to us by the National Science Foundation and the National Institutes of Health, and once our research began, the inconvenience of moving was forgotten and we found Davenport to be a warm and pleasant place to work. We continued our genetic analysis of the loci involved in the control of cell type and developed a yeast DNA cloning vector that allowed us to isolate the DNA from the mating-type locus and to determine the nature of the DNA alteration associated with the changes in cell type.

By the spring of 1979, several of our immediate research goals had been achieved, but it was time to move out for the summer courses once again. This time, space was made available in Demerec laboratory for a limited continuation of research. The summer of 1979 was a hectic one and included the organization of a large and productive meeting on yeast molecular biology, attended by over 400 scientists from around the world. We also began the organization of a book for the Cold Spring Harbor Monograph Series, which will provide an overview of yeast molecular biology, including many of the observations presented at the yeast meeting.

Active research in Davenport began again in September, but without the presence of Jim Broach, who left Cold Spring Harbor Laboratory to take a faculty

position at the State University of New York, Stony Brook. Happily, we have maintained a strong collaboration between our groups and expect to continue working together in the future. This fall also marked the initiation of plans to make a structural addition to the Davenport building in order to provide office and laboratory space for the yeast group during the summer months. We look forward to this addition and to the continuation of our work at Cold Spring Harbor.

Research Summary

The genes that control the cell type in yeast reside in transposable genetic elements, the *a* and α cassettes that occupy three different loci on chromosome III. At *HML* and *HMR* the cassettes are normally silent. The cassette residing at *MAT* (*MATa* or *MAT α*) is expressed and controls the cell type by regulating the expression of *a*- and α -specific mating functions encoded elsewhere in the genome. Homothallic yeast can change efficiently (about 50% of the cell divisions) between the *a* and α mating types and does so in an orderly pattern in cell pedigrees. These changes of cell type reflect interconversion of the *MATa* and *MAT α* alleles and, as described below, involve replacing the cassette at *MAT* with a replica of the unexpressed cassette at *HML* or *HMR* (Fig. 1). The genetic and physical experiments supporting these conclusions are described below.

Switching can heal *mat* mutations

The cassette model was based on the pattern of involvement of the various *HML* and *HMR* alleles and on the observation that mutants of *MAT* (*mat α 1*) could be switched to *MATa* and then to *MAT α* . Most laboratory strains have the *HML α* allele that provides a function required for the switch from *MATa* to *MAT α* and it was

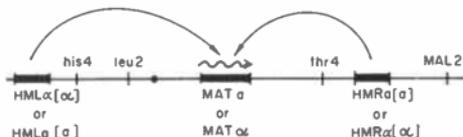


Figure 1

Schematic diagram of yeast chromosome III showing the cassette model for mating-type switching. Heavy lines indicate the *a* and α mating-type cassettes, which are sequentially transposed from the silent *HML* and *HMR* loci to *MAT* under the influence of the *HO* (homothallicism) gene. Only the cassette occupying the *MAT* locus is expressed (wavy arrow) in normal strains.

proposed that *HML* α was the site of unexpressed α information (an α cassette) that could be transposed to *MAT* as part of the switching-and-healing process. Similarly, most laboratory strains have the *HMRa* allele that provides a function required for switching *MAT* α to *MATa*, the *a* cassette. The transposition-substitution process includes a replication step, i.e., the *HML* and *HMR* alleles are not altered or consumed by the switching process. A prediction of this cassette model is that all *mat* $^-$ mutations in the transposable part will be healable. We were able to confirm this prediction both for additional *mata* α mutants and for *mata* mutants (Strathern et al., *Proc. Natl. Acad. Sci.* 76:3425 [1979]; Klar et al., *Genetics* 92:159 [1979]).

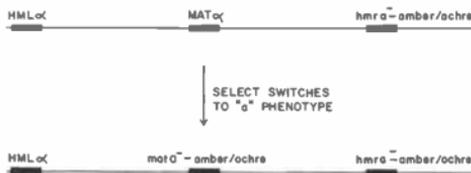


Figure 2

Selected transposition of mutant *a* $^-$ information from *HMR* to *MAT* showing that, during switching, the *MAT* DNA sequence is replaced by an exact copy of the cassette at *HMR*.

The *MAR1* hypothesis and the isolation of mutations of the *HML* and *HMR* loci.

On the basis of genetic studies, a locus designated *MAR1* (mating-type regulator) has been proposed to act as a repressor of the *HML* and the *HMR* loci (Klar et al., *Genetics* [1979]). The mating phenotype of a strain containing the mutant *mar1* $^-$ allele depends on the genetic content of the *HML*, *HMR*, and *MAT* loci, i.e., a strain of genotype *HML* α *MAT* α (or *MATa*) *HMRa* *mar1* $^-$ is nonmating, a phenotype similar to that of the *MAT* α /*MATa* diploids. On the other hand, a strain *HML* α *MAT* α *HMRa* *mar1* expresses an α phenotype and a strain *HMLa* *MATa* *HMRa* *mar1* expresses an *a* phenotype. It was suggested that in cells containing the *mar1* mutation all copies of the mating-type alleles are expressed, including the ordinarily silent information proposed to exist at *HML* and *HMR*. The nonmating phenotype of *mar1* *HML* α *MAT* α *HMRa* provided us with a novel selection for mutations in the *a* information at *HMRa*, since elimination of *a* should make such a cell mate as an α . Likewise, selection of mating-type-*a* cells from sterile *HML* α *MATa* *HMRa* *mar1* should yield mutations in the α information expressed at *HML*. Using this scheme, we have obtained a large number of mutations, including the nonsense *amber* and other types, of the *HML* and *HMR* loci.

Transposition of genetic information from *HMR* and *HML* to *MAT* during switching.

A critical prediction of the specific cassette model is that strains with mutations in the "storage" loci *HML* and *HMR* should generate correspondingly defective *MAT* alleles during the switching process. We observed that strains containing the *amber* and other mutations within the *HMRa* locus yield corresponding *amber* and other mutant *mata* $^-$ loci (Fig. 2). Similarly, the *hml* α $^-$ mutant strain generates *mata* $^-$ mutant alleles, i.e., specific mutations from the *HMRa* and *HML* α are transmitted to *MAT* (Klar, *Genetics* [1980]). The loci *HMRa* and *HML* α remain unaltered; there-

fore, a replica of the mating-type information originating from these loci is transmitted to *MAT* where it replaces the existing information.

Recombination between *MAT* and *HML* or *HMR*.

In many yeast strains, the *MATa* and *MAT* α alleles (and hence the *a* and α cell types) are very stable. In these stable, or heterothallic, strains, switches from *MATa* to *MAT* α occur at rates of about 10^{-6} per cell division. At similar rates, rearrangements of chromosome III occur, resulting in a change of cell type but creating a recessive lethal at *MAT*. We had proposed that the switch from *MAT* α to lethal *MATa* was the result of an intrachromosomal recombination between *MAT* and *HMRa* deleting the α cassette and the portion of chromosome III between *MAT* and *HMRa* and activating the *a* cassette. Similarly, we interpreted the switch from *MATa* to lethal *MAT* α as an intrachromosomal recombination between *MAT* and *HML* α to form a ring chromosome with an activated α cassette. The genetic analysis was consistent with this interpretation. Furthermore, we were able to identify and isolate this ring chromosome, thus providing the first physical evidence supporting the cassette model (Strathern et al., *Cell* 18:309 [1979]).

Transformation and DNA cloning vectors for yeast

The ability to establish the biological function encoded by a piece of DNA by cloning it and reintroducing it into the organism opens particularly powerful forms of genetic and physical analysis. These techniques, as applied to yeast, were in their fledgling stages when we first came to Cold Spring Harbor. To exploit more fully the ability to transform yeast, we constructed a series of cloning vectors that were hybrids between the bacterial vector pBR322 and the selectable yeast gene *LEU2*. The particularly useful vector *yep13* combines pBR322, *LEU2* and an origin of replication from the yeast plasmid (2- μ m circle or *Scpl*). The *yep13* plasmid can replicate in yeast or bacteria, has markers that can be selected in yeast (*LEU2*) and bacteria (*amp* r and *tet* r), and has unique

restriction sites into which additional DNA to be tested for function can be placed. From a collection of yep13 clones containing additional yeast DNA, we were able to identify several DNAs carrying genes for nutritional enzymes, the arginine permease (Broach et al., *Gene*, 8: 121 [1979]) and the α cassette as described below.

The 2- μ m circle

The construction of these vectors and the development of transformation not only provided a means to isolate *MAT*, it also provided an opportunity to study the replication of and the functions provided by the 2- μ m circle plasmid. In this context, hybrid 2- μ m-circle:*LEU2* plasmids were introduced into a *leu2⁻* strain that did not contain the normal plasmid. The ability of these hybrid plasmids to replicate was dependent on the portion of 2- μ m plasmid included. In this fashion, the origin of replication was defined as the region required in *cis* for the replication of the hybrid. In addition, we identified a function encoded by 2- μ m plasmid required for the replication of the hybrid but which can be supplied in *trans*.

The 2- μ m circles have the interesting property of existing in two forms that can interconvert by an internal recombination in a region of repeated DNA. The transcription map is consistent with a requirement for both forms to express all of the messages. This site-specific recombination-mediated activation of genes has intellectual similarity to interconversion of *MAT*.

The isolation of the α cassette

We surveyed 7000 *Bam*HI restriction endonuclease fragments of yeast DNA for having a functional α cassette by transforming those DNAs (cloned in Yep13) into a *mat⁻ leu2⁻* strain. These transformants were screened for having obtained the α phenotype. One of these transformants clearly had the α phenotype and that phenotype was dependent on the presence of the plasmid. We isolated this plasmid from yeast and showed that it could again confer this phenotype when again introduced into a *mat⁻* strain. We found that the portion of the DNA that conferred this α phenotype hybridized to only three of the

thousands of fragments produced by restriction endonuclease cleavage (*Eco*RI, *Bam*HI, *Hind*II, or *Pst*) of the whole yeast genome. Using natural variants of the genomic fragment sizes, we were able to identify these bands as *HML*, *HMR*, and *MAT*. Using the α clone as a probe, we were able to show that the α cassette is larger than the α cassette; *HML α* , *HMR α* , and *MAT α* are all approximately 150 bp larger than their respective alleles *HMLa*, *HMRa*, and *MATa*. Furthermore, we were able to demonstrate that, as predicted by the cassette model, the switch from *MATa* to *MAT α* in a homothallic strain involves replacing the α cassette at *MAT* with a replica of α cassette from *HML α* or *HMR α* (Hicks et al., *Nature* 282:478 [1978]).

Details of the switching process

The cross-homology between the α clone and genomic *HMLa*, *HML α* , *HMRa*, *HMR α* , *MATa*, and *MAT α* sequences enabled us to use it as a probe to screen phage or plasmid libraries of yeast DNA and to isolate clones carrying each of the genes. The identification of the clones was made on the basis of restriction endonuclease mapping done on whole genomic DNA probed with α clone.

To examine the nature of the homology between *HMLa*, *HML α* , *HMRa*, *HMR α* , *MATa*, and *MAT α* , electron microscopic analyses of heteroduplexes formed between cloned DNA fragments corresponding to these loci were made. The α and α cassettes differ by a DNA substitution corresponding to about 600 bp in α and about 750 bp in α . This difference was observed at *HML*, *HMR*, and *MAT*. Flanking these substitutions are DNA sequences shared by all alleles of *HML*, *HMR*, and *MAT* corresponding to about 700 bp on one side and 250 bp on the other (Strathern et al., *Proc. Natl. Acad. Sci.* [1980]). There are additional regions of homology between *HML* and *MAT*, so that the long homology extends to about 1450 bp and the short homology is about 300 bp (Fig. 3). The function of this structural difference between *HML* and *HMR* is not yet understood. It is tempting to invoke a role for this difference in defining the pattern of cell-type switches in pedigrees of homothallic cells.

The mechanism of cassette transposition is still un-

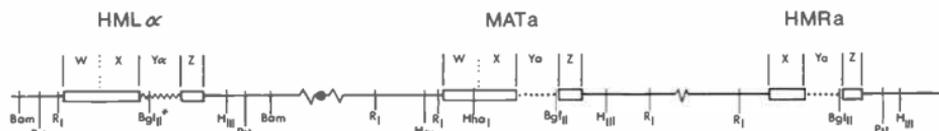


Figure 3
Partial restriction map of the mating-type cassettes showing their orientation on the chromosome. (□) Sequences common to more than one locus (W, X, and Z); the W region is lacking at HMR in all strains tested so far. (.....) Sequences unique to α and α cassettes, respectively.

known. We know that DNA sequences from *HMR* and *HML* must be replicated and eventually substituted for the sequences at *MAT*. This could be accomplished by replication of diffusible cassettes from *HML* and *HMR* followed by a substitution reaction at *MAT*. Alternatively, substitution might be accomplished by homologous pairing of *HML* or *HMR* with *MAT* followed by transfer of a strand from the storage loci to *MAT*. The heteroduplex formed at *MAT* would be resolved in the direction of the donated strand and the gap at the donor locus would be filled in by replication. The regions of homology between *MAT* and *HML* or *HMR* on each side of the transposed region provide an opportunity to initiate and terminate the recombination events required in either mechanism. Which mechanism is involved has not yet been determined. However, we have not detected sequences homologous to the cassettes that are not associated with the chromosomes. Furthermore, we have shown that pairing and reciprocal recombination occur between these loci to form chromosome rearrangements at low frequency in heterothallic strains. It is therefore reasonable that such pairing followed by nonreciprocal gene conversion could occur at high frequency in the presence of a site-specific recombination enzyme in *HO* strains.

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MAMMALIAN CELL GENETICS

M. WIGLER, D. KURTZ, C. Fraser, M. Hallaran, D. Hanahan, D. Levy, L. Lipsich, C. Nicodemus, C. Perucho, M. Perucho

Certain cultured cell lines can readily be transformed by exogenous DNA when given as a calcium phosphate coprecipitate. Although stable transformants, detected by biochemical selection, can arise from exposure of 10^6 cells to as little as 50 μg of a purified gene, such efficient gene transfer requires the use of vast excesses of carrier DNA. Cells that incorporate a selectable marker, such as thymidine kinase, are also likely to incorporate significant amounts of carrier DNA, a process we have called cotransformation. By adding well-defined DNA sequences to the carrier, it is possible to construct cell lines containing virtually any defined DNA sequence. This method is a potentially powerful tool in the study of animal cell genetics and gene expression. In our laboratory we are attempting to increase our understanding of this tool. To exploit this understanding in specific ways, we are (1) attempting to determine the physical state of transforming elements in the transformed host, (2) designing more general animal cell vectors, (3) studying the expression of transforming elements, (4) collaborating with the Tumor Virology Section to engineer new mutant-specific host cells that will be useful in the development and analysis of new viral mutants, and (5) utilizing the method of plasmid rescue to isolate cellular genes coding for selectable markers.

Transfer and amplification of an altered dhfr gene

Successful transfer of the cellular genes coding for thymidine kinase (tk) and adenine phosphoribosyl transferase (aprt) has been demonstrated using total genomic DNA from vertebrate species as donor. Isoelectric focusing of enzymatic activity in transformants has shown this activity to be donor-derived. These findings suggest that it may now be possible to transfer any cellular gene that codes for a selectable marker. More recently, we have demonstrated the transfer to mouse cells of a mutant gene from hamster cells (A29), which codes for a dihydrofolate reductase (dhfr) with altered binding affinity of methotrexate (Mtx). This gene confers resistance to high concentrations of Mtx. Murine recipients were shown to contain the hamster dhfr gene by blot hybridization, using as probe a cDNA clone of dhfr mRNA (kindly provided by R. Schimke, Stanford University). When the Mtx concentration was raised slowly from 0.1 $\mu\text{g}/\text{ml}$ to 10 $\mu\text{g}/\text{ml}$, the hamster dhfr gene resident in surviving mouse cells was shown to undergo a commensurate increase in copy number (amplification). Moreover, if genomic A29 DNA is ligated to a defined genetic element prior to transformation and the transformants

are subsequently amplified, the ligated genetic marker is also often amplified. In this way we have constructed mouse cells containing up to 50 copies of a yeast suppressor tRNA gene (see below).

Cloning the HSV-1 tk gene

Herpes simplex virus type 1 (HSV-1) codes for a thymidine kinase activity that can complement tk^- animal cells. We have cloned the HSV-1 tk gene as a 3.5-kb fragment inserted into the *Bam*I site of the prokaryotic plasmid pBR322. This prokaryotic/eukaryotic chimeric molecule (ptk-2) transforms tk^- mutant mouse cells (Ltk⁻) with the same efficiency as the HSV tk gene purified from viral DNA. When ptk-2 is cotransformed into Ltk⁻ aprt⁻ cells in a nonselective manner (using a cellular aprt gene as the selectable marker), eight of nine cotransformants express tk, suggesting that the cloned HSV-1 tk gene carries its own promoter.

Plasmid rescue

It is possible to cotransform animal cells with the prokaryotic antibiotic resistance plasmid pBR322. These bacterial sequences are found integrated into the high-molecular-weight nuclear DNA of the host. The integrated pBR322 sequences often include a contiguous origin of plasmid replication and an antibiotic resistance factor. In such cases, we have demonstrated that the pBR322 sequences can act as a transducing element from the animal host to *Escherichia coli*. Host DNA is digested with restriction endonucleases and the resulting restriction fragments are then ligated with T4 DNA ligase under cyclization conditions. Those cyclized molecules containing pBR322 and its flanking host sequences can be used to transform *E. coli* to antibiotic resistance. We refer to this process as plasmid rescue.

Cointegration of cotransforming DNA

Cotransforming elements become genetically linked in the transformed host. This has been demonstrated in a variety of ways. We have used prokaryotic sequences such as pBR322 and $\phi\text{X}174$ RF DNA as the cotransforming markers and ptk-2 as the selectable marker. tk^- revertants can be selected from tk^- transformants. Revertants that have deleted the tk gene will also often delete the cotransforming markers as well. In addition, when the tk gene resident in a transformed host is transferred to tk^- recipient cells by chromo-

some-mediated transfer, cotransforming markers are also cotransferred. Finally, when cells are transformed with A29 dhfr and cotransformed with pBR322 derivatives, amplification of the dhfr gene will often result in amplification of pBR322 sequences. We used the method of plasmid rescue to demonstrate that the flanking sequences of cotransforming pBR322 derive not from the endogenous host sequences but rather from carrier sequences. These observations have led us to hypothesize that transforming elements do not integrate directly into the chromosomes of the host cell but, rather, are first ligated to carrier DNA into a single unit, which we have called the pekelaosome. We do not know at present if pekelaosomes are sometimes or usually stably integrated into host chromosomes. Thus, the picture that emerges of transformation in animal cells differs markedly from that in yeast or bacteria.

Expression of rat α_{2u} globulin gene in rat hepatoma cells

The α_{2u} globulin is a protein with a molecular weight of 20,000 that is synthesized in the liver of mature male rats, secreted into their serum, and excreted in their urine. The hepatic synthesis of this protein is under complex hormonal control involving the participation of the sex hormones, glucocorticoids, thyroid hormone, and pituitary growth hormone. The cDNA for this protein has been cloned into pBR322, and this clone is being used to screen a genomic rat library. Several library isolates have been obtained that show nucleotide homology to the probe, but they have not yet been mapped. At the same time, a rat hepatoma cell line has been obtained that synthesizes α_{2u} , and its hormonal responses have been characterized. Using this cell line and DNA-mediated transformation, it may be possible to localize genomic sequences responsible for hormonal control by site-specific mutagenesis of the gene and insertion into an expressing cell.

Expression of transformed genetic elements in host cells

Work in progress in our laboratory and in others indicates that the phenotypic pattern of expression of transformed elements in genetically transformed cells is very complex. In collaboration with M. Botchan and D. Lane of the Tumor Virology Section, we have used the method of cotransformation to examine the expression of SV40 T antigen in mouse cells transformed nonselectively with the early region of SV40 DNA. Some transformants express T antigen constitutively. With other transformants, subclones arise spontaneously, at high frequency, and do not express T antigen. These subclones, in turn, can spontaneously give rise to cells that express T antigen. Despite this varied

phenotypic expression, the genotypic content of these cells remains constant as determined by Southern blotting. Several transformants were obtained that contain multiple copies of the entire SV40 early region but express no detectable T antigen. This perhaps represents an example of either *cis*- or *trans*-acting coordinate regulation.

Construction of mutant-specific host cells

Much effort in viral genetics centers around the isolation of conditional lethal mutants. For animal cells, temperature sensitivity is the most commonly used conditional selection, whereas host range is used much less frequently. In collaboration with T. Grodzicker and D. Klessig of the Tumor Virology Section, we have constructed a number of human cell lines that have stably incorporated various regions of the adenoviral genome. These cells can now complement adenovirus mutations that map at those loci. The use of these cells should greatly facilitate the development and analysis of new adenoviral mutants. In addition, in collaboration with J. Broach, now at the State University of New York, Stony Brook, we have constructed mouse cell lines with greater than 50 copies of a specific yeast suppressor tRNA gene. We are preparing to examine this mouse cell line for the proper expression of these genes. Suppressor animal cells provide another avenue to the development of mutant-specific host cells.

Isolation of cellular genes coding for selectable markers by the method of plasmid rescue

We have previously demonstrated that cellular genes coding for selectable markers can be transferred to appropriate recipient cells using total genomic DNA as donor. We have coupled this observation with the method of plasmid rescue to devise a means for the cloning of the cellular gene coding for thymidine kinase on bacterial plasmids. To this end we ligate restriction-endonuclease-cleaved, total genomic DNA to bacterial plasmid pBR322 prior to transformation of tk^- animal cells. DNA from transformed animal cells is then used to transform *E. coli* to ampicillin resistance. The chicken *tk* gene is now the first vertebrate gene isolated by purely genetic means. It also represents the first "household" gene cloned. Our isolation scheme can be applied equally as well to any dominant-acting gene coding for a selectable marker. Included in this category are various "putative" genes that code for the malignant phenotype, as well as a variety of biochemically well-defined genetic loci.

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CELL BIOLOGY

After several years of being scattered throughout the Cold Spring Harbor Laboratory, the groups working on motility, architecture, and protein composition of animal cells are now united in the McClintock building. Remodeling of the various laboratories, offices, and cold rooms in this building was essentially completed by the summer of 1979; after moving into this new facility, the groups began to operate again. Naturally, there was inevitable interruption and disturbance of each group's work, causing some delay in progress; however, we are now fully operational.

We gratefully acknowledge the financial support received from the National Science Foundation and the Robertson Research Fund. Jack Richards, head of the Lab's Building and Grounds Department, and his crew are thanked for the excellent work they put into our building. We also extend to all the other groups at Cold Spring Harbor Laboratory our appreciation of their tolerance and patience during this time, when Laboratory funds and manpower were largely occupied by our reorganization.

Dr. William Gordon left the group and science altogether in January 1979. We lost in him a quite promising young cell biologist who had already contributed significant work on animal cell architecture.

Newcomers to our group are Dr. E. Birgitte Lane, Dr. Jim Lin, and Dr. James Feramisco. We were happy to have with us Dr. Ian Buckley, from the John Curtin School of Medical Research, Canberra, Australia, who spent last year on sabbatical with our group.

The detailed report on published work by the groups is outlined below.

CELL MOTILITY

G. ALBRECHT-BUEHLER, S. Blose, I. Buckley, A. Bushnell, A. Calasso, E. B. Lane, T. Lukralle, M. Schwartz, M. Szadkowski

The ultrastructure of primary cilia in quiescent 3T3 cells

Primary (rudimentary, solitary) cilia and their biological function are still an enigmatic and often unknown aspect of animal cell physiology. They are defined as single cilia that grow out of one of the centrioles during interphase in otherwise unciliated animal cells. Before mitosis, the cilium shaft is disassembled and only unciliated centrioles are found at the spindle poles. Many of the primary cilia remain, for the most part, inside the cell body. In contrast to normal, motile cilia with the well-known $9 + 2$ pattern, primary cilia show a $9 + 0$ pattern. Although discovered in 1898, primary cilia were first carefully investigated in 1961 and 1962 by Barnes and Sorokin, who described them in mouse hypophysis, as well as in fibroblasts and smooth muscle cells of neonatal chicken (Barnes, *J. Ultrastr. Res.* 5:453 [1961]; Sorokin, *J. Cell Biol.* 15:363 [1962]). By 1967, Scherft and Daems (*J. Ultrastr. Res.* 19:546) had listed 36 avian and mammalian tissues containing primary cilia. In 1971 Wheatley (*J. Anat.* 105:351) described such cilia in cultured cell lines such as BHK-21 and 3T6. It seems, therefore, that primary cilia are quite common centriolar specializations in vivo and in vitro.

The biological function of primary cilia is unknown. Since retinal rods, olfactory flagellae, and kinocilia may be considered specializations of primary cilia, even though only retinal rods show the $9 + 0$ pattern, one may suspect them of having a sensory function, perhaps that of a chemoreceptor. It was also noted that ciliation of centrioles correlated with a reduction of mitotic activity. Furthermore, it was found that blocking mitosis with Colcemid induced primary cilia in CHO cells. These findings led to the suggestion that primary cilia may be involved in control of the cell cycle, although this interpretation has been challenged.

Aside from these possibilities, using indirect immunofluorescence, we recently found that the primary cilia in migrating 3T3 cells were oriented parallel to the substrate and predominantly pointing in the direction of migration. In addition, we found in migrating 3T3 cells one centriole oriented preferentially parallel, and another oriented perpendicular, to the substrate. The parallel centriole could be ciliated and, thus, was easily observable by immunofluorescence. It seems that these observations add an aspect of spatial directionality to the suspected sensory function of primary cilia.

Electron microscopy was used to investigate primary cilia in quiescent 3T3 cells. As in the case of

primary cilia of other cell types, their basal centriole was found to be a focal point of numerous cytoplasmic microtubules that terminate at the basal feet. There are also intermediate filaments that appear to converge at the basal centriole. Cross-striated fibers of microtubular diameter, reminiscent of striated rootlets of ordinary cilia, appear associated with the proximal end of the basal centriole. Usually less than nine cross-banded feet surround the basal centriole in a well-defined plane perpendicular to the centriolar axis. The ciliary shaft was found to be entirely enclosed in the cytoplasm of fully flattened cells. In rounded cells, it could be found extending to the outside of the cell. Periodic striations along the entire shaft were observed. The tip of the shaft showed an electron-dense specialization. Several unusual forms of primary cilia were observed, reminiscent of olfactory flagella or retinal rods.

Through the use of tubulin antibody for indirect immunofluorescence, a fluorescent rod is visible in the cells, which we demonstrate is identical to the primary cilium.

The relationship of 10-nm filaments and microtubules in interphase cells and during mitosis

Guinea pig vascular endothelial cells in culture arrange the majority of their 10-nm filaments into a perinuclear ring (Blose and Chacko, *J. Cell Biol.* 70:459 [1976]; Blose et al., *Proc. Natl. Acad. Sci.* 74:662 [1977]). This arrangement greatly facilitates the observation of these filaments during mitosis. We have recently shown that the array of 10-nm filaments remains stable during mitosis, and that cytokinesis is needed to cleave the filaments into daughter cells (Blose, *Proc. Natl. Acad. Sci.* 76:3372 [1979]). Observations from this study have demonstrated that during mitosis the spindle was contained within the 10-nm filament ring. This raised the following questions: (1) Does the spindle apparatus insert into the 10-nm filament ring? (2) In interphase cells, how does the cytoplasmic microtubule network structure itself relative to the 10-nm filament ring? To answer these questions, a double-label immunofluorescence study was conducted to simultaneously visualize 10-nm filaments and microtubules in the same cell. Antibodies against chick brain tubulin were made in guinea pigs, and the previously described (Blose, *Proc. Natl. Acad. Sci.* 76:3372 [1979]) rabbit anti-10-nm antibody was employed. Endothelial cells were fixed and stained simultaneously with antitubulin and anti-10-nm filament antibodies, followed by staining with fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG and

tetramethylrhodamine isothiocyanate (TMRITC)-labeled goat anti-guinea pig IgG. In interphase cells, the cytoplasmic microtubules radiate away from the juxtannuclear cytocentrom and extend to the cell's border. The cytocentrom is located within the ring of 10-nm filaments. As cells enter mitosis, the most striking change is observed in the microtubule pattern. The cytoplasmic microtubules disassemble and reappear in the mitotic spindle. Throughout mitosis the spindle poles remain in the same plane and closely applied to the 10-nm filament ring. As shown in Figure 1 a-c the 10-nm filaments are excluded from the spindle.

The immunofluorescent pattern of microtubules throughout mitosis is very similar to previous reports on cultured cells. However, the relationship between the 10-nm filaments and microtubules during mitosis seems special: (1) The organizing centers for microtubules, i.e., the cytocentrom in interphase cells, or the spindle poles in mitosis, remain within the ring of 10-nm filaments. (2) The spindle poles are in the same approximate plane and in close apposition to the 10-nm filament ring. (3) The 10-nm filaments precede

and are excluded from the spindle during mitosis. This relationship suggests that the spindle poles might be suspended from the 10-nm filament scaffold during mitosis. This could aid in orienting the spindle apparatus and the whole process of mitosis parallel to the substrate. The overall consequence of this orientation might be to direct both daughter cells to remain attached to the substrate during mitosis. *In vivo*, this would have an advantage of assuring that daughter cells remain attached to the vessel wall and not be swept away in the bloodstream. From these and earlier studies, it was found that the 10-nm filament ring moves ahead of the spindle. The motive force that moves these filaments remains obscure, although two possibilities are entertained: (1) The 10-nm filaments generate the motive force for their movement, or (2) the mitotic spindle apparatus pushes the filaments into daughter cells.

This study illustrates the divergent behavior of two elements of the cytoskeleton during mitosis. The 10-nm filaments remain intact and the microtubules recycle. We have recently suggested (Bloise, *Proc.*

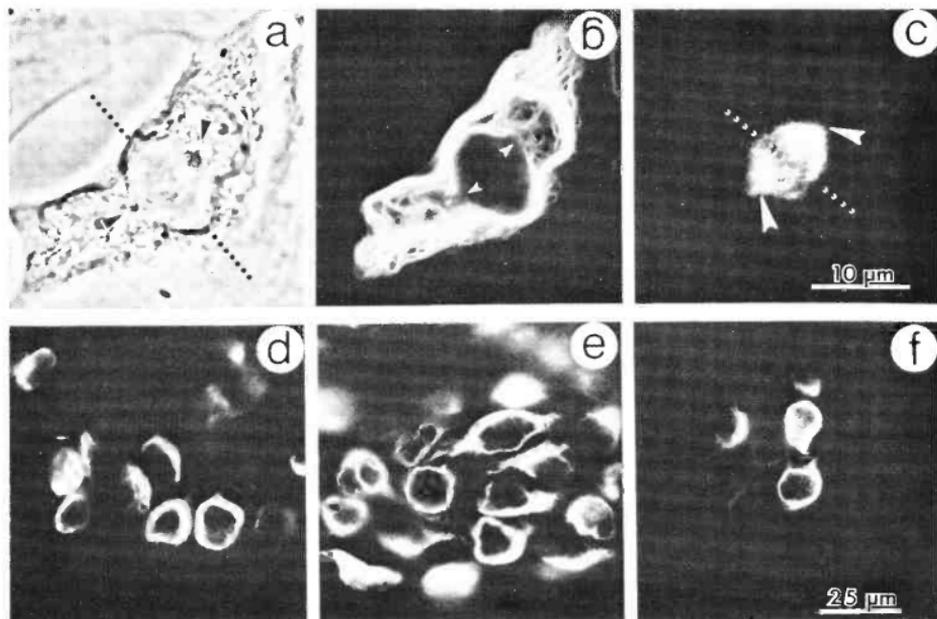


Figure 1

Phase (a) and fluorescence micrographs of the same dividing endothelial cell in metaphase stained for 10-nm filaments with antibodies labeled with fluorescein (b) and for tubulin with antibodies labeled with rhodamine (c). The chromosomes have migrated to the equatorial plate (dotted line; a and c). The 10-nm filaments remain assembled (b) and contain the mitotic spindle (c). The spindle poles (arrows, b and c) are in close apposition to the filament ring. *In situ* staining (d-f) of the inner surface of the guinea pig aorta with antibodies to 10-nm filaments reveal intact perinuclear rings.

Natl. Acad. Sci. 76:3372 [1979]) that the 10-nm filaments provide topological information about the relative position of fibrous structures and organelles within the microtrabecular lattice. It is possible that these filaments provide a framework for orienting the assembly of the mitotic spindle and cytocentrum in newly restructuring daughter cells. Current studies are directed at determining if this association might occur through the centriole region.

In situ demonstration of the endothelial 10-nm filament cytoskeleton

Studies now in progress seek to correlate the perinuclear ring of 10-nm filaments found in cultured guinea pig endothelial cells to their *in vivo* counterparts. Guinea pigs, 1 month old and 1 year old, were sacrificed and their thoracic aortas were rapidly removed. After the blood was washed away, vessels were opened along their longitudinal axis; the endothelial surface was pinned down face up and fixed with 3.7% Formalin in buffered saline for 30 minutes. The vessels were extracted in absolute acetone at -20°C , and the surface endothelium stained with antibodies to 10-nm filaments. When the surface endothelium was examined by epifluorescence, 70% of the cells exhibited intact perinuclear rings of 10-nm filaments (Fig. 1d-f). The other 30% were either very distorted or did not fall in the plane of focus, hence making observation of the ring impossible. This exciting observation confirms our *in vitro* findings. Future studies are directed at determining how widespread the ring of 10-nm filaments is in blood vessels of various species, including man.

Monoclonal antibodies to study the cytoskeleton

In the last few years the contribution of immunological markers to the study of cell motility systems has been prodigious, and a large part of our present understanding of actomyosin systems, microtubules, and intermediate filaments comes directly from immunofluorescence studies. Some aspects of this field of research, however, are not approaching limits imposed by ancillary technology; with a long-term view to expanding these technological limits, a project has been initiated to raise monoclonal antibodies to cytoskeletal components. Based on the cell hybridization procedure of Köhler and Milstein (*Eur. J. Immunol.* 6:511 [1976]), plasma cells of an immunized mouse are immortalized by fusion to a myeloma cell line, and the resulting "hybridoma" cell lines secrete immunoglobulin of a definable specificity in theoretically unlimited quantities. From such fusions involving mice immunized with crude epithelial cytoskeleton preparations, we have obtained and stabilized, by repeated cloning, several cell lines, rec-

ognizing different aspects of tonofilament proteins, or cytokeratins, as well as a number of other previously unidentified antigens. The various monoclonal antibodies give different immunofluorescent staining patterns (Fig. 2) which, although subtle in the original antigenic epithelial cell line, are often striking in other cell lines; the patterns obtained by immunoradiography on SDS-PAGE of the immunizing antigen (Burridge, *Proc. Natl. Acad. Sci.* 73:4457 [1976]) suggest that these antibodies might be alternately recognizing sites in either constant or variable regions of tonofilament protein molecules, in keeping with the structure proposed for keratin molecules by Steinert (*J. Mol. Biol.* 123:49 [1978]).

These monoclonal antibodies are proving to be excellent for use as intracellular markers, giving a very low background, and in view of their cross-reactivity with different epithelial cell types (in which the cytokeratins appear to have different molecular weights), they may become useful as standardized reagents, providing a more functional framework for defining cytoskeletal components. This approach is expected to be particularly powerful when combined with high-resolution two-dimensional gel and electron microscope technology in this laboratory.

Organelle movements and the distribution of filamentous actin in nonmuscle cells

It has for a long time been known that, although the cortical cytoplasm of nonmuscle cells is rich in thin filaments that bind heavy meromyosin (HMM), the subcortical cytoplasm contains few of these HMM-binding thin filaments. Here in this deeper cytoplasm, the predominant filament type is intermediate in size and fails to bind HMM. It thus appeared possible that the subcortical cytoplasm (where organelles reside and move) contained too little filamentous actin to provide for an actin/myosin-based system of organelle movement. As an alternative interpretation, it had to be considered whether these intermediate-size filaments contained filamentous actin closely associated with proteins that could block the binding of HMM. Investigating this possibility, various types of cultured cells (that had been extracted with glycerol) were treated with low doses of trypsin before being labeled with HMM. Initially, these cells were prepared for electron microscopy as either negatively stained or critical-point-dried whole-cell mounts. In such preparations, all filaments remaining after trypsin treatment labeled clearly with HMM; in control preparations that had had the trypsin but not the HMM, it appeared that most intermediate filaments had survived the trypsin treatment. From this it was initially concluded that most intermediate filaments of the subcortical cytoplasm contained a central core of filamentous actin. However, when similarly pretreated cultured

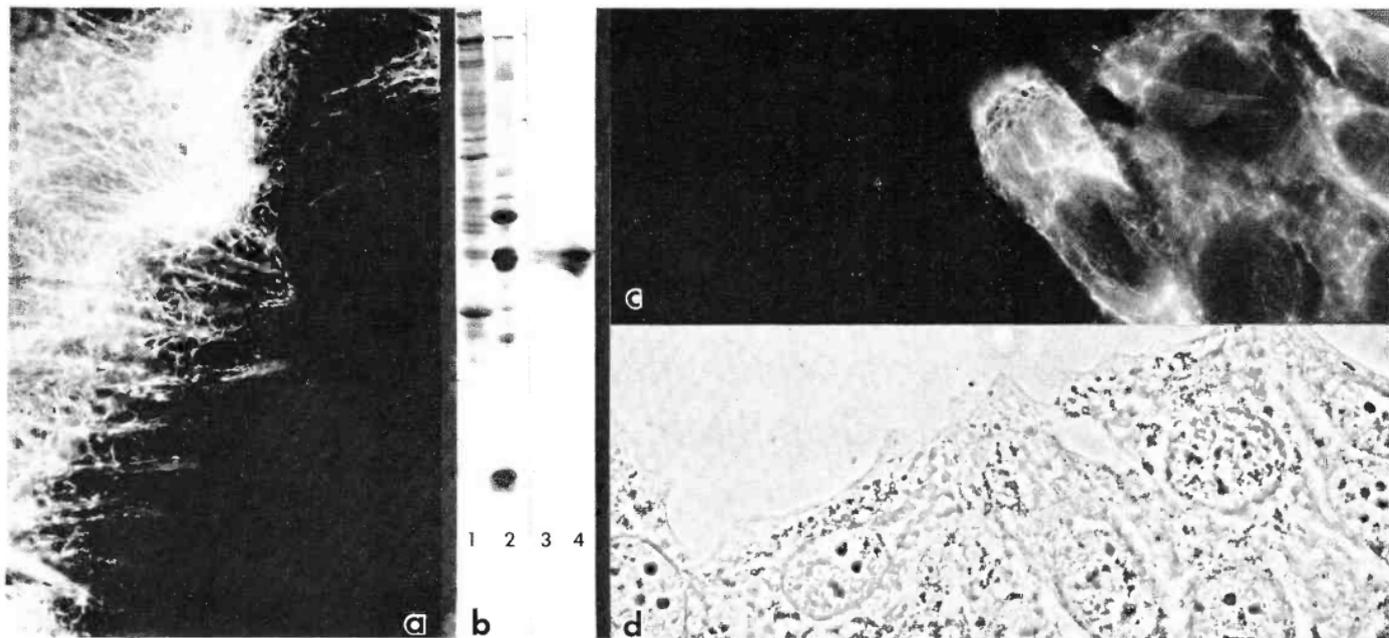


Figure 2

(a) Staining of spreading ptK1 cell margins by a monoclonal antibody. (b) Detergent-soluble (1) and insoluble (2) (= immunizing antigen) fractions of PtK1 cells and binding of this monoclonal antibody to two gel bands (3,4) on the same gel revealed by autoradiography. Staining of HeLa cells by the same antibody is heterogeneous (c). (d) Phase contrast of same field as c.

cells were prepared for electron microscopy by resin embedding and sectioning, it became clear that the effect of the trypsin was to largely eradicate the predominating intermediate filaments of the subcortical cytoplasm. Further analysis of these sectioned cells suggested that the seemingly positive results from the whole-cell-mount preparations were due to the occurrence of arrays of converging and closely apposed thin filaments that, surviving the trypsin treatment and labeling with HMM, were able to simulate the HMM labeling of what seemed to be typical intermediate filaments. It thus appears that the predominating intermediate-size filaments of the subcortical cytoplasm are devoid of filamentous actin and that there may indeed be very little of this form of actin available to generate the motion of cytoplasmic organelles.

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The efforts of our group are primarily directed toward understanding the functions and interactions of structural proteins in higher cells, particularly those proteins involved in generating cell movements.

α -Actinin in nonmuscle cells

One protein that continues to command our attention is α -actinin. Originally it was found in striated skeletal muscle where it was localized exclusively to the Z-line structures. It is into these regions that actin filaments insert, and because α -actinin will bind actin *in vitro*, it has seemed likely that it is involved in the attachment of actin to these structures. With the discovery of α -actinin in nonmuscle cells several years ago, it was suggested that α -actinin might function similarly and mediate the attachment of actin to membranes. By immunofluorescence microscopy, it has been found in locations that are consistent with such a function. In last year's report we described some preliminary experiments testing whether α -actinin does function in the attachment of actin to plasma membranes. We demonstrated that α -actinin is a prominent component of the plasma membranes isolated from several different cell types, but our attempts to extract selectively either α -actinin or actin were inconclusive. More recently, we have found relatively mild conditions of ionic strength and pH that favor the preferential release of α -actinin from membrane preparations. After several cycles of extraction, we have obtained membranes almost devoid of α -actinin but still rich in actin. Such experiments strongly suggest that α -actinin does not function in linking actin to the plasma membrane, although it may function indirectly to promote or stabilize attachments as a result of its ability to cross-link actin filaments.

We have continued to study the biochemistry of both muscle and nonmuscle α -actinins. A traditional problem in working with α -actinin has been that it is difficult to purify, and the conventional procedures are very time-consuming. Realizing the need for a better and more rapid purification, we have developed a new procedure that is both fast and simple (Feramisco and Burridge, *J. Biol. Chem.*, in press [1980]). Besides its relative convenience, our new purification scheme has several advantages. One of these has been that when applied to smooth muscle it also gives rise to pure filament and a protein with a molecular weight of 130,000. This 130K protein is most interesting and will be described in more detail below. With minor modifications, the purification of α -actinin has been applied to smooth and skeletal muscles as well as to nonmuscle tissues. This has permitted us to study the

nonmuscle form of α -actinin and compare its structure and biochemistry to the muscle types. The nonmuscle form is a distinct protein, although definite regions of homology are conserved and shared particularly with the α -actinin from smooth muscle.

Microinjection of fluorescently labeled proteins into living cells

During the last 5 years, the localization of structural proteins within cells has been revolutionized by the use of specific antibodies against these proteins and by the technique of immunofluorescent microscopy. This powerful technique, however, requires that the cells be fixed and permeabilized—procedures that prevent the observation of living cells. To overcome this problem and as a complementary approach to immunofluorescence, we have used the technique of microinjection to introduce into living fibroblasts fluorescently labeled structural proteins. This approach to studying the distribution and behavior of structural elements in higher cells such as fibroblasts has been developed independently in our laboratory (Feramisco, *Proc. Natl. Acad. Sci.* 76:3967 [1979]) and by others (Kreis et al., *Proc. Natl. Acad. Sci.* 76:3814 [1979]) and derives from the work of Taylor and Wang (*Proc. Natl. Acad. Sci.* 75:857 [1978]), who microinjected fluorescently labeled structural proteins into comparatively large protozoans. The very flat cells we have been using have well-organized stress fibers (unlike the large amoebae injected by Taylor and Wang), and as such they provide a better system for high-resolution analysis of the location of injected proteins within the living cells.

Because of our interest in the protein α -actinin, it was the first protein we studied by microinjection. When the native protein was covalently labeled with a fluorescent dye and microinjected into living fibroblasts, the fluorescent protein incorporated into the actin microfilament bundles and polygonal networks of the living cells (Feramisco, *Proc. Natl. Acad. Sci.* 76:3967 [1979]) (Fig. 1). On the other hand, when the α -actinin was first denatured by boiling and then injected, it incorporated into vesicular structures (probably lysosomes) around the nucleus. Further analysis of the injected "native" α -actinin in fibroblasts in relation to other endogenous structural proteins (viewed by conventional immunofluorescence) showed that the injected protein was (1) incorporated at least everywhere that the cells' endogenous α -actinin was found, (2) distributed along virtually all discernible actin bundles, and (3) found in antiperiodic arrangement with respect to tropomyosin along

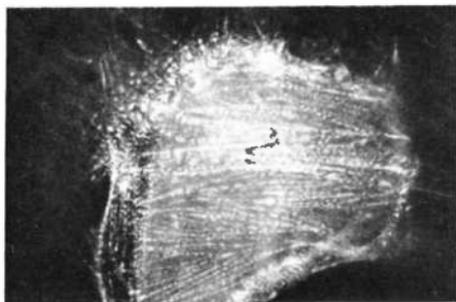


Figure 1
Incorporation of rhodamine-labeled α -actinin into a living fibroblast. A fully spread gerbil fibroblast cell was microinjected with rhodamine-labeled α -actinin and photographed 16 hr later with fluorescence optics. Note the periodicities of the injected protein along the actin microfilament bundles.

the actin bundles. These results suggest that the injected native protein will incorporate faithfully into the supramolecular structures of the living cell.

An interesting sidelight of this study showed that the distribution of fluorescently labeled α -actinin seen in the living cell was not significantly altered by chemical fixation and permeabilization of the cells—treatments that are normally used for the preparation of cells for immunofluorescent localization of many proteins. This information combined with our observation that, in the fixed and permeabilized cell, the distribution of the injected α -actinin was virtually identical to the distribution of α -actinin as seen by immunofluorescence in the same cell offers, for the first time, some evidence indicating the validity of the immunofluorescence technique in the localization of α -actinin in cultured cells.

Microinjection affords us a unique opportunity to follow specific proteins within the living cell. We have recently succeeded in injecting simultaneously two different proteins, each labeled with a different fluorescent dye, into the same cell. Also, we have connected a highly light-sensitive television camera to our fluorescence microscope, which will let us record the dynamics of the fluorescently labeled proteins within the cell as movement occurs. It is hoped that studies of this kind will help us unravel some of the complex molecular events and interactions of cell motility.

A new structural protein

In developing the new purification scheme for α -actinin from smooth muscle, we also obtained a pure protein with a molecular weight of 130,000 (Feramisico and Burridge, *J. Biol. Chem.*, in press [1980]). We were studying the interactions of this pro-

tein with other structural proteins, such as actin, when we learned at the Cold Spring Harbor meeting on the cytoskeleton this summer that the same protein had also been isolated by Geiger, who had localized it to the tips of actin microfilament bundles by immunofluorescence (Geiger, *Cell* 18:193 [1979]). Clearly, this was an interesting protein, and we decided to study its behavior and distribution both by microinjection into living cells and by immunofluorescence. Visualization of fluorescence in living fibroblasts 2–4 hours after microinjection of the protein conjugated with a fluorescent dye revealed two major distributions (Burridge and Feramisico, *Cell*, in press [1980]). One distribution was as bright, focal patches on the lower surface of the cells, similar to the distribution described by Geiger using immunofluorescence. This prominent focal distribution prompted Jim Watson to name the protein "Focin," a name that we now use in the laboratory and that we have proposed for the protein (Burridge and Feramisico, *Cell*, in press [1980]). Double-label fluorescent microscopy of cells injected with the protein and stained for actin by indirect immunofluorescence 2 hours later has confirmed that this focal distribution is at the ends of actin microfilament bundles. A second major distribution has also been seen for the injected protein with a streaky or fibrillar appearance more commonly on the top of the cells, but sometimes also on the bottom. This distribution was reminiscent of the patterns of fibronectin on the cell surface. Using antibodies against fibronectin and double-label fluorescence microscopy, we have shown that the distribution of the injected protein does indeed correlate closely with the distribution of surface fibronectin on these cells (Burridge and Feramisico, *Cell*, in press [1980]) (Fig. 2). Where the fibronectin extended beyond a cell, the 130K protein remained strictly cellular and often appeared concentrated as if in an anchorage point. The distribution of this protein both at the tips of the actin microfilament bundles and underlying the matrix of fibronectin suggests that it may be one element involved in the linkage between the actin cytoskeleton and the extracellular matrix.

Monoclonal antibodies

Specific antibodies have been powerful tools for analyzing the distribution of structural proteins in higher cells. However, to raise an antiserum by conventional means, it is usually necessary to have a pure protein for immunization. This is a major limitation since many potentially interesting proteins are difficult to purify or cannot be obtained in sufficient quantity for immunization. The production of monoclonal antibodies can eliminate this problem since immunization can be with a crude antigenic mixture and there can be subsequent selection of cells producing a pure antibody by cell cloning. Since his arrival in the labo-

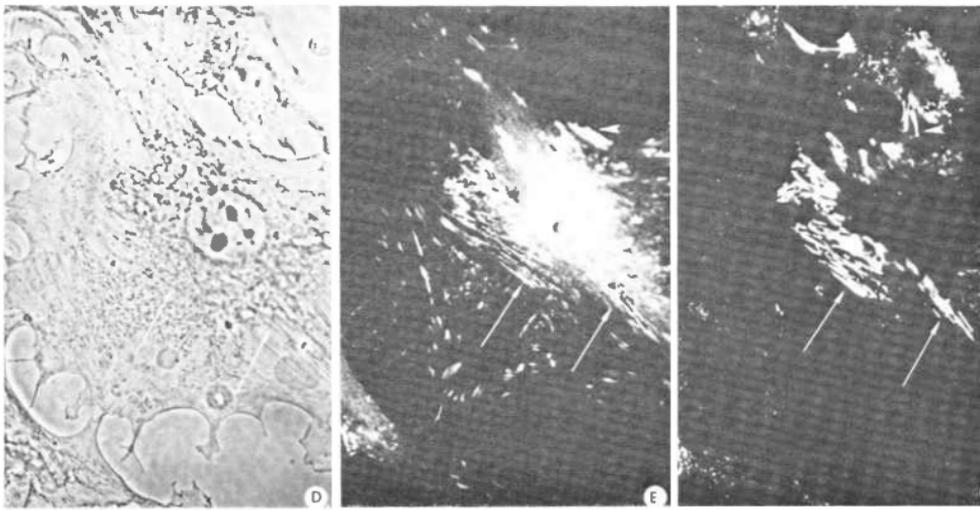


Figure 2

Phase and fluorescent micrographs of a fibroblast microinjected with the gizzard 130K protein labeled with fluorescein and indirectly stained for fibronectin with antibodies labeled with rhodamine. Two hours after the living cell was microinjected with the labeled 130K protein, it was fixed and stained for fibronectin. (D) Phase micrograph of the cell; (E, F) fluorescent micrographs viewing the fluorescein and rhodamine fluorescence, respectively; (E) two distributions of the microinjected protein can be seen: one appears as "local" patches of fluorescence on the bottom of the cell and the other appears as a fibrillar or streaky distribution (marked with arrows). This fibrillar distribution coincides with the distribution of fibronectin on the surface of the cell revealed in F.

ratory, J. Lin has begun to apply this strategy to raise antibodies against the cytoskeleton, with the aim to raise monoclonal antibodies against minor or previously unidentified components that by other means could not be approached immunologically. Antibodies against such components will be powerful reagents and will also provide a potential means of purifying the target proteins through the use of affinity chromatography on the immobilized antibodies. Although this work is still at an early stage, already several interesting monoclonal antibodies have been raised that react with components of the microfilament bundles or with intermediate filaments. The proteins binding these antibodies are currently being identified by antibody staining of SDS gels.

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QUEST-2D GEL LABORATORY

J. GARRELS, C. Burwell, J. Farrar, E. Kelly, T. Kelly

In the past year, we have designed and set up a new laboratory for analyzing the proteins of the cell by two-dimensional gel electrophoresis. We have also developed computer programs for quantitation of the proteins detected by this method and for assigning a set of standard coordinates to each detectable protein. The name we use to describe our new laboratory and computer facility stands for QUantitative Electrophoresis STandardized in 2 Dimensions (QUEST-2D).

Our aim has been to make the detection and quantitation of several thousand proteins from any cellular or subcellular sample an easy and routine procedure. At the same time, we want to ensure that our gels will have consistently high resolution and reproducibility so that we can use our computer to automatically match the proteins of each new gel to a standard protein map stored in the computer. The proteins detected on each new gel will be assigned standard map coordinates and will be stored along with their intensities in a computer data bank. This data bank should within a few years contain information about the occurrence of thousands of proteins in cells of many differentiated types and states of growth. This information will be searched and correlated in many ways to determine which proteins are found in all cells, which are tissue-specific, which are growth-specific, and which are abnormal in particular conditions of disease or transformation. We also hope to use these methods to determine the rates of synthesis, rates of degradation, posttranslational modifications, and subcellular localizations for thousands of proteins in muscle cells as they undergo differentiation in culture. These studies should help us to determine which of the many unknown proteins we should focus on in future efforts to purify and characterize proteins that regulate gene expression.

The new laboratory

It was clear to us from the start that a specialized laboratory would be needed if we expected to produce enough two-dimensional gels for in-depth analyses of differentiation in cultured cells. It was also clear from previous work that to consistently produce gels of high resolution and high reproducibility the whole procedure should be as convenient and routine as possible. The lab that was designed and built during 1979 provides for a throughput of 24 two-dimensional gels each day. The steps that must be carried out each day include the casting and loading of first-dimension gels; running of the first-dimension gels; removal of

first-dimension gels from their tubes, equilibration, and placement onto the second-dimension gels; running the second-dimension gels; processing the second-dimension gels in stains or chemicals for fluorography; drying the gels; exposure of gels to film; and film development. To make these steps easier and more reproducible, we have designed and built the following pieces of equipment: (1) A simple plastic apparatus has been built for casting many first-dimension gels quickly and reproducibly. (2) A simple clamping and sealing device has been built for holding and casting the large slab gels. (3) Special sink/benches have been installed for slab gel electrophoresis. Each sink is covered with a porous grating so that after electrophoresis, the large gel apparatus can be washed in place using sprayer hoses mounted on the wall nearby. (4) Electrode buffer, which is used in large amounts, is pumped through filters from a central reservoir to an outlet near each gel bench. (5) Special microprocessor-controlled power supplies allow any current-voltage scheme to be preprogrammed (such as constant wattage). During the run, current and voltage are monitored and logged and the power is automatically shut off when the ion front passes through the gel. (6) A dipping system has been installed for automatic staining and destaining of gels and for automatic processing of gels through chemicals for fluorography. This apparatus, which is enclosed in a hooded floor-sink, consists of a mechanical assembly for lifting and transporting the dipping baskets, the dipping baskets themselves, and large chemical tanks. Each aluminum dipping basket holds 36 perforated metal shelves on which the gels are placed. The baskets are lowered and gently agitated up and down in the tanks by the lifting mechanism. The electronic controls (microprocessor-based) provide for automatic transfer of the dipping baskets from one tank to another. (7) Large gel dryers have been built with integral heaters and with a connection to a high-vacuum system. These dryers can dry all 24 gels at once. (8) Special light-tight boxes have been built for holding the gels next to film for the duration of the exposure period. Films are developed with our automatic X-Omat film processor.

None of the above equipment could have been built without the help of the lab shop personnel, namely, Gus Dulis, Rocco Soccolich, and Steve Humenick. Gus built all the electrophoresis chambers, the gel dryers, the gel-casting apparatus, and numerous other useful items. Rocco did all the machine work for the dipping baskets and the lifting mechanism. Steve designed and built all the electronic controls.

Midway through the year, Tom Kelly joined us and began training as the technician in charge of daily operation of the gel lab. He also has participated in its construction and has become experienced in its operation.

Carter Burwell, normally in the computer room, has also helped to get the gel lab ready for operation. He has written a set of programs that are run from a computer terminal in the gel lab to help us keep track of all the samples, gels, and films we produce. Since quality control is so important, we will also use these programs to keep careful records of each solution that we make for gel electrophoresis, including lot numbers, exact formulation, pH, and conductivity.

The computer facility

The development of the gel lab has gone hand-in-hand with the development of a set of computer programs for the analysis of our gels. Our computer and scanning equipment were installed on the second floor of the library at the end of 1978 (see the Annual Report for that year). During 1979 we were able to write a set of basic programs for scanning and aligning the film images, for subtraction of background streaks and film noise, for merging data from different exposures of the same gel, and for automatically matching the pattern of spots on each new gel to a standard spot pattern.

John Farrar, who has written many of our basic scanning and display programs, as well as our background subtraction routine and our backup programs, left in August to return to medical school. Carter Burwell, who joined us in January, has developed our alignment and merging programs and matching programs, and he has considerably enhanced our data-display programs. As mentioned above, Carter has also written a powerful record-keeping system that will be used not only to record laboratory data, but also to keep records on the scanning and calibration of each film.

In the design of our image-processing programs, we have put primary emphasis on rigorous matching of data from many different gels. We want to establish a standard may in which a unique set of coordinates are assigned to each detectable protein within a species, such as rat or mouse. The proteins of each new pattern will then be matched to the proteins of the standard map. Since the patterns become quite crowded after long exposures, we know that we cannot describe each protein unambiguously by only the two coordinates of size and charge. Fortunately, we can use our gel system to obtain more information about each protein, and our matching programs have been designed to make use of this additional information.

Our major source of additional information for identification of the proteins will come from double-label experiments. Many of the cells we study will be

labeled with two radioactively labeled amino acids, one containing ^3H and the other containing ^{35}S . The gels obtained by electrophoresis of these double-labeled samples will be exposed soon after they are run and again 4–6 weeks later, when a substantial amount of the ^{35}S will have decayed. By scanning both exposures and asking the computer to compare the two images, we can obtain the ratio of the two amino acids across the entire gel. Within a protein spot the amino acid ratio is constant, but between neighboring spots, the ratio of amino acids, such as methionine and proline, can vary considerably. If we display this ratio information in terms of color on our TV display, we will be able to visualize the three parameters of size, charge, and amino acid ratio. In the matching programs, the ratio information will be considered as well as size and charge in determining which spots should be matched.

In addition to the amino acid ratio data, there is another source of additional information that we will often use for more rigorous matching. We know that if we compare the pattern of spots detected on a 7.5% second-dimension gel and the pattern detected on a 10% second-dimension gel, there are numerous small shifts in the relative positions of the spots. This occurs because the relationship between mobility and pore size is slightly different for each protein. Therefore, once we know where a protein runs at two different slab gel concentrations, we can look for it in both types of gels before we confirm its identity in a new sample. This parameter will also help us to detect proteins that are overlapped by neighboring spots at one acrylamide concentration, but not at another. Our matching programs have been designed to take into account this type of information for samples that have been run at two or more acrylamide concentrations.

In the programs that have been written during the past year, we have tested the basic concepts and have found that our approach is feasible. What remains for us to do in the future is to refine these programs and to connect them together into a finished system for routine gel scanning. With the development work nearly complete, we will in the next year begin to establish our data base for the proteins in differentiated cells and to pursue our special interests into the details of muscle differentiation as a model system.

Publications

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NEUROBIOLOGY

The Neurobiology Section has progressed from just a summer program into a year-round activity at Cold Spring Harbor Laboratory. Not only does research continue all year long, but meetings and workshops also occur during the spring, autumn, and winter months. Some of this off-season activity reflects the old spirit of Cold Spring Harbor, when scientists visited the laboratory for a few weeks or months to do experimental work. A good example is George Augustine. After taking the Synapse Course in 1978, George came back the following spring with Linda Beres, Bill Higgins, Patrick Riordan, Cynthia Scott, and Winsor Watson to collaborate on common projects.

The number of our neurobiology courses has been steadily increasing since 1972, and we now have a total of seven courses projected for 1980. Of the six courses given in 1979, a new one, the Neurobiology of Behavior, was taught by Eric Kandel, John Koester, Fernando Nottebohm, and Keir Peirson. The Mammalian Central Nervous System Course had a radical change in leadership, with only Peter Kirkwood continuing on as instructor and David Hubel, Joseph Malpel, and Helen Sherk as new instructors. The Leech Course, taught for many years by John Nicholls and Ken Muller, gained Itzack Parnas as a new instructor. Courses that continued largely under their old leadership were Electrophysiological Methods for Cellular Neurobiology, taught by Jacsue Kehoe, Dante Chiarandini, Enrique Stefani, and Ray Kado, and Synaptic Structure and Function, taught by Rami Rahamimoff, U. Jack McMahan, Charles Stevens, and Dale Purves. We would like to thank Jennifer and Matthew LaVail for developing the Neuroanatomical Methods Course; this course will have new leadership in 1980.

Every year, new concepts are taught in our courses and new technologies are incorporated. Two examples for 1979 were (1) the immunocytochemical techniques introduced by Elyn Glazer and Allan Basbaum into the Neuroanatomical Methods Course and the Pain Workshop and (2) the principles of the use of digital computers in data collection and analysis taught by Rami Rahamimoff in the Exploratory Synapse Workshop. Workshops involving a MINC computer (donated by the Digital Equipment Corporation) will also become part of the 1980 Synapse Course curriculum.

During the past year, much effort was put into acquiring fellowship monies for the participants in our summer courses. In previous years, half the students in each course received half-fellowships deriving largely from the Marie Robertson Fund and a National Institutes of Health grant. In addition, for the next three years, we are fortunate to have secured full scholarships from the Grass Foundation and the Stiftung Volkswagenwerk.

The Neurobiology Laboratory uses the leech as a model system to study the nervous system. The advantage of using an invertebrate preparation like the leech lies in the possibility of studying identified neurons rather than a large population, which is usually what is done with vertebrate brains. The importance of working with precisely identified neurons becomes obvious if one realizes that the two nociceptive (or N) cells in a leech ganglion, with their similar axoneuritic geometries and electrophysiological properties, are functionally different. The N cell sensing pain in the leech belly has different membrane receptors and integrates into different neural networks than the N cell that senses pain in the leech back.

During the past year, we worked on the following three different projects using the leech nervous system: (1) the neural network subserving mating behavior, (2) an analysis of the mode of action of the opioid peptide enkephalin, and (3) the isolation of hybridomas producing monoclonal antibodies against the leech nervous system.

Neuronal interactions in the mating network

The basic unit of the leech central nervous system is a 400-neuron ganglion that is repeated 34 times to in-

nervate the animal's segmental body. The total number of leech nerve cells exceeds 34×400 because extra neurons are added to a few ganglia to subservise such specialized functions as reproductive behavior. In the *Haemopsis* leech, the sex ganglia, 5 and 6, are almost twice as large as the standard midbody ganglion. This size increase is due, in part, to the addition of two pairs of large neurons.

Much of our past work centered on these two large, easily identified pairs of neurons. This year a number of other sex neurons have been mapped by nerve backfilling, which we recently were able to adapt to the leech. Backfilling a nerve permits the identification of neuronal elements that project their axons through this common pathway. If the nerve subserves a specialized function, as is true for the male sex-nerve feeding into ganglion 6 (Fig. 1), the backfill can reveal major sensory and motor neurons. The smaller cells seen in Figure 1 are all newly identified neurons, whereas, of the three large cells, the middle one is the serotonergic Retzius cell and the other two are the two motor neurons, the R and L cells, that we originally studied.

The two motor neurons interact electrotonically across a high junctional resistance. In spite of this relative electrical insulation, a strong voltage coupling



Figure 1
Cell bodies in ganglion 6 backfilled with HRP from the black-stained sex nerve at the upper right.

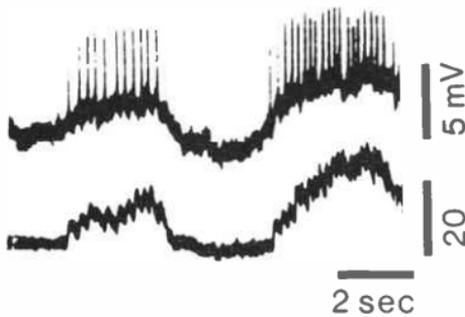


Figure 2
Electrical interactions between L and R motor neurons. Action potentials in an L cell (upper trace) elicit depolarizing synaptic potentials in an R cell (lower trace.).

exists for low-frequency depolarizing of potential changes transmitted from the L cell to the R cell. The explanation lies in the voltage-dependent membrane resistance of the R cell. The input resistance of the R cell increases tenfold as its membrane is depolarized from resting potential to threshold. The resulting electrotonic interactions are asymmetrical, almost like a unidirectional chemical synaptic transmission.

The L cell also excites the R cell by means of a fast electrotonic postsynaptic potential (PSP). The stoichiometry of the PSP generation led to the hypothesis of a pair of interneurons connecting the R and L cells electrically. The data are as follows: An L-cell action potential can evoke PSPs of two discrete amplitude distributions in the other L cell and in the pair of R cells. Since the smaller PSP is half the size of the larger one, they are considered to be single and double events. Synchronous action potentials occurring in

both L cells similarly only elicit single- or double-size PSPs or failures, but not events that are three- or four-fold in size. PSPs that are blocked by Tetrodotoxin are explained as reflections of action potentials from a pair of interneurons connecting symmetrically to the L and R cells via gap junctions.

The hypothetical interneurons appear to be key decision-making elements in the network, since L cells can only fire R cells with the help of the electrotonic PSPs. If the single- and double-size PSPs are generated with a high probability, the L cells can assume a wavy bursting pattern (Fig. 2), which is characteristic of vertebrate and invertebrate neuron-activating behaviors.

Further evidence for the existence of the interneurons came from an experiment in which the L cells were injected with Lucifer yellow, a fluorescent dye that crosses gap junctions (Fig. 3). A bilateral pair of small neurons (arrows) that lie in a different focal plane than the brightly fluorescent L cell showed more dye coupling than did the homologous L or the pair of R cells. This raises the possibility that even the low-frequency electrotonic coupling might occur via the interneurons.

Opioid peptides

The small endogenous peptides, Met and Leu enkephalins, bind to the same specific receptors as opiate analgesics. They, together with the larger endorphin peptides, have been widely found in mammals, where they appear to play a significant role in neuronal pathways mediating pain and emotional behavior. The earthworm is the only invertebrate presently known to have enkephalinlike substance.

This year, an enkephalinlike activity was localized

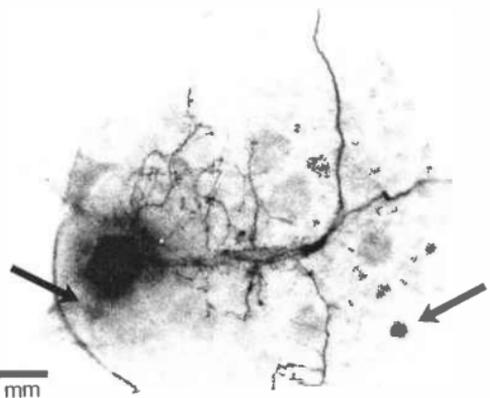


Figure 3
Localization of a bilateral pair of neurons through fluorescent-dye coupling.



Figure 4
Identification of a specific leech neuron immunoreactive to enkephalin.

in the leech nervous system using immunocytochemical techniques (Fig. 4). Rabbit anti-enkephalin that had reacted with leech tissue was linked to a peroxidase-antiperoxidase complex (PAP; Sternberger) which then produced an insoluble brown reaction product upon the addition of 3,3'-diaminobenzidine and H.O. We learned this immunocytochemical technique at the Cold Spring Harbor Neuroanatomy Course last summer.

We are now in the process of studying further the enkephalin-containing neuron. Its cell body invariably appears between a pair of neurons called the "nuts" and the giant serotonergic Retzius cell as a member of a small set of three to four neurons with 30- μ m somatas. Since all leech neurons have their particular electrical parameters, it should be possible to reliably characterize the enkephalin-containing neuron electrophysiologically.

Monoclonal antibodies against the leech nervous system

In collaboration with Ron McKay, monoclonal antibodies against the leech nervous system are being made by injecting neural tissue into mice. Figure 5



Figure 5
A monoclonal antibody reacting with a small subset of cells in a 400-neuron midbody ganglion.

shows a leech ganglion reacted with our first interesting monoclonal antibody. On the ventral surface (see Fig. 5), nine cell bodies are stained to various degrees by an HRP reaction product obtained by linking mouse antibodies with protein A to the PAP complex. Eight of the stained somatas seem to be bilaterally symmetrical, whereas the ninth soma lies medially on top of stained axons running vertically across the ganglion. The medial glial package to which this neuron belongs has identified unilateral cells, whereas the lateral glial package to which the other neurons belong contains bilaterally symmetrical sensory and motor neurons.

Publications

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 ———. 1980. Identification of specific leech neurons immunoreactive to enkephalin. *Nature* (in press).
 ———. 1979. Identifiable neurons controlling penile eversion in the leech. *J. Neurophysiol.* 42:455.
 ———. 1979. Voltage-modulated membrane resistance in coupled leech neurons. *J. Neurophysiol.* 42:465.
 ———. 1979. Quantal electric psp. In *Abstracts of Society for Neuroscience Ninth Annual Meeting.*

INVERTEBRATE NEUROBIOLOGY WORKSHOP, March 12 – March 31

ORGANIZER

Augustine, George J., B.S., University of Maryland, College Park

Many nervous system functions are performed by discretely localized networks of neurons. These networks, although presumed to occur in all animals, are frequently studied in the somewhat simpler nervous systems of invertebrates. In the winter workshop this year, the participants examined how the properties of localized neural networks of several invertebrates could be influenced by neurotransmitters, neurohormones, and other exogenous influences.

Experiments were performed on three different systems; the cardiac ganglion of the horseshoe crab *Limulus polyphemus*, the penile eversion network of the leech *Haemopsis marmorata*, and the visceral ganglion of the soft-shell clam *Mya arenaria*. Most attention was focused on the *Limulus* cardiac ganglion. W. Watson and G. Augustine examined the efferent innervation of the ganglion to determine the structure and physiological properties of the neurons responsible for modulating its rhythmic output. W. Higgins and C. Scott determined the action of putative neurotransmitters and neurohormones on the ganglion and the possible mediation of the long-lasting effects of these substances via cyclic nucleotides. P. Riordan, B. Zipser, and G. Augustine developed a modified technique for labeling cardiac ganglion neurons with the electron-dense marker horseradish peroxidase, and they used this technique to examine the anatomy of these neurons with light and electron microscopy.

In the leech, W. Higgins and B. Zipser considered how several neurotransmitters could interact to regulate the activity of muscles responsible for copulation. L. Beres worked with the clam nervous system, to determine how the salinity of the clam's environment could alter the characteristics of its nervous system.

PARTICIPANTS

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Higgins, William J., Ph.D., University of Maryland, College Park
Riordan, G. Patrick, M.S., Georgetown University, Washington, DC
Scott, Cynthia, B.S., University of Maryland, College Park
Watson, Winsor H., Ph.D., University of New Hampshire, Durham

EXPLORATORY SYNAPSE WORKSHOP, July 24 – August 22

ORGANIZER

Rahamimoff, Rami, M.D., Hebrew University Medical School, Jerusalem, Israel

The purpose of the Exploratory Synapse Workshop was to teach electrophysiological and computational methods while at the same time examining new approaches and techniques in the study of the cellular basis of neuronal function. Workshop participants learned how to program a small lab computer for On-Line data collection and analysis using the muscle endplate potential as a neurobiological signal.

The projects were:

1. the use of MINC-11 digital computer for On-Line Data Acquisition and analysis (Maehlen, McManus, Meiri, Pickett, Rahamimoff, Stewart)
2. the use of heavy water in a study of subthreshold and suprathreshold synaptic potentials (Maehlen, Pickett, Rahamimoff)
3. time series analysis of electromyographic data of patients with muscle weakness (Pickett)
4. the action of purified small neurotoxins on neuromuscular transmission (McManus)
5. generic and environmental control over shape and electrical behavior on nerve cells (Feder, Stewart)
6. oscillations in evoked release and neuronal facilitation (Meiri, Pickett)
7. the action of the sodium ionophore monensin on transmitter release (Meiri, Erulkar, Rahamimoff)
8. catecholamine modulation of tetanic potentiation (Nussinovitch)

The workshop was made possible through the gift of a MINC-11 system from the Digital Equipment Corporation.

PARTICIPANTS

Erulkar, Sol, D.Sc., Ph.D., University of Pennsylvania Medical School, Philadelphia
Feder, Ned, M.D., National Institutes of Health, Bethesda, Maryland
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Pickett, Jack, M.D., University of California, San Francisco
Rivera, Amelia, M.S., University of Puerto Rico Medical School, San Juan
Rahamimoff, Rami, M.D., Hebrew University Medical School, Jerusalem, Israel
Stewart, Walter, B.A., National Institutes of Health, Bethesda, Maryland

COMPUTER MODELING OF SPECIFIC NEURAL NETWORKS, August 5 – August 11

ORGANIZER

Zipser, David, Ph.D., Cold Spring Harbor Laboratory, New York

The building of models holds a central position in the explanation of natural phenomena. Models enable science to relate masses of complex data at one level of organization to processes at another. Nowhere is this more clearly needed than at the interface between neuro and cognitive science. This year's workshop was devoted to the problems of constructing and understanding computer models of neuronal phenomena. The goal of the workshop was to examine some of the existing neuro models, see how they might be expanded to meet more significant challenges, and examine in detail their structure and logical significance. While computer modeling was emphasized and the computer was hovering in the background at all times, many of the models were conceptual or mathematical.

The general organization of the workshop involved first investigating techniques that could be applied to relatively simple, usually invertebrate systems in which neural network architecture is well known. We then discussed a variety of intermediate systems, moving on finally to presentations related to eye movement. The eye movement system is perhaps the most tractable neurobiology area involving the interface between cognitive and neurophysiological studies. The system involves the ability to trace processes from input through output in a complex neuromuscular system in which the cognitive correlates can be derived from behavior in a fairly straightforward fashion. The whole operation takes place in the head, thus circumventing a good deal of complexity usually brought in by spinal processing.

Although our workshop was only a beginning, barely scratching the surface of many of these problems, it demonstrated the rich rewards to be obtained by an intensive study of neuro modeling paradigms.

PRESENTATIONS

- Bahill, T., Carnegie Mellon University, Pittsburgh, Pennsylvania: *Validation, linearization, and sensitivity analysis of homomorphic model for eye movement control system.*
- Eckmiller, R., University of Dusseldorf, Germany: *New models of the neural control system for pursuit eye movements.*
- Edwards, D., Stanford University, California: *Formulating and organizing computer models of neuronal systems.*
- Feldman, J., University of Rochester, New York: *Computer-science-motivated approaches to representing neural network function.*
- Friesen, O., University of Virginia, Charlottesville: *Analysis of rhythm generation in leech swimming with analog circuits.*
- Just, M., Carnegie-Mellon University, Pittsburgh, Pennsylvania: *Eye movements and psychological processes.*
- Lara, R., University of Massachusetts, Amherst: *Visual motor coordination in the frog and toad.*
- Llinas, R., New York University School of Medicine, New York: *Use of models in neurobiology.*
- Merickel, M., Baylor College of Medicine, Houston, Texas: *Motivation and techniques for modeling a neural network.*
- Mulloney, B., University of California, Davis: *Cellular and synaptic basis of stomatogastric motor patterns: What and when is enough?*
- Optican, L., National Institutes of Health, Bethesda, Maryland: *Adaptive control mechanisms for saccadic eye movements.*
- Pellionisz, A., New York University School of Medicine, New York: *A model of the cerebellum.*
- Raphan, T., Mt. Sinai School of Medicine, New York: *The role of the integrator in modeling the visual-vestibular interaction.*
- Stark, L., University of California, Berkeley: *Modeling neurological generation of controller signals for eye movements.*
- Von der Malsburg, C., Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany: *Self organization in the visual system.*

ORGANIZER

Basbaum, Allan I., Ph.D., University of California, San Francisco

ASSISTANT

Goldstein, Ron, B.S., Columbia University, New York

Because analysis of the mechanisms of pain generation and modulation inherently requires an interdisciplinary approach, pain itself is an ideal topic for a workshop that brings together physiological, anatomical, and behavioral approaches to solving a common problem. The Pain Workshop at Cold Spring Harbor Laboratory was specifically organized to include researchers from several disciplines, each of whom has a long-standing interest in the problems of pain and pain control. The physiological focus was provided by Gary Bennett and Ron Dubner, who made intracellular recordings from "pain-responsive" neurons of the primate spinal cord. These neurons were intracellularly marked for subsequent anatomical reconstruction. Tissue of one animal was examined by both immunohistochemical and autoradiographic procedures for localizing CNS amines and peptides (including the endogenous opiate leucine enkephalin). Of particular interest were the interactions that apparently take place between these compounds and how this might underlie some endogenous pain-control mechanisms. Additional anatomical studies of the pain pathways were examined with transport methods using horseradish peroxidase (HRP) and various lectins. The anatomical studies were carried out by Ellyn Glazer, Joe Coulter, Susan Hockfield, and M.A. Ruda. A more global analysis of the neural substrate of pain included the use of the 2-deoxyglucose method to map the CNS metabolic changes generated by a pain-producing stimulus. The latter studies, under the direction of Peter and Carol Hand, were directed toward locating the heretofore elusive thalamic and cortical structures that subserve the perception of pain. In addition, guest lectures by Ann-Judith Silverman and Tom Jessel contributed to the scientific interchange that was an important part of the workshop. It is our hope that the results of these studies will bring fresh approaches to the problem of pain, a problem that clearly will not be solved without similar interactions.

PARTICIPANTS

Basbaum, Allan, Ph.D., University of California, San Francisco
Bennett, Gary, Ph.D., National Institutes of Health, Bethesda, Maryland
Coulter, Joe, Ph.D., Marine Biomedical Institute, Galveston, Texas
Dubner, Ronald, Ph.D., National Institutes of Health, Bethesda, Maryland
Glazer, Ellyn, Ph.D., University of California, San Francisco
Hand, Carol, B.S., University of Pennsylvania, Philadelphia
Hand, Peter, Ph.D., University of Pennsylvania, Philadelphia
Hockfield, Susan, Ph.D., University of California, San Francisco
Ruda, M., Ph.D., National Institutes of Health, Bethesda, Maryland

PRESENTATIONS

Jessel, Tom, M.D., Harvard University, Cambridge, Massachusetts
Silverman, Ann-Judith, Ph.D., Columbia University, New York

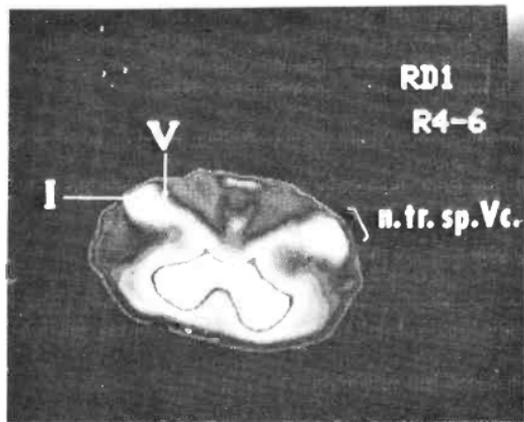


Figure 1

A computer-enhanced color display of [^{14}C] deoxyglucose (2DG) metabolic labeling in a transverse section of the medulla oblongata of the rhesus monkey. Color differences indicate optical densities of labeling and thus of differences in glucose utilization and neural activity. In this awake, flaxedilized animal (RD1), which was under nitrous oxide analgesia, noxious heat ($40\text{--}50^\circ\text{C}$) was pulsed through a probe applied to the left upper lip. Observe the two foci of increased 2DG labeling in the left spinal trigeminal subnucleus caudalis (N. tr. sp. Vc). The spot labeled I (arrow) is localized to the maxillary representation in lamina I and the larger area labeled to lamina V of N. tr. sp. Vc.

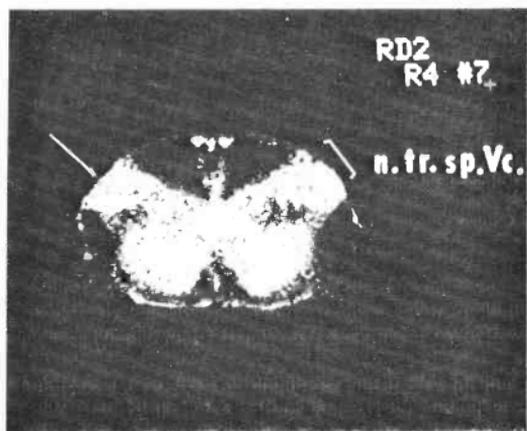


Figure 2

A computer-enhanced color display of 2DG labeling in a transverse section of the medulla oblongata of the rhesus monkey. The level of this section was similar to that in Fig. 1. In this animal (RD2), under the same experimental conditions as RD1, a heat probe was placed on the left upper lip, but no thermal stimulus was applied. Note that labeling was most dense in the left N. tr. sp. Vc (arrow) and was not focalized as in RD1.

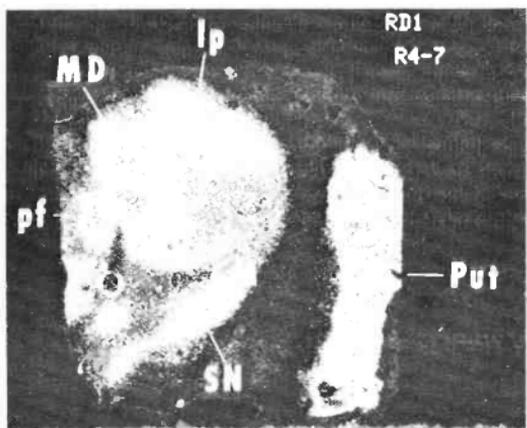


Figure 3

A color display of 2DG labeling in a transverse section of the right thalamus of RD1, which had noxious heat applied to its left upper lip (see Fig. 1). At this thalamic level, dense labeling is observed in the following contralateral nuclei: parafascicularis (pf); centrum medianum (CM); medialis dorsalis (MD); lateral posterior (lp). Labeling in the subthalamic nucleus (SN) appears not to be specific to the noxious heat stimulus, as it has been found in a number of control animals.

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

VIRAL ONCOGENES, May 30–June 6

In the early days of tumor virus research, few people had any illusions that any final answers would soon be forthcoming. The nature of viruses themselves was still in doubt, and the necessity of working with living animals meant that many months, if not years, would be needed to carry out successfully a given experiment. Then, in the early 1960s, Temin, Rubin, Dulbecco, Sachs, and Stoker successfully brought cell culture into tumor virology, and for the first time, thinking at the molecular level could begin. Cell culture procedures, however, are necessarily expensive, and the prospect of doing extensive studies on molecular biology of any tumor virus seemed to demand amounts of money that only a few lucky labs might be able to command.

And so, when in 1969 research on SV40 commenced here, we saw a long uphill fight to achieve meaningful answers at the DNA level. Fortunately, however, we did not long remain effectively bored, as the discovery of the restriction enzymes, together with the newer methodologies for separating DNA fragments and blotting them to RNA, led to an explosion of meaningful facts about the genomes of the DNA tumor viruses. Simultaneously, through the discovery of reverse transcriptase, the RNA tumor viruses also were open to meaningful analysis. By 1974 we could hold our June Symposium on tumor viruses, and though long and trying, it was at all times exciting.

Since then, the pace of tumor virus research has quickened even further. The arrival of the recombinant DNA technology, together with powerful new ways of sequencing DNA, allows us now to directly focus on those viral genes that are directly involved in the conversion of normal cells into their malignant counterparts. "Viral oncogenes" are no longer theoretical constructs but real objects of intensive research, which soon should profoundly affect the way we think about cancer cells.

We thus felt it appropriate again to bring together for a Symposium the now frequently separate worlds of the DNA tumor viruses and the RNA tumor viruses. In doing so, we hoped that many key insights might turn out to be common to both disciplines. In arranging this Symposium, Joe Sambrook, Mike Botchan, and I called many friends for advice, and, in particular, we thank David Baltimore, Mike Bishop, and Phil Sharp for providing lists of key speakers. Again limitations in time kept us from inviting all we wished, and the formal program was necessarily somewhat arbitrary. However, we did have about 140 scheduled speakers and a number of short, impromptu presentations. Our audience was correspondingly large, with a registration of 411.

We acknowledge the much needed financial support given by the



National Institutes of Health, the National Science Foundation, and the Department of Energy. It allowed us to provide the travel expenses of many foreign visitors, in addition to covering the costs of all our invited speakers while they were at Cold Spring Harbor.

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

Opening Address: H. TEMIN, McArdle Laboratory, University of Wisconsin, Madison

Session 1

L. CRAWFORD, Imperial Cancer Research Fund, London, England: Synopsis—The transforming genes of DNA tumor viruses.

P. DUESBERG, University of California, Berkeley: Synopsis—The transforming genes of retroviruses.

Session 2: DNA Tumor Viruses—Early RNAs and Early Proteins

Chairperson: P. BERG, Stanford University, California

M. PIATAK,* P. K. GHOSH,† V. B. REDDY,* J. SWINSCOE,* P. LEBOWITZ,† J. MERTZ,‡ T. SHENK,§ K. N. SUBRAMANIAN,‡ and S. M. WEISSMAN,* *Department of Human Genetics, and †Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut; ‡McArdle Laboratory, University of Wisconsin, Madison; §Microbiology Department, University of Connecticut Health Center, Farmington; ‡Microbiology Department, University of Illinois Medical Center, Chicago: The structure of SV40 mRNA.

G. KHOURY, J. C. ALWINE, R. DHAR, P. GRUSS, C. -J. LAI, S. SEGAL, and I. SEIF, NCI, National Institutes of Health, Bethesda, Maryland: Regulation of SV40 gene expression through RNA splicing.

R. KAMEN, J. FAVALORO, J. PARKER, L. LANIA, and A. MELLOR, Imperial Cancer Research Fund, London, England: Comparison of polyoma virus early gene expression in productively infected mouse cells and transformed rodent cell lines.

M. GRIGORYAN, P. CHUMAKOV, and E. LUKANIDIN, Institute of Molecular Biology, USSR Academy of Sciences, Moscow: Transcription of SV40 DNA in transformed rat cells.

S. J. FLINT, G. A. BELTZ, J. STÄDLER, J. LIESCH, and H. W. WEINTRAUB,* Department of Biological Sciences, Princeton University, New Jersey; *Fred Hutchinson Cancer Research Center, Seattle, Washington: Mechanisms of expression of viral genes in cells transformed by SV40 and adenovirus type 2.

R. TJIAN and A. ROBBINS, Department of Biochemistry, University of California, Berkeley: The SV40 tumor antigen is an enzyme.

D. LIVINGSTON, J. GRIFFIN, D. SHALLOWAY, and G. SPANGLER, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Enzymatic and DNA binding properties of large SV40 T antigen.

C. PRIVES, M. OREN, D. GIDONI, H. SHURE, and Y. BECK, Weizmann Institute of Science, Rehovot, Israel: Interactions of SV40 T antigen with DNA.

W. ECKHART, M. A. HUTCHINSON, and T. HUNTER, Tumor Virology Laboratory, Salk Institute, San Diego, California: Polyoma T antigens.

A. SMITH, B. SMITH, B. GRIFFIN, and M. FRIED, Imperial Cancer Research Fund, London, England: Protein kinase activity associated with polyoma virus middle-T.

Session 3: src. I.

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

R. L. ERIKSON, M. S. COLLETT, E. ERIKSON, J. S. BRUGGE, and A. F. PURCHIO, Department of Pathology, University of Colorado Medical Center, Denver: The transforming protein of avian sarcoma viruses and its homolog in uninfected cells.

J. M. BISHOP, G. CALOTHY,* A. D. LEVINSON, L. LEVINTOW, H. OPPERMANN, D. SIEINNESS, R. SWANSTROM, and H. E. VARMUS, University of California, San Francisco; *Institut Curie, Orsay-Paris, France: The origin and function of avian retrovirus transforming genes.

T. HUNTER, B. SEFTON, and K. BEEMON, Tumor Virology Laboratory, Salk Institute, San Diego, California: Analysis of avian sarcoma virus pp60^{src}.

- J. M. BUCHANAN, W. H. BERG, J. G. BURR, J. KAMINE, S.-R. LEE, and M.-H. PARK, Department of Biology, Massachusetts Institute of Technology, Cambridge: Studies on the characterization, processing and isolation of transformation-specific proteins.
- P. MANESS, H. ENCESER, M. GREENBERG, M. O'FARRELL, and G. M. EDELMAN, Department of Developmental and Molecular Biology, Rockefeller University, New York, New York: Isolation and assays for activity of the *src* gene product.
- E. TSEN, B. DAMIANI, P. HSIEH, M. WALSH, and L. B. CHEN, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Inhibitors for protein kinase activity associated with *src* gene product.
- R. RADKE and G. S. MARTIN, Department of Zoology, University of California, Berkeley: Synthesis and phosphorylation of cellular polypeptides in chick embryo fibroblasts transformed by Rous sarcoma virus.
- C. CALOTIY, POIRIER, and B. PESSAC, Institut Curie-Biologie, Orsay, France: Expression of viral oncogenes in differentiating chick embryo neuroretinal (NR) cells infected with avian tumor viruses.

Session 4: T Antigens

Chairperson: B. HIRT, Swiss Institute for Experimental Cancer Research, Lausanne

- Y. ITO and N. SPURR, Imperial Cancer Reserach Fund, London, England: T-antigens expressed in polyoma-virus-transformed cells.
- P. TEGTMEYER and T. SPILLMAN, Department of Microbiology, State University of New York, Stony Brook, New York: Purification of SV40 small-T antigen.
- F. MCCORMICK, F. CHAUDRY, A. E. SMITH, and E. PAUCHIA,* Translation Laboratory, Imperial Cancer Research Fund, London; *Department of Biochemistry, Imperial College, London, England: Characterization of different tumor antigens present in cells transformed by SV40.
- L. V. CRAWFORD, D. P. LANE,* D. T. DENHARDT, E. E. HARLOW, P. M. NICKLIN, K. OSBORN, and D. C. PIM, Imperial Cancer Research Fund, London, England; *Cold Spring Harbor Laboratory, New York: Characterization of the complex between SV40 large-T antigen and the 53K host protein in transformed mouse cells.
- P. MAY, M. KRESS, M. LANGE, and E. MAY, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Study of the 55K protein(s) expressed in SV40-transformed cell lines and immunoprecipitable by an anti-SV40 tumor serum.
- J. A. MELERO, D. S. GREENSPAN, S. TUR, K. MUELLO, and R. B. CARROLL, Department of Pathology, New York University School of Medicine, New York: T antigen-associated proteins, which are induced by SV40 transformation.
- D. I. H. LINZER, W. MALTZMAN, and A. J. LEVINE, Princeton University, New Jersey: Characterization of a murine cellular SV40 tumor antigen in SV40 transformed cells and uninfected embryonal carcinoma cells.
- W. DEPERT* and R. HENNING,† *Max-Planck-Institute for Biophysical Chemistry, Göttingen; †Department of Biochemistry, University of Ulm Medical School, Germany: SV40 T-antigen related molecules on the surface of adenovirus 2-SV40-hybridvirus-infected HeLa cells and on SV40 transformed cells.
- S. S. TEVETHIA, R. S. GREENFIELD, D. FLYER, and M. J. TEVETHIA, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: SV40 transplantation rejection antigen—Relationship to SV40 specific proteins.
- G. KLEIN, Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden: Transformation by lymphotropic herpesviruses—Nuclear antigen studies.

Session 5: The Genomes and Proteins of Retroviruses. 1.

Chairperson: R. WEISS, Imperial Cancer Research Fund, London, England

- D. DONOGHUE, M. GOLDFARB, C. TRENT, and R. A. WEINBERG, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Transformation by Moloney sarcoma virus.
- S. AARONSON, E. CANAANI, K. ROBBINS, P. ANDERSEN, and S. TRONICK, NCI, National Institutes of Health, Bethesda, Maryland: Cellular origin of the transforming gene of Moloney murine sarcoma virus.
- G. VANDE WOUDE, M. OSKARSSON, L. ENQUIST, P. FISCHINGER, W. McCLEMENTS, S. NOMURA, D. BLAIR, and M. SULLIVAN,* NCI and *NICHHD, National Institutes of Health, Bethesda, Maryland: Characterization of integrated proviruses of Moloney sarcoma viruses and host flanking sequences cloned in bacteriophage λ .

- T. G. WOOD,* J. P. HORN,* D. G. BLAIR,† J. ROBFY,† and R. B. ARLINGHAUS,* *University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston; †NCI, National Institutes of Health, Bethesda, Maryland: Detection of a 85,000-dalton polypeptide at the permissive temperature in transformed cells infected with a mutant murine sarcoma virus (MuSV) that is temperature sensitive for transformation.
- L.-H. WANG, P. SNYDER, T. HANAFUSA, C. MOSCOVICI,* and H. HANAFUSA, Rockefeller University, New York, New York; *Tumor Virus Laboratory, Veterans Administration Hospital, Gainesville, Florida: Comparison of cellular and viral transforming sequences by oligonucleotide fingerprinting.
- R. E. KARESS, W. S. HAYWARD, and H. HANAFUSA, Rockefeller University, New York, New York: Transforming protein encoded by the cellular information in the genome of recovered avian sarcoma virus.
- H. M. TEMIN, E. KESHET, S. WEILER, I. CHEN, and J. O'REAR, McArdle Laboratory, University of Wisconsin, Madison: Relationship of numbers and structures of avian retrovirus DNAs to virus production and cell killing.
- E. M. SCOFNICK, H. A. YOUNG, and T. Y. SHIH, NCI, National Institutes of Health, Bethesda, Maryland: Different rat-derived transforming retroviruses code for an immunologically related intracellular phosphoprotein.
- H. W. SNYDER, JR., M. ESSEX,* A. H. SLISKI,† W. D. HARDY, JR.,‡ and J. RHIM,† *Harvard University School of Public Health, Boston, Massachusetts; †National Institutes of Health, Bethesda, Maryland; ‡Memorial Sloan-Kettering Cancer Center, New York, New York: Feline oncornavirus-associated cell membrane antigen.

Session 6: The Transforming Genes of DNA Tumor Viruses and Their Arrangement in Transformed Cells

Chairperson: M. BOTCHAN, Cold Spring Harbor Laboratory, New York

- L. VISSER, M. VAN MAARSCHALKERWEERD, T. H. ROZIJN, A. WASSENAAR, A. REEMST, and J. S. SUSSENBACH, Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands: Presence and expression of viral sequences in 15 adenovirus-transformed cell lines.
- W. DOERFFER, S. STABEL, R. NEUMANN, D. SUTTER, H. IJELGAUFTS, J. GRONEBERG, R. DEURING, and U. WINTERHOFF, Institute of Genetics, University of Cologne, Germany: Selectivity in the integration of adenovirus DNA in infected and transformed cells.
- J. SAMBROOK, J. STRINGER, S.-L. HU, R. GREENE, and M. BOTCHAN, Cold Spring Harbor Laboratory, New York: The arrangement and expression of integrated sequences of SV40 and adenovirus DNA.
- M. A. MARTIN, M. A. ISRAEL, K. CHOWDHURY, J. M. RAMSEUR,* and W. P. ROWE,* NIAID, National Institutes of Health, Bethesda, Maryland: Characterization of the polyoma virus DNA and polyoma virus proteins present in hamster tumors induced by virions and viral DNA.
- L. LANIA, D. GANDINI-ATTARDI, and M. FRIFO, Imperial Cancer Research Fund, London, England: The structure and function of polyoma virus DNA in transformed and normal cells.
- A. GRAESSMANN, M. GRAESSMANN, and C. MUELER, Institut für Molekularbiologie und Biochemie der Freien Universität, Berlin, Germany: Transformation of monkey and rat cells by microinjection of an early SV40 DNA fragment.
- B. BASILICO, D. ZOUZIAS, S. GATTONI, G. DELLA VALLE, V. COLANTUONI, and R. FENTON, Department of Pathology, New York University School of Medicine, New York: Integration and excision of polyoma virus genomes.
- E. WINOCOUR,* M. F. SINGER,† and E. L. KUFF,† *Department of Virology, Weizmann Institute of Science, Rehovot, Israel; †NCI, National Institutes of Health, Bethesda, Maryland: Host-substituted SV40 variants.
- G. R. RYFES, R. LAFEMINA, S. D. HAYWARD, and G. S. HAYWARD,* Department of Pharmacology, Johns Hopkins Medical School, Baltimore, Maryland: Evidence for two distinct transforming regions in the DNA of herpes simplex virus.

Session 7: Proviruses and Their Expression

Chairperson: J. M. BISHOP, University of California Medical Center, San Francisco

- S. H. HUGHES,* P. K. VOGT,† E. J. STUBBLEFIELD,‡ H. ROBINSON,§ J. M. BISHOP,* and H. E. VARMUS,* *Department of Microbiology, University of California, San Francisco; †Department of Microbiology, University of Southern California, Los Angeles; ‡Department of Biology, University of Texas Cancer Center, Houston; §Worcester Institute for Experimental Biology, Shrewsbury, Massachusetts: Organization of endogenous and exogenous avian viral and linked nonviral sequences.

- J. M. TAYLOR, T. W. HSU, C. YEATER, and W. S. MASON, Institute for Cancer Research, Fox Chase Center, Philadelphia, Pennsylvania: Avian RNA tumor virus DNA.
- A. SKALKA, W. MCCLEMENTS, P. DEBONA, and F. HISHINUMA, Roche Institute of Molecular Biology, Nutley, New Jersey: Avian endogenous proviral DNA—relationship between structure and expression.
- S. M. ASTRIN,* H. L. ROBINSON,† L. B. CRITTENDEN, and W. S. HAYWARD,‡ *Institute for Cancer Research, Philadelphia, Pennsylvania; †Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; ‡Regional Poultry Research Laboratory, East Lansing, Michigan; §Rockefeller University, New York, New York: Ten genetic loci in the chicken that contain structural genes for endogenous avian leukosis viruses.
- W. S. HAYWARD, S. B. BRAVERMAN, and S. M. ASTRIN,* Rockefeller University, New York, New York; *Institute for Cancer Research, Philadelphia, Pennsylvania: Transcriptional products of endogenous and exogenous oncovirus genes.
- H. L. ROBINSON and M. N. PEARSON, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Subgroup E avian leukosis virus associated disease in chickens.
- D. R. LOWY, E. RANDS, S. K. CHATTOPADHYAY, and G. R. HAGER, NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning of infectious integrated AKR murine leukemia virus DNA genomes.
- G. L. HAGER, L. A. DONEHOWER, D. S. BERARD, and R. G. WOLFORD, NCI, National Institutes of Health, Bethesda, Maryland: Construction and characterization of molecular clones containing integrated mouse mammary tumor virus sequences.
- N. E. HYNES and B. GRONER, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: Genomic integration sites of endogenous and acquired MMTV proviral genes.
- G. M. COOPER, N. G. COPELAND, A. D. ZELENETZ, and T. KRONTIRIS, Sidney Farber Cancer Institute, Boston, Massachusetts: Transformation of mouse cells by avian retrovirus DNAs.

Session 8: The Genetics of Transformation by DNA Tumor Viruses

Chairperson: P. TEGMEYER, State University of New York, Stony Brook

- T. BENJAMIN, B. S. SCHAFFHAUSEN, and G. CARMICHAEL, Pathology Department, Harvard Medical School, Boston, Massachusetts: The hr-t gene of polyoma virus.
- B. E. GRIFFIN, Y. ITO, N. SPURR, U. NOVAK, S. DILWORTH, and N. SMOLAR, Imperial Cancer Research Fund, London, England: Early mutants of polyoma virus which have altered transformation properties.
- J. M. PIPAS, S. P. ADLER, S. LAZAROWITZ, K. PEDEN, D. SIORTLE, and D. NATHANS, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Mutants of SV40 that affect the structure or synthesis of viral tumor antigens.
- G. DIMAYORCA,* D. PINTEL,* N. BLOUCK,* B. SWERDLOW,† and T. E. SHENK,‡ *Department of Microbiology, University of Illinois Medical Center, Chicago; †Department of Microbiology, University of Connecticut Health Center, Farmington: *dIA 1499* is a new early region mutant of SV40 which is heat sensitive for lytic growth and cold sensitive for transformations.
- R. G. MARTIN, V. P. SETLOW, A. B. CHEPELINSKY, and R. SEIF, NCI, National Institutes of Health, Bethesda, Maryland: Roles of the tumor antigens in transformation by SV40.
- R. J. FRISQUE, W. C. TOPP, and D. B. RIFKIN,* Cold Spring Harbor Laboratory; *Department of Cell Biology, New York University Medical School, New York: The genetics of transformation of nonpermissive cells by SV40.
- M. RASSOULZADEGAN, E. MOUGNEAU, B. PERBAL, F. BIRG,* and F. CUZIN, Centre de Biochimie du CNRS, Nice; *Unité 119 INSERM, Marseille, France: Analysis of a homogeneous set of polyoma and SV40 transformed rat cells—temperature dependence of tsA transformants, integration sites, levels of expression of the transformed phenotype.
- J. HISCOTT, M. STEINBERG, and V. DEFENDI, Department of Pathology, New York University School of Medicine, New York: Viral and cellular control of the transformed phenotype.
- J. WILLIAMS,* R. S. GALOS,* M. H. BINGER,† and S. J. FLINT,‡ *Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania; †Department of Biochemical Sciences, Princeton, New Jersey: Location of additional early sequences within the left quarter of the adenoviral genome.
- T. SHENK, N. JONES, and W. COLBY, Department of Microbiology, University of Connecticut Health Center, Farmington: Adenovirus type 5 deletion mutants defective for transformation of rat embryo cells.

Session 9: *The Transformed Phenotype and Differentiation*

Chairperson: M. LINIAL, Fred Hutchinson Cancer Center, Seattle, Washington

- N. M. TEICH, M. BOSS, T. CURRAN, and T. M. DEXTER,* Imperial Cancer Research Fund, London; *Paterson Laboratories, Manchester, England: Murine leukemia viral gene expression in transformed hematopoietic cells.
- D. HOUSMAN, R. LEVENSON, V. VOLLOCH, A. TSIFISOGLIOU, J. GUSELLA, D. PARKER, J. KERNEN, A. MITRANI, V. WEEKS, O. WITTE, and P. BESMER, Biology Department and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Control of proliferation and differentiation in Friend virus transformed cells.
- W. C. RASCHKE, Developmental Biology Laboratory, Salk Institute, San Diego, California: Transformation by Abelson leukemia virus—Properties of the transformed cells.
- R. RISSER, D. GRUNWALD, P. JELEN, and C. SINARKO, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Cell surface antigens of Abelson or Friend MuLV and of hematopoietic differentiation.
- C. MORONI, J. STOYKE, J. DELAMARTER, F. JAY, J. JONGSTRA, and G. SCHUMANN,* Friedrich Miescher-Institut, Basel; *Pharmaceuticals Division, Ciba-Geigy Ltd., Basel, Switzerland: Correlation of endogenous C-type virus expression and B-cell differentiation—Implications for immune cell function and leukemogenesis.
- D. STEHELIN, S. SAUJE, M. ROUSSEL, C. LAGROU, and C. ROMMENS, INSERM, Pasteur Institute, Lille, France: Three new types of transforming genes in acute defective avian leukemia viruses. I. Specific viral nucleotide sequences correlating with distinct phenotypes of virus-transformed cells.
- T. GRAF, H. BEUG, A. V. KIRCHBACH, and M. HAYMAN,* Deutsches Krebsforschungszentrum, Heidelberg, Germany; *Imperial Cancer Research Fund, London, England: Three new types of transforming genes in acute defective avian leukemia viruses. II. Biological, genetical, and biochemical evidence.
- R. EISENMAN, M. GROUNDINE, and M. LINIAL, Fred Hutchinson Cancer Research Center, Seattle, Washington: Analysis of avian oncovirus-related polyproteins in uninfected hematopoietic cells and in cells transformed by the acute leukemia viruses MC29 and AEV.
- D. BOETTIGER and E. DURBAN, Department of Microbiology, University of Pennsylvania, Philadelphia: Infection and transformation of progenitor cell populations by Rous sarcoma virus and avian myeloblastosis virus.

Session 10: *The Genomes and Proteins of Retroviruses. II.*

Chairperson: T. AUGUST, Johns Hopkins University, Baltimore, Maryland

- P. DUESBERG,* K. BISTER,* L. EVANS,* C. MOSCOVICI,† P. VOGT,‡ D. TROXLER,§ and E. SCOLNICK,§ *Department of Molecular Biology, University of California, Berkeley; †Veterans Administration Hospital, Gainesville, Florida; ‡Department of Microbiology, University of Southern California, Los Angeles; §NCI, National Institutes of Health, Bethesda, Maryland: Gene structure of avian and murine leukemia viruses.
- K. TOYOSHIMA,* T. KAMAHORA,* H. SUGIYAMA,* M. OWADA,* A. NOMOTO,† and M. YOSHIDA,‡ *Research Institute for Microbial Diseases, Osaka University; †School of Pharmaceutical Sciences, Kitasato University, Tokyo; ‡Cancer Institute, Tokyo, Japan: Analysis of RNA specific for the transforming agent of R strain of avian erythroblastosis virus.
- D. BALTIMORE, A. SHIELDS, G. ORTO, and S. GOFF, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Structure of the A-MuLV genome.
- O. N. WITTE,* N. E. ROSENBERG,† and D. BALTIMORE,* *Massachusetts Institute of Technology, Cambridge; †Tufts University School of Medicine, Boston, Massachusetts: Syngeneic tumor regressor serum reactive with the unique region of the Abelson MuLV p120 protein recognizes a normal cellular protein in hematopoietic tissues.
- N. ROSENBERG,* O. N. WITTE, and D. BALTIMORE, Massachusetts Institute of Technology, Cambridge; *Tufts University School of Medicine, Boston, Massachusetts: Characterization of A-MuLV isolates differing in p120 expression.

- J. R. STEPHENSON, W. J. M. VAN DE VEN, A. S. KHAN, and F. H. REYNOLDS, JR., Frederick Cancer Research Center, National Cancer Institute, Frederick, Maryland: Mammalian RNA type C transforming viruses—Characterization of viral-coded polyproteins containing phosphorylated nonstructural components with possible transforming function.
- Y. IKAWA,* M. YOSHIDA,* M. OBINATA,* F. HARADA,† and H. YOSHIKURA,‡ *Department of Viral Oncology, Cancer Institute, Tokyo; †Virology Division, National Cancer Center Research Institute, Tokyo; ‡Department of Genetics, Institute of Medical Science, University of Tokyo, Japan: RNA sequences and proteins specific to Friend strain of spleen focus-forming virus (SFFV).
- A. S. BERKOWER, R. KAUL, F. LILLY, F. PIATA, R. SOEIRO, and R. A. STEEVES, Departments of Genetics and Medicine, Albert Einstein College of Medicine, Bronx, New York: Progressive shutdown of viral gene expression in Friend virus-induced erythroleukemia cells.
- T. W. MAK,* A. BERNSTEIN,* C. GAMBLE,* M. MACDONALD,* and P. BRANTON, † *Ontario Cancer Institute, University of Toronto; †McMaster University, Ontario, Canada: Genetic control of the expression of Friend erythroleukemia virus-specific sequences in normal and leukemic mouse tissues.
- R. C. NOWINSKI, Fred Hutchinson Cancer Research Center, Seattle, Washington: Monoclonal antibodies against murine leukemia viruses.

Session 11: The Transforming Genes of Adenoviruses and Their Expression

Chairperson: P. GALLIMORE, University of Birmingham, England

- A. J. VAN DER EB, P. I. SCHRIER, H. VAN ORMONDT, H. JOCHEMSEN, J. MAAT, P. J. VAN DEN EISEN, J. H. LUPKER, R. DIJKEMA, R. J. DELEYS, C. P. VAN BEVEREN, and A. DE WAARD, Sylvius Laboratories, University of Leiden, The Netherlands: Organization and structure of the transforming genes of human adenoviruses.
- L. T. CHOW, J. B. LEWIS, and T. R. BROKER, Cold Spring Harbor Laboratory, New York: The spliced RNAs of adenovirus-2 early regions 1A and 2B.
- C. C. BAKER and E. B. ZIFF, Department of Molecular Cell Biology, Rockefeller University, New York: 5' termini of Ad2 early messenger RNAs.
- P. A. SHARP, A. J. BERK, F. K. LEE, J. WILLIAMS, and T. HARRISON, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Regulation of early adenovirus mRNA synthesis.
- D. J. SPECTOR, D. N. HALBERT, L. D. CROSSLAND, and H. J. RASKAS, Department of Pathology, Washington University School of Medicine, St. Louis, Missouri: Expression of genes from the transforming region of adenovirus.
- M. C. WILSON and J. E. DARNELL, Department of Molecular Cell Biology, Rockefeller University, New York: Metabolism of viral mRNAs in Ad2-transformed and lytically infected cells.
- M. GREEN, W. S. M. WOLD, K. BRACKMANN, and M. CARTAS, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Studies on human adenovirus early and transformation proteins.
- N. J. LASSAM,* S. T. BAYLEY,* and F. L. GRAHAM,*† Departments of *Biology and †Pathology, McMaster University, Hamilton, Ontario: Transforming proteins of human adenoviruses—Studies with infected and transformed cells.
- J. B. LEWIS, J. SMART, M. HARTER, H. ESCHÉ, and M. B. MATHEWS, Cold Spring Harbor Laboratory, New York: Studies of mRNAs and proteins encoded by the leftmost 30 percent of the genome of group C adenoviruses.
- S. KVIST, H. PERSSON, L. ÖSTBERG, O. KÄMPE, P. PETERSON, and L. PHILIPSON, Department of Cell Research, Wallenberg Laboratory, and Department of Microbiology, Biomedical Center, Uppsala University, Sweden: Molecular association between transplantation antigens and the early adenovirus glycoprotein E3-19K.
- K. FUJINAGA,* Y. SAWADA,* Y. UEMIZU,* H. SHIMOJO,† K. SHIROKI,† H. SUGISAKI,‡ and M. TAKANAMI,‡ *Cancer Research Institute, Sapporo Medical College; †Institute of Medical Science, University of Tokyo; ‡Institute for Chemical Research, Kyoto University, Japan: Transforming DNA sequences of highly oncogenic human adenovirus type 12—Their integrations and transcriptions in transformed cells.
- K. SHIROKI,* K. SEGAWA,* I. SAITO,* H. SHIMOJO,* K. FUJINAGA,† Y. MAETA,‡ and C. HIMADA,‡ *Institute of Medical Science, University of Tokyo; †Sapporo Medical College; ‡Niigata University School of Medicine, Japan: Products of the adenovirus 12 transforming genes and their functions.

Chairperson: R. ERIKSON, University of Colorado, Denver

- A. R. GOLDBERG, J. G. KRUEGER, and E. WANG, Rockefeller University, New York, New York: Localization and characterization of the transforming protein of Rous sarcoma virus.
- R. R. FRIIS, B. BOSCHKE, B. JOCKUSCH,* A. ZIEMIECKI, H. RÜBSAMEN, and H. BAUER, Institut für Virologie, Giessen; *European Molecular Biology Laboratories, Heidelberg, Germany: Microfilaments as immediate targets of the RSV *src* gene product.
- L. R. ROHRSCHEIDER, Fred Hutchinson Cancer Research Center, Seattle, Washington: Localization of pp60^{src} in NRK cells infected with temperature-sensitive mutants (t-class) of Rous sarcoma virus.
- I. PASTAN, M. C. WILLINGHAM, N. REICHERT, P. DAVIES, and G. JAY, NCI, National Institutes of Health, Bethesda, Maryland: Ultrastructural localization of the *src* gene product to the plasma membrane and characterization of the immune complex kinase from Schmidt-Ruppin avian sarcoma virus-transformed cells.
- D. ANDERSON,* L. ROHRSCHEIDER,† and M. WEBER,* †Fred Hutchinson Cancer Center, Seattle, Washington; *Department of Microbiology, University of Illinois, Urbana: Properties of some new transformation mutants of Rous sarcoma virus.
- F. LIPMANN and S. DECKER, Rockefeller University, New York, New York: Isolation of a glucose transport protein from normal chicken fibroblasts that is two to three times more abundant in Rous sarcoma virus-transformed cells.
- M. L. BREITMAN, R. VIGNE, and P. K. VOGT, Department of Microbiology, University of Southern California School of Medicine, Los Angeles: Focus formation by *src* deletion mutants of avian sarcoma viruses.
- A. LAU,* M. COLLETT,† R. KRZYZEK,* R. ERIKSON,† and A. FARAS,* *Department of Microbiology, University of Minnesota Medical School, Minneapolis; †Department of Pathology, University of Colorado Medical School, Denver: Biologically active *src* gene product is present in morphological reverted yet tumorigenic Rous sarcoma virus-transformed field vole cells.
- J. A. WYKE,* J. A. BEAMAND,* and H. E. VARMUS,† *Imperial Cancer Research Fund, London, England; †University of California Medical School, San Francisco: Factors affecting transformation of rat cells by avian sarcoma virus and their subsequent reversion.
- S. J. SINGER, J. F. ASH, R. C. CARROLL, and E. BALL, Department of Biology, University of California, San Diego: The reversibility of the transformed phenotype in cells infected with a temperature-sensitive Rous sarcoma virus.

Session 13: *The Transformed Cell*

Chairperson: L. B. CHEN, Sidney Farber Cancer Center, Boston, Massachusetts

- G. J. TODARO and J. E. DE LARCO, NCI, National Institutes of Health, Bethesda, Maryland: Mouse sarcoma virus (MSV)-transformed cells produce a polypeptide hormone, sarcoma growth factor (SGF), that specifically binds to membrane receptors for epidermal growth factor (EGF).
- D. R. SENGER, D. F. WIRTH, C. BRYANT, and R. O. HYNES, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Transformation-dependent secretion of specific proteins, phosphoproteins, and protein kinase.
- G. JAY,* A. B. DELEO,† E. APPELLA,* L. W. LAW,* G. KHOURY,* and L. J. OLD,† *NCI, National Institutes of Health, Bethesda, Maryland; †Memorial Sloan-Kettering Cancer Center, New York, New York: A common transformation-related protein in murine sarcomas and leukemias of different etiologies.
- D. B. RIFKIN, New York University Medical School, New York: Studies on the control of plasminogen activator production by transformed cells.
- W. F. MANGEL, D. C. LIVINGSTON,* J. R. BROCKLEHURST,* H.-Y. LIU, G. A. PELTZ, J. F. CANNON, S. P. LEYTUS, J. A. WEHRLY, B. L. SALTER and J. L. MOSHER, Department of Biochemistry, University of Illinois, Urbana; *Imperial Cancer Research Fund, London, England: A new assay for the plasminogen activator activity of transformed cells.
- R. E. POLLACK, B. M. STEINBERG, and A. LO, Department of Biological Sciences, Columbia University, New York, New York: The in vitro tumorigenic syndrome.
- K. K. JHA,* E. G. GURNEY,† L. A. FELDMAN,‡ and H. L. OZER,* *Hunter College, City University of New York, New York; †Department of Biology, University of Utah, Salt Lake City; ‡Department of Microbiology, College of Medicine and Dentistry of New Jersey: Expression of transformation in cell hybrids.

- S. SHIN, A. S. KLEIN, P. KAHN, and R. SIMON, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Tumor formation in nude mice by transformed cells.
- P. H. GALLIMORE and C. PARASKEVA, Department of Cancer Studies, University of Birmingham Medical School, England: A study to determine the reasons for differences in the tumorigenicity of adenovirus 2 and adenovirus 12 transformed rat cell lines.

Session 14: Viruses of Thymic Leukemia

Chairperson: E. SCOLNICK, National Institutes of Health, Bethesda, Maryland

- P. V. O'DONNELL, E. STOCKERT, Y. OBATA, A. B. DELEO, and L. J. OLD, Memorial Sloan-Kettering Cancer Center, New York, New York: MuLV-related cell surface antigens as serological markers of AKR ecotropic, xenotropic, and dualtropic MuLV.
- W. P. ROWE, M. W. CLOYD, and J. W. HARTLEY, National Institutes of Health, Bethesda, Maryland: Status of the association of MCF viruses with leukemogenesis.
- M. LILUNG,* J. ROMMELAERE,* N. HOPKINS,* J. W. HARTLEY,† and W. P. ROWE,† *Biology Department and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; †National Institutes of Health, Bethesda, Maryland: Analysis of the genomes of MCF viruses—A progress report.
- J. G. SUTCLIFFE, T. M. SHINNICK, R. A. LERNER, and I. VERMA,* Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla; *Salk Institute, San Diego, California: The nucleic acid sequence of parental and recombinant retroviral envelope (env) genes.
- E. FLEISSNER, N. FAMULARI, J.-S. TUNG, and P. V. O'DONNELL, Laboratory of Viral Oncology, Sloan-Kettering Institute for Cancer Research, New York, New York: Murine leukemia virus env gene expression in preleukemic thymocytes and leukemia cells of AKR mice.
- M. S. MCGRATH, E. PILLEMER, E. PILLEMER, D. A. KOOISTRA, and I. L. WEISSMAN, Department of Pathology, Stanford University Medical Center, California: Thymotropic MuLV's and T-cell receptors.

Summary: P. SHARP, Massachusetts Institute of Technology, Cambridge

SUMMER MEETINGS



POXVIRUS-FROGVIRUS 3, April 23 – April 26

arranged by

Allan Granoff, *St. Jude Children's Research Hospital, Memphis, Tennessee*

Enzo Paoletti, *New York State Department of Health, Albany*

40 participants

Session 1: DNA Structure

Chairperson: C.C. RANDALL, *University of Mississippi, Jackson*

D.L. PANICALI and E. PAOLETTI, *New York State Department of Health, Albany:* Heterogeneity in the DNA content of vaccinia virus (WR strain).

C.F. GARON, E. BARBOSA, and B. MOSS, *NIAID, National Institutes of Health, Bethesda, Maryland:* Analysis of the DNA sequence heterology among several poxvirus genomes.

M. MACKETT and L.C. ARCHARD, *Department of Virology, St. Mary's Hospital Medical School, London, England:* Restriction endonuclease analysis of orthopoxvirus genome structure.

R. WITTEK, H. CHAN, and B. MOSS, *NIAID, National Institutes of Health, Bethesda, Maryland:* Cloning of vaccinia virus DNA restriction fragments in coliphage lambda.

E. VIÑUELA, J. KUZNAR, J. ORTIN, M.L. SALAS, A.L. CARRASCOA, and L. ENJUANES, *Centro de Biología Molecular (CSIC), Universidad Autónoma, Madrid, Spain:* Structural components of African swine fever (ASF) virus.

Session 2: DNA Replication

Chairperson: J.R. KATES, *State University of New York, Stony Brook*

B.G.T. POGO and M. O'SHEA, *Center for Experimental Cell Biology, Mount Sinai School of Medicine, New York, New York:* Studies on vaccinia DNA replication.

G. MCFADDEN and S. DALES, *Department of Microbiology and Immunology, University of Western Ontario, Canada:* Biogenesis of vaccinia: Characterization of DNA-ts vaccinia mutants.

Session 3: DNA Replication

Chairperson: J.R. KATES, State University of New York, Stony Brook

- M.D. CHALLBERG and P.T. ENGLUND, Department of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Vaccinia virus DNA polymerase.
- J.A. HOLOWCZAK, College of Medicine and Dentistry, Rutgers Medical School, Piscataway, New Jersey: Electron microscopic and biochemical studies of Milker's nodule virus.
- D. FOJGELSONG, E. RESSNER, J. KATES, and W. BAUER, School of Basic Health Sciences, State University of New York, Stony Brook: Purification and characterization of a vaccinia virus topoisomerase.
- D. WILLIS, R. GOORHA, and A. GRANOFF, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Nongenetic reactivation of purified frog virus 3 DNA.

Session 4: Transcription

Chairperson: W.J. JOKLIK, Duke University, Durham, North Carolina

- E. BARROSA, C.F. GARCÓN, and B. MOSS, NIAID, National Institutes of Health, Bethesda, Maryland: Transcription of the terminal repetition in vaccinia virus DNA.
- E. PACOLETTI and B.R. LIPINSKAS, Division of Laboratories and Research, New York State Department of Health, Albany: Synthesis of low-molecular weight RNA molecules by vaccinia virus in vitro.

Session 5: Transcription

Chairperson: W.J. JOKLIK, Duke University, Durham, North Carolina

- E. SPENCER, D. LÖRING, and J. HURVITZ, Albert Einstein College of Medicine, Bronx, New York: The role of ATP in RNA synthesis by permeabilized vaccinia virus.
- B.M. BAROUDY and B. MOSS, NIAID, National Institutes of Health, Bethesda, Maryland: Purification and characterization of DNA-dependent RNA polymerase from vaccinia virions.
- S. VENKATESAN and B. MOSS, NIAID, National Institutes of Health, Bethesda, Maryland: Modification of the 5'-end of mRNA: Association of RNA triphosphatase activity with purified vaccinia virus RNA guanylyltransferase-RNA (guanine-7-)-methyltransferase complex.
- M. SILVER, G. MCFADDEN, and S. DALFS, Department of Microbiology and Immunology, University of Western Ontario, Canada: Biogenesis of vaccinia: A host transcriptional function is essential for producing mature virus.
- D.E. HRUBY and J.R. KATES, Health Sciences Center, State University of New York, Stony Brook: Vaccinia virus replication requires active involvement of the host cell nucleus.
- R. GOORHA, St. Jude's Children's Research Hospital, Memphis, Tennessee: Frog virus 3 requires RNA polymerase II for its replication.

Session 6: Translation

Chairperson: B. MOSS, National Institutes of Health, Bethesda, Maryland

- D.E. HRUBY, D.L. LYNN, and J.R. KATES, Health Sciences Center, State University of New York, Stony Brook: Identification of a viral-coded protein in the nucleus of vaccinia virus-infected cells.
- R.M. ELLIOTT and D.C. KELLY, NERC Unit of Invertebrate Virology, Oxford, England: Induction and subcellular location of frog virus 3 specific polypeptides.
- R.M. ELLIOTT and D.C. KELLY, NERC Unit of Invertebrate Virology, Oxford, England: Cascade regulation of frog virus 3 induced polypeptides.
- D.C. KELLY, R. BRAVO, and R.M. ELLIOTT, NERC Unit of Invertebrate Virology, Oxford, England: Acidic and basic two-dimensional gel electrophoresis of frog virus 3 induced polypeptides.

- A.N. AUBERTIN, O. CORDIER, and A. KIRN, INSERM U 74 and Laboratory of Virology, Strasbourg, France: Influence of temperature on polypeptide synthesis in frog virus 3 infected CHO cells.
- R. RAGHOW and A. GRANOFF, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Translation regulation of frog virus 3 mRNAs in vitro.
- K. ESSANI and S. DALES, Department of Microbiology and Immunology, University of Western Ontario, Canada: Biogenesis of vaccinia: Evidence for more than 100 polypeptides in the virion.
- J. COOPER and B. MOSS, NIAID, National Institutes of Health, Bethesda, Maryland: Characterization and gene mapping of RNA from vaccinia virus-infected cells by translation in vitro.

Session 7: Genetics

Chairperson: S. DALES, University of West Ontario, London, Canada

- R.W. MOYER, Microbiology Department, Vanderbilt University, Nashville, Tennessee: Genetic and biochemical examination of the "white pock" host range mutants of rabbitpox virus.
- D.E. HRUBY,* D.L. LYNN,* R.C. CONDIT,[†] and J.R. KATES,[†] *Health Sciences Center, State University of New York, Stony Brook; [†]Schools of Medicine and Dentistry, State University of New York, Buffalo: Studies on the mechanism of host range restriction of vaccinia virus.
- M.J. ENSINGER, College of Physicians and Surgeons, Columbia University, New York, New York: Isolation and complementation analysis of temperature-sensitive mutants of vaccinia virus.
- R. DRILLIEN, F. KOEHLER, and A. KIRN, INSERM U 74 and Laboratory of Virology, Strasbourg, France: Preliminary characterization of conditional lethal mutants of vaccinia virus.

Session 8: Virus-Cell Interactions/Miscellaneous

- M. SCHROM* and R. BABLANIAN,[†] *Department of Microbiology and Immunology, Albany Medical College, New York; [†]Department of Microbiology and Immunology, Downstate Medical Center, State University of New York, Brooklyn: Studies on the inhibition of protein synthesis by vaccinia virus.
- E. PETERHANS, D. SCHÜMPEL, and R. WYLER, Institute of Virology, University of Zurich, Switzerland: Ion regulation in poxvirus-infected cells.
- L.G. PAYNE, Virusinstitutionen, Karolinska Institutet, Stockholm, Sweden: The biology of extracellular vaccinia virus.
- J. ESPOSITO, E. PALMER, J. OBJIESKI, E. BORDEN, A. HARRISON, and F. MURPHY, Center for Disease Control, DHEW, Atlanta, Georgia: Studies on the poxvirus *cotia*.

C. elegans, May 10 -- May 13

arranged by

Robert Edgar, University of California, Santa Cruz

Robert Herman, University of Minnesota, St. Paul

John E. Sulston, Medical Research Council, Cambridge, England

William B. Wood, University of Colorado, Boulder

128 participants

Opening Remarks: J. E. SULSTON, Medical Research Council, Cambridge, England

Session 1: Genes and Development. I.

Chairperson: H. R. HORVITZ, Massachusetts Institute of Technology, Cambridge

- E. SCHIERENBERG and G. VON EHRENSTEIN, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Embryonic cell lineages and development in *C. elegans*.
- R. CASSADA, E. ISNENGI, M. CULOTTI, E. SCHIERENBERG, and G. VON EHRENSTEIN, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Some recent results with ts embryonic mutants.
- E. SCHIERENBERG, J. MIWA, and G. VON EHRENSTEIN, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Cell lineages and developmental defects of temperature-sensitive embryonic arrest mutants in *C. elegans*.
- P. BAZZICALUPO, J. LAUFER, and W. B. WOOD, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: The mosaic nature of early development in *C. elegans*.

Session 2: Genes and Development. II.

Chairperson: R. H. WATERSTON, Washington University School of Medicine, St. Louis, Missouri

- J. KIMBLE, J. SULSTON, and J. WHITE, MRC Laboratory of Molecular Biology, Cambridge, England: Regulation in post-embryonic lineages.
- M. CHALFIE,* H. R. HORVITZ,[†] and J. E. SULSTON,* *MRC Laboratory of Molecular Biology, Cambridge, England; [†]Department of Biology, Massachusetts Institute of Technology, Cambridge: Mutations which cause reiteration in cell lineages.
- C. FERGUSON and H. R. HORVITZ, Department of Biology, Massachusetts Institute of Technology, Cambridge: Vulva development in *C. elegans*.
- J. COX, M. KUSCH, J. POLITZ, and B. EDGAR, Thimann Laboratories, University of California, Santa Cruz: Biochemical and genetic studies of the *C. elegans* cuticle.
- M. HUSTA and R. M. HECHT, Department of Biophysical Sciences, University of Houston, Texas: Gene-enzyme systems in *C. elegans*.
- M. M. SWANSON and D. L. RIDDLE, Division of Biological Sciences, University of Missouri, Columbia: Temperature-sensitive periods of dauer constitutive mutants.
- P. M. MENEELY and R. K. HERMAN, Department of Genetics and Cell Biology, University of Minnesota, St. Paul: X-linked lethals, steriles and deficiencies.

Session 3: Poster Session

- N. ABDULKADER and J. BRUN, Laboratoire de Génétique Physiologique et Nématologie, Université Lyon-I, and Département de Biologie Générale et Appliquée, CNRS, Villeurbanne, France: Mutations affecting the caudal extremity of the *C. elegans* hermaphrodite.
- Y. ARGON, G. NELSON, T. ROBERTS, and S. WARD, Carnegie Institution of Washington, Baltimore, Maryland: Genetics and morphology of *fer* mutants sperm.
- D. L. BAILLIE, T. M. ROGALSKI, and R. ROSENBLUTH, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada: Genetic organization in *C. elegans*:—Lethal analysis of the *unc-23* region.
- J. A. BOLLINGER and J. D. WILLET, Chemistry Department, University of Idaho, Moscow: Establishing age synchrony in xenic populations of *C. elegans*—Problems and solutions.
- U. CERTA, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Changes in metabolism of *C. elegans* from egg to adult.
- M. CHALFIE and J. N. THOMSON, MRC Laboratory of Molecular Biology, Cambridge, England: Microtubule organization in *C. elegans* neurons.
- J. G. DUCKETT,* R. K. HERMAN[†] and R. L. RUSSELL,* *Department of Biological Sciences, University of Pittsburgh, Pennsylvania; [†]Department of Genetics and Cell Biology, University of Minnesota, St. Paul: Gene dosage and dosage compensation effects studied with mutations affecting acetylcholinesterase.
- D. B. DUSENBERY, School of Biology, Georgia Institute of Technology, Atlanta: Responses of *C. elegans* to controlled chemical stimulation.
- M. K. EDWARDS and W. B. WOOD, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: In situ hybridization techniques.
- A. FODOR, P. DEÁK, and I. KISS, Institute of Genetics, Biological Research Center of Hungarian Academy of Sciences, Szeged: Competition of insect juvenoid ZR515 and precocene 2 in *C. elegans*.

- A. FODOR and P. DEÁK, Institute of Genetics, Biological Research Center of Hungarian Academy of Sciences, Szeged: Isolation of new balancer chromosomes of *C. elegans*.
- P. GOLDSTEIN, Department of Genetics, North Carolina State University, Raleigh: Nematode chromosome analysis by 3-D reconstruction of serial sections and electron microscopy.
- P. GOLDSTEIN, Department of Genetics, North Carolina State University, Raleigh: Molecular characterization of *Ascaris suum* DNA and of chromatin diminution.
- L. GOSSETT and R. M. HECHT, Department of Biophysical Sciences, University of Houston, Texas: Squash technique for visualization of individual nuclei during embryogenesis.
- I. GREENWALD and H. R. HORVITZ, Department of Biology, Massachusetts Institute of Technology, Cambridge: E1500—A new muscle mutant with unusual genetic properties.
- J. S. HAEMER, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: New map distances from old map data.
- E. HEDGECOCK, J. SULSTON, M. CHALFIE, and M. DEW, MRC Laboratory of Molecular Biology, Cambridge, England: Fluorescence microscopy of plant lectin receptors in *C. elegans*.
- B. HENNIG and G. VON EHRENSTEIN, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Two isocytichromes c in *C. elegans*.
- R. K. HERMAN,* R. L. RUSSELL,† M. R. CULOTTI,† and J. G. CULOTTI,† *Department of Genetics and Cell Biology, University of Minnesota, St. Paul; †Department of Biological Sciences, University of Pittsburgh, Pennsylvania: Identification of the *ace-1* gene by recombination away from a simultaneously occurring *unc-3* allele.
- E. J. HESS and H. R. HORVITZ, Department of Biology, Massachusetts Institute of Technology, Cambridge: Mutants defective in the migrations of the ventral cord precursor cells.
- H. R. HORVITZ, Department of Biology, Massachusetts Institute of Technology, Cambridge: Suppression studies of cell lineage mutants.
- E. ISNENGI, R. CASSADA, M. CULOTTI, E. SCHIERENBERG, and G. VON EHRENSTEIN, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Further characterization of temperature-sensitive embryonic arrest mutants of *C. elegans* (G set).
- T. E. JOHNSON, M. MCAFEE, and W. B. WOOD, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: The generation of genetic mosaics.
- I. S. KASS, J. P. WALROND, A. O. W. STRETTON, and C. C. WANG,* Department of Zoology, Madison, Wisconsin; *Merck Institute, Rahway, New Jersey: The action of avermectin B1a on *Ascaris*.
- F. R. KHAN and B. A. MCFADDEN, Program in Biochemistry/Biophysics, Washington State University, Pullman: The glyoxylate cycle in *C. elegans*.
- R. G. KNOWLTON, M. LAUTH, and W. B. WOOD, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: cDNA probes for *C. elegans* messenger RNA sequences.
- N. C. LU, G. M. BRIGGS, and E. L. R. STOKSTAD, Department of Nutritional Sciences, University of California, Berkeley: "Two-carbon" fragments as a growth factor for *C. elegans* in a completely chemically defined medium.
- J. MADL and B. HERMAN, Department of Genetics and Cell Biology, University of Minnesota, St. Paul: Polyploids and sex determination.
- D. MITCHELL, R. ROSENBERG and L. KOUMJIAN, Boston Biomedical Research Institute, Boston, Massachusetts: Effects of X-irradiation on longevity and X-ray resistance in *C. elegans*.
- J. MIWA, E. SCHIERENBERG, and G. VON EHRENSTEIN, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Genetics, parental effects, and terminal phenotypes of temperature-sensitive mutations arresting embryonic development in *C. elegans*.
- J. RAND, Department of Biological Sciences, University of Pittsburgh, Pennsylvania: Choline acetyltransferase mutants of *C. elegans*.
- D. RIDDLE, Division of Biological Sciences, University of Missouri, Columbia: A search for cold-sensitive dauer-constitutive mutants.
- D. RIDDLE, M. M. SWANSON, and P. S. ALBERT, University of Missouri, Columbia: Summary of mapped dauer-constitutive and dauer-defective genes.
- T. ROBERTS, Y. ARGON, and S. WARD, Carnegie Institution of Washington, Baltimore, Maryland: Monoclonal antibodies against surface antigens on *C. elegans* sperm.
- R. L. RUSSELL, C. D. JOHNSON, M. R. CULOTTI, and J. G. CULOTTI, Department of Biological Sciences, University of Pittsburgh, Pennsylvania: Procedure for enzymatic assays with small numbers of worms.
- M. SAMOILOFF,* D. BURKE,† S. SCHULZ,* K. DENICH,* and Y. JORDAN,* *Department of Zoology, University

of Manitoba, Winnipeg, Canada; †Department of Biology, Johns Hopkins University, Baltimore, Maryland: The X-chromosome of *P. redivivus*.

- D. SCHMITT, B. YODER, T. COLE, C. KRIEG, E. SCHIERENBERG, R. CASSADA, and G. VON EHRENSTEIN, Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany: Computer-aided analysis for combining light- and electron microscopic data.
- D. SCHOMER, J. ORO, E. V. HUNGERFORD,* A. BARTEL, and R. M. HECHT, Departments of Biophysical Sciences and *Physics, University of Houston, Texas: Zygote defective mutants studied with the flow microfluorometer.
- S. S. SIDDIQUI,* G. VON EHRENSTEIN,* and P. BABU,† *Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany; †Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India: Genetic mosaics in *C. elegans* using fluorescent mutants.
- S. S. SIDDIQUI,* G. VON EHRENSTEIN,* and P. BABU,† *Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany; †Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India: Fluorescent mutants as a tissue specific marker for the intestinal cells of *C. elegans*.
- T. TRANQUILLA, R. CORTESE, D. MELTON, and J. D. SMITH, MRC Laboratory of Molecular Biology, Cambridge, England: Cloning and characterization of nematode tRNA.
- R. H. WATERSTON and T. R. LANE, Departments of Anatomy and Neurobiology and of Genetics, Washington University School of Medicine, St. Louis, Missouri: Suppressors in *C. elegans*.
- B. M. ZUCKERMAN and M. A. GEIST, Laboratory of Experimental Biology, University of Massachusetts, East Wareham: Influence of cultural regime on development and aging of *C. briggsae* and *C. elegans*.

Session 4: Sex

Chairperson: D. L. RIDDLE, University of Missouri, Columbia

- J. HODGKIN, MRC Laboratory of Molecular Biology, Cambridge, England: Sex determining genes in *C. elegans*.
- J. B. KIRSCHBAUM, K. K. LEW, and T. O. FOX, Department of Neuropathology, Harvard Medical School, and Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts: Steroid hormones and *C. elegans*.
- D. G. ALBERTSON,*† O. C. NWAROGU,† and J. E. SULSTON,* *MRC Laboratory of Molecular Biology, Cambridge, England; †Department of Clinical Veterinary Medicine, University of Cambridge, England; ‡Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Sex determination by chromatin diminution in *Strongyloides papillosus*.
- S. WARD, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: The acquisition of cellular asymmetry.
- G. NELSON, Carnegie Institution of Washington, Baltimore, Maryland: Motility studies of *C. elegans* sperm.
- Y. ARGON, Carnegie Institution of Washington, Baltimore, Maryland: Lectin interaction with sperm.
- M. R. KLASS, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: A developmentally regulated sperm protein in *C. elegans*.

Session 5: Neurobiology

Chairperson: D. G. ALBERTSON, University of Colorado, Boulder

- H. R. HORVITZ,* J. SULSTON,† and J. WHITE,* *Department of Biology, Massachusetts Institute of Technology, Cambridge; †MRC Laboratory of Molecular Biology, Cambridge, England: The structure and function of the *C. elegans* egg-laying system.
- J. A. LEWIS, Department of Biological Sciences, Columbia University, New York: Levamisole-resistant mutants.
- J. E. DONMOYER, P. A. DESNOYERS, and A. O. W. STRETTON, Department of Zoology, University of Wisconsin, Madison: Synaptic interactions in the ventral nerve cord of *Ascaris*.
- J. P. WALROND, J. E. DONMOYER, P. A. DESNOYERS, and A. O. W. STRETTON, University of Wisconsin, Madison: How single excitatory motoneurons control zones of inhibition.
- C. D. JOHNSON, Department of Zoology, University of Wisconsin, Madison: Localization of separable forms of acetylcholinesterase in *Ascaris* tissues.

- C. D. JOHNSON and D. WHEATON, Department of Zoology, University of Wisconsin, Madison: Localization of choline acetyltransferase in *Ascaris* tissues.
- J. G. CULOTTI,* C. D. JOHNSON,[†] R. L. RUSSELL,[†] and G. VON EHRENSTEIN,* *Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany; [†]Department of Life Sciences, University of Pittsburgh, Pennsylvania: Acetylcholinesterase mutants of *C. elegans*.
- R. L. RUSSELL, J. B. RAND, J. G. DUCKETT, W. M. BLACKBURN, and J. G. CULOTTI, Department of Biological Sciences, University of Pittsburgh, Pennsylvania: Further genetic and physical separation of multiple acetylcholinesterase forms.

Session 6: Muscle

Chairperson: D. B. DUSENBERY, Georgia Institute of Technology, Atlanta

- R. H. WATERSTON,* S. PLEURAD,* and J. N. THOMSON,[†] *Departments of Anatomy and Neurobiology and of Genetics, Washington University School of Medicine, St. Louis, Missouri; [†]MRC Laboratory of Molecular Biology, Cambridge, England: Genes affecting muscle structure.
- R. H. WATERSTON and K. C. SMITH, Departments of Anatomy and Neurobiology and of Genetics, Washington University School of Medicine, St. Louis, Missouri: Organization of the *unc-54 I* myosin heavy chain gene.
- J. M. ZENDEL, S. HOUTS, D. C. REIN, and H. F. EPSTEIN, Department of Neurology, Baylor College of Medicine, Houston, Texas: A genetic analysis of 118 new body-wall muscle-defective mutants of *C. elegans*.
- P. ANDERSON and S. BRENNER, MRC Laboratory of Molecular Biology, Cambridge, England: A positive selection for *unc-54* myosin heavy chain mutants—Deletion analysis of the surrounding chromosomal region.
- D. G. MOERMAN, D. POPHAM, and D. L. BAILLIE, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada: An analysis of the *unc-22* region in *C. elegans*.
- A. M. ROSE and D. L. BAILLIE, Department of Bioscience, Simon Fraser University, Burnaby, British Columbia, Canada: Analysis of the region around the gene for paramyosin.

Session 7: DNA and RNA

Chairperson: D. L. BAILLIE, Simon Fraser University, Burnaby, British Columbia, Canada

- S. EMMONS and M. KLASS, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Comparison of DNA sequences from *C. elegans* var *Bristol* germ-line and somatic line cells, and from *C. elegans* var *Bergerac* and *C. briggsae*.
- S. EMMONS, S. CARR, K. JOHNSON, and D. HIRSH, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Placing restriction fragments on the genetic map.
- J. KARN, S. BRENNER, and G. CESARENI, MRC Laboratory of Molecular Biology, Cambridge, England: Construction of phage and plasmid libraries representing the *C. elegans* genome.
- J. FILES, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Ribosomal DNA of *C. elegans*.
- A. R. MACLEOD and J. KARN, MRC Laboratory of Molecular Biology, Cambridge, England: Characterization of the *unc-54* myosin heavy chain messenger RNA.
- M.-A. GIBERT and J. STARCK, Laboratoire de Génétique Physiologique et Nématologie, and Département de Biologie Generale et Appliquée, CNRS, Université Lyon-I, Villeurbanne, France: RNA synthesis during *C. elegans* oogenesis.

THE CYTOSKELETON: MEMBRANES AND MOVEMENT, May 16 – May 20

arranged by

John Condeelis, Albert Einstein College of Medicine, New York, New York

Peter Satir, Albert Einstein College of Medicine, New York, New York

Keith Burridge, Cold Spring Harbor Laboratory, New York

255 participants

Session 1: Dynein and Ciliary Motility

Chairperson: P. SATIR, Albert Einstein College of Medicine, New York, New York

- F. D. WARNER and D. R. MITCHELL, Department of Biology, Syracuse University, New York: Dynein cross bridges and force production in *Tetrahymena* cilia.
- Y. YANO, H. MOHRI,* and T. MIKI-NOUMURA, Department of Biology, Ochanomizu University, Tokyo; *Department of Biology, University of Tokyo, Japan: Recovery of tubule-extrusion ability in the axonemes recombined with extracted dynein 1 after removal of outer arms or both arms.
- J. J. BLUM and M. HINES, Department of Physiology, Duke University School of Medicine, Durham, North Carolina: Some properties of a self-consistent three-state model of dynein cross bridges.
- V. PALLINI, Institute of Zoology, University of Siena, Italy: Studies on mammalian dynein.
- B. BACCETTI, Institute of Zoology, University of Siena, Italy: Comparative study of the relationship between dynein HMW polypeptides and axonemal structures.
- R. HIEBSCH and D. MURPHY, Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland: Evidence for a dynein-like ATPase on cytoplasmic microtubules.
- E. T. ROSENTHAL* and R. W. LINCK,† *Cell and Developmental Biology Program, and †Department of Anatomy, Harvard Medical School, Boston, Massachusetts: Sequence-specific patterns of doublet microtubule sliding in dynein-mediated disintegration of rat sperm flagella.
- K. TAKAHASHI, S. KAMIMURA, and Y. MOGAMI, Zoological Institute, University of Tokyo, Hongo, Japan: Cinematographic analysis of microtubule sliding in cilia and flagella.
- S. F. GOLDSTEIN, C. BESSE, and J. SCHRÉVEL, Laboratoire de Zoologie et Biologie Cellulaire et SGMEAB, Poitiers Cedex, France: Motility of a "6 + 0" flagellum.
- S. TAMM, Laboratory of Molecular Biology and Department of Zoology, University of Wisconsin, Madison: Calcium-dependent ciliary reversal in ctenophore larva—Relation to axonemal structures.
- C. K. OMOITO and C. KUNG, Laboratory of Molecular Biology, University of Wisconsin, Madison: Central pair of microtubules in paramecium cilia—Evidence for rotation and twist during the beat cycle.
- R. W. TUCKER and A. B. PARDEE, Sidney Farber Cancer Institute, Boston, Massachusetts: Centriole ciliation is related to quiescence and DNA synthesis in 3T3 cells.
- D. SANDOZ, E. BOISVIEUX-ULRICH, and B. CHALLEY, Centre de Cytologie Expérimentale, CNRS, Ivry, France: Cytochemical characterization of intramembrane particles associated with microfilaments linked to microtubules in cilia.

Session 2: Microtubules—Polarity and Nucleation

Chairperson: B. R. BRINKLEY, Baylor College of Medicine, Houston, Texas

- J. C. BULINSKI and G. G. BORISY, Laboratory of Molecular Biology, University of Wisconsin, Madison: Comparison of microtubule protein in mitotic and interphase cells.
- T. C. S. KELLER III and L. I. REBHUN, Department of Biology, University of Virginia, Charlottesville: Studies of tubulin from mitotic apparatuses isolated with and without glycerol.
- M. DE BRABANDER,* G. GEUENS,* J. DE MEY,* and M. JONIAU,† *Janssen Pharmaceutica, Beerse; †Catholic University of Leuven, Kortrijk, Belgium: Nucleated assembly of microtubules in living mitotic PTK²-cells.
- L. G. BERGEN, R. KURIYAMA, and G. G. BORISY, Laboratory of Molecular Biology, University of Wisconsin, Madison: Polarity of centrosomal microtubules.
- B. R. BRINKLEY, S. M. COX, and D. A. PEPPER, Department of Cell Biology, Baylor College of Medicine, Houston, Texas: Cytoplasmic microtubule assembly sites in mammalian cells.
- B. M. SPIEGELMAN,† M. LOPATA,* D. RING,* and M. KIRSCHNER,* *Department of Biochemistry and Biophysics, University of California, San Francisco; †Department of Biology, Massachusetts Institute of Technology, Cambridge: Initiation sites for microtubule assembly in interphase mammalian cultured cells.
- R. VALLEE, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Isolation of the portion of map-2 that promotes microtubule assembly.
- A. H. LOCKWOOD, Cancer Research Center and Department of Anatomy, Cell Biology Laboratories, University of North Carolina Medical School, Chapel Hill: Purification and relationship of several assembly proteins present in microtubules.
- R. L. MARGOLIS and L. WILSON, Department of Biological Sciences, University of California, Santa Barbara: Regulation of the microtubule steady state in vitro by ATP.

- P. B. SCHIFF and S. B. HORWITZ, Departments of Cell Biology and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York: Taxol stabilizes cytoplasmic microtubules in mouse fibroblasts.
- J. M. CARON and R. D. BERLIN, University of Connecticut Health Center, Farmington: Interaction of microtubule proteins with phospholipid vesicles.
- I. GOZES and U. Z. LITTAUER, Neurobiology Department, Weizmann Institute of Science, Rehovot, Israel: The α -subunit of tubulin is preferentially associated with brain synaptic membranes.
- D. SOIFER and H. CZOSNEK, Institute for Basic Research in Mental Retardation, Staten Island, New York: Tubulin synthesis on membrane-bound polyribosomes—Incorporation of newly synthesized tubulin into microsomal membranes.

Session 3: Actin Polymerization

Chairperson: F. OOSAWA, Osaka University, and Institute of Molecular Biology, Nagoya University, Japan

- J. PARDEE and J. A. SPUDICH, Sherman Fairchild Center, Stanford University School of Medicine, California: Role of ions and nucleotide in the conformation and assembly of actin from *Dictyostelium discoideum*.
- H. HARRIS, J. BAMBURG, and A. G. WEEDS, MRC Laboratory of Molecular Biology, Cambridge, England: Depolymerization of actin filaments by extracellular and intracellular proteins.
- L. CARLSSON, F. MARKEY, I. BLUKSTAD, T. PERSSON, and U. LINDBERG, Wallenberg Laboratory, Uppsala University, Sweden: Reorganization of actin in platelets stimulated by thrombin, as measured by the DNase inhibition assay.
- L.-E. NYSTRÖM, B. MALM, and U. LINDBERG, Wallenberg Laboratory, Uppsala, Sweden: Properties of the profilin:actin complex.
- S. LIN, Department of Biophysics, Johns Hopkins University, Baltimore, Maryland: Motility-related high-affinity cytochalasin binding complexes are powerful inducers of actin polymerization.
- M. GRUMET, Department of Biophysics, Johns Hopkins University, Baltimore, Maryland: Regulation of actin polymerization induced by high affinity cytochalasin binding complex from platelets.

Session 4: Actin Cytoskeleton

Chairperson: K. R. PORTER, University of Colorado, Boulder

- K. T. EDDS, Marine Biological Laboratory, Woods Hole, Massachusetts: Coelomocyte cytoskeletons vs egg actin gels—Similar components?
- R. R. WEIHING, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Cytoskeletal proteins and plasma membranes of HeLa cells.
- J. CONDEELIS, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Isolation of two gelation factors from the amoeboid stage of *Dictyostelium discoideum*.
- M. H. ELLISMAN* and K. R. PORTER,† *Department of Neurosciences, University of California, San Diego; †Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: New techniques for revealing the cytoskeleton of axoplasm and the effects of Ca^{++} upon its organization.
- W. IP and D. A. FISCHMAN, Department of Anatomy and Cell Biology, State University of New York Downstate Medical Center, Brooklyn: Analysis of cytoskeletal networks by high resolution scanning electron microscopy.
- J. M. MACKENZIE, JR. and H. F. EPSTEIN, Departments of Neurology and Biochemistry, Baylor College of Medicine, Houston, Texas: A novel approach to electron microscopy of myosin filaments.
- J. HEUSER* and M. KIRSCHNER,† *Department of Physiology, and †Department of Biochemistry and Biophysics, University of California, San Francisco: 3D visualization of the filamentous cytoskeleton in triton-extracted freeze dried fibroblasts.
- R. D. ALLEN, L. R. ZACHARSKI, S. WIDIRSKY, and L. ZAITLIN, Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire: Cytoskeletal structure and platelet motility.
- N. S. ALLEN and G. C. RUBEN, Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire: Cryopreservation of the cytoskeleton of *Nitella lurcata*.
- S. HIGASHI-FUJIME, Institute of Molecular Biology, Nagoya University, Japan: Active movement in vitro of cytoplasmic fibers isolated from *Nitella*.
- F. OOSAWA, Department of Biophysical Engineering, Osaka University, and Institute of Molecular Biology, Nagoya University, Japan: Motility of actin filaments.

- I. M. HERMAN, N. CRISONA, and T. POLLARD, Department of Cell Biology and Anatomy, Johns Hopkins University Medical School, Baltimore, Maryland: Simultaneous localization of actin and myosin by double antibody staining.
- D. DRECKHAHN, Department of Anatomy, University of Kiel, Germany: Immunocytochemical localization of myosin and tropomyosin in various epithelial cells of ectodermal and endodermal origin.
- J. R. FALLON and V. T. NACHMIAS, Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia: Skeletal and cytoplasmic myosins in myotubes.

Session 5: Regulation of Cytoplasmic Structure

Chairperson: M. CLARKE, Albert Einstein College of Medicine, New York, New York

- J. M. SCHOLEY and J. KENDRICK-JONES, MRC Laboratory of Molecular Biology, Cambridge, England: Myosin linked regulation of actin-myosin interaction in bovine thymus and thymocytes.
- S. CHACKO, Department of Pathobiology, University of Pennsylvania, Philadelphia: Phosphorylation and actin-activated ATPase activity of vascular smooth muscle myosin.
- A. SEN and G. J. TODARO, National Cancer Institute, Bethesda, Maryland: Mouse sarcoma virus particles contain a low molecular weight (~15K) protein kinase that is cAMP-independent and that binds to actin.
- L. J. VAN ELDIK, Rockefeller University, New York, New York: Phosphorylation and calmodulin-stimulated activities.
- D. K. JEMIOLO, T. C. S. KELLER III, W. H. BURGESS, and L. I. REBHUN, Department of Biology, University of Virginia, Charlottesville: Tubulin-CDR interactions.
- W. H. BURGESS, D. K. JEMIOLO, and R. H. KRETSINGER, Department of Biology, University of Virginia, Charlottesville: Interaction of calmodulin and Ca_2^+ in the presence of SDS.
- M. CLARKE, S. C. KAYMAN, and W. L. BAZARI, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Isolation and characterization of a calcium-dependent regulator protein from *Dictyostelium discoideum*.
- W. DRABIKOWSKI and J. KUZNICKI, Department of Nervous System and Muscle, Nencki Institute of Experimental Biology, Warsaw, Poland: Characterization of calmodulin from *Physarum polycephalum*.
- E. ZUBRZYCKA, B. KORCZAK, L. KUZNICKI, and W. DRABIKOWSKI, Nencki Institute of Experimental Biology, Warsaw, Poland: Properties of microsomal fraction from *Physarum polycephalum*.
- M. S. RUNCE,* P. B. HEWLEY,† D. PUETT,† and R. C. WILLIAMS, JR.,* *Department of Molecular Biology, and †Department of Biochemistry, Vanderbilt University, Nashville, Tennessee: Cyclic nucleotide phosphodiesterase activity in neurofilament preparations from bovine brain.
- W. W. SCHLAEFFER, Department of Pathology, University of Pennsylvania, Philadelphia: The breakdown of mammalian neurofilaments and its mediation by calcium and a soluble tissue factor.
- P. A. M. EAGLES and D. S. GILBERT, Kings College, London University, England: The phosphorylation of neurofilament proteins.
- F. CABRAL and M. M. GOTTESMAN, National Institutes of Health, Bethesda, Maryland: Phosphorylation of 10 nm filament protein in CHO cells.

Session 6: Poster Sessions

Actin Cytoskeleton

- P. J. ANDERSON, Department of Biochemistry, University of Ottawa, Ontario, Canada: Determination of actin and tubulin in cells and tissues.
- R. A. BADLEY, J. R. COUCHMAN and D. A. REES, Unilever Research, Colworth House, Bedford, United Kingdom: Cytoskeletons, adhesion, and cell locomotion.
- R. D. GOLDMAN and J. TALIAN, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Adhesion plaques of nonmuscle cells are analogous to z-lines—The localization of an α -actinin-like protein.
- A. MILSTEID, S. E. HITCHCOCK, and R. D. GOLDMAN, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Alterations in contractile protein organization and cell locomotion in aging cells.
- S. S. BROWN and J. A. SPUDICH, Sherman Fairchild Center, Stanford University School of Medicine, California: Role of ATP in the nucleation of actin assembly by polylysine-coated beads.

- P. B. BELL, JR. and J.-P. REVEL, Division of Biology, California Institute of Technology, Pasadena: Cytochalasin-B-induced changes in the distribution of the triton-insoluble cytoskeleton.
- S. T. BRADY,* and W. O. MCCLURE,† *Department of Anatomy, Case Western Reserve University, Cleveland, Ohio; †Department of Biological Sciences, University of Southern California, Los Angeles: Antimitotic drugs and axonal transport in the absence of microtubules.
- F. C. S. RAMAEKERS,* A. M. E. SELTEN-VERSTEEGEN,* E. KATZ,† and H. BLOEMENDAL,* *Department of Biochemistry, University of Nijmegen, The Netherlands; †Department of Biology, University of Stony Brook, New York: Cytoskeletal elements in elongating cultured bovine lens cells—Structural and biosynthetic aspects.
- M. PEARL,* and A. TAYLOR,† *Department of Physiology, Mt. Sinai School of Medicine; †Department of Physiology, Cornell Medical College, New York, New York: Actin-like protein in toad bladder epithelium and in situ labeling with HMM.
- D. PAULIN and M. YANIV, Institut Pasteur, Paris, France: Changes of cytoskeleton organization upon differentiation of mouse embryonic cells in vitro and in vivo.
- J. TANNENBAUM, Biology Department, Massachusetts Institute of Technology, Cambridge: Effect of cytochalasin D (CD) on the distribution of cytoskeletal proteins in chick embryo fibroblasts (CEF).
- R. W. RUBIN, R. H. WARREN, and C. L. LEONARDI, Department of Anatomy, University of Miami, Florida: Cytoskeleton-associated proteins in normal and transformed cells.
- E. BELL, C. MERRILL, and B. IVARSSON, Department of Biology, Massachusetts Institute of Technology, Cambridge: A tissue-equivalent assay system for studying fibroblast contractility.
- T. M. PRESTON and C. A. KING, Department of Zoology, University College London, England: The use of reflexion interference microscopy in the study of amoeboid locomotion and cell-substrate interactions.
- D. E. KELLY and A. M. KUDA, Department of Anatomy, University of Southern California School of Medicine, Los Angeles: Freeze-fracture analysis of desmosomal and triadic junctions.
- Y. FUKUI and J. T. BONNER, Department of Biology, Princeton University, New Jersey: Dimethyl-sulfoxide-induced multinucleation and novel nuclear division in *Dictyostelium* cells.
- M. WALSH, L. JOHNSON, and L. B. CHEN, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Use of Rhodamine-mit, a mitochondria-specific probe, to reflect possible cytoskeletal alteration in living cells.
- K. WANG, Clayton Foundation Biochemical Institute and Department of Chemistry, University of Texas, Austin: Novel myofibrillar proteins of striated muscles.

Genetic Approaches and Morphogenesis

- J. M. ZENGEL, S. HOUTS, D. C. REIN, and H. F. EPSTEIN, Department of Neurobiology, Baylor College of Medicine, Houston, Texas: 118 body-wall muscle-defective mutants of *C. elegans*.
- F. CABRAL and M. M. GOTTESMAN, National Institutes of Health, Bethesda, Maryland: β -tubulin mutants of CHO cells.
- B. R. OAKLEY and N. R. MORRIS, Department of Pharmacology, Rutgers Medical School, Piscataway, New Jersey: Genetic evidence that nuclear movement in *Aspergillus* is microtubule-dependent.
- N. R. MORRIS, Department of Pharmacology, Rutgers Medical School, Piscataway, New Jersey: The genetics of resistance to antimitotic drugs in *Aspergillus nidulans*.
- D. L. KIRK, Department of Biology, Washington University, St. Louis, Missouri: Cytoskeletal mediation of morphogenesis in *Volvox*—I. Introduction.
- K. J. GREEN, Department of Biology, Washington University, St. Louis, Missouri: Cytoskeletal mediation of morphogenesis in *Volvox*—II. Cleavage.
- G. I. VIAMONTES, Department of Biology, Washington University, St. Louis, Missouri: Cytoskeletal mediation of morphogenesis in *Volvox*—III. Inversion.
- W. S. LYNN, C. MUKHERJEE, and B. FREEMAN, Department of Biochemistry, Duke University, Durham, North Carolina: Hydrophobic anion transport and leukocyte motility.
- G. THOMAS, M. SIEGMANN, A. M. KUBLER, J. GORDON, and L. JIMINEZ DE ASUA, Friedrich Miescher-Institut, Basel, Switzerland: Ribosomal protein S6 phosphorylation, protein synthesis and cAMP.

Intermediate Filaments

- E. FUCHS and H. GREEN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Multiple keratins of cultured human epidermal cells are translated from different messenger RNA molecules.

- M. S. RUNGE,* W. W. SCHLAEPFER,[†] and R. C. WILLIAMS, JR.,* *Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee; [†]University of Pennsylvania, Philadelphia: Isolation of neurofilaments from mammalian brain.
- F.-C. CHIU, B. KOREY, J. E. GOLDMAN, and W. T. NORTON, Albert Einstein College of Medicine, Bronx, New York: Intermediate filament proteins from mammalian nervous system.
- E. WANG and S. C. SILVERSTEIN, Rockefeller University, New York, New York: The distribution of 10 nm filaments and lysosomes in phorbol myristate acetate treated mouse peritoneal macrophages.
- E. BORENFREUND, P. J. HIGGINS, and A. BENDICH, Memorial Sloan-Kettering Cancer Center, New York, New York: In vitro studies of a cytoskeletal abnormality in rat liver cells induced in vivo by a carcinogen.
- B. ASCH,* C. LEONARDI,[†] N. BURSTEIN,* and R. RUBIN[†] *Department of Pathology, Beth Israel Hospital, and Harvard Medical School, Boston, Massachusetts; [†]Department of Anatomy, University of Miami School of Medicine, Florida: Are cytoskeletal models from different nonmuscle cell types identical or unique?
- K. RYBICKA, Cardiology Department, US Public Health Service Hospital, Staten Island, New York: Association of 100 Å filaments with glycosomes in the heart.
- H. CZOSNEK, D. SOIFER, and H. M. WISNIEWSKI, Institute for Basic Research in Mental Retardation, Staten Island, New York: The biosynthesis of filamentous proteins in rabbit spinal cord: Cell-free and in vivo studies.
- D. L. GARD, P. B. BELL, and E. LAZARIDES, Division of Biology, California Institute of Technology, Pasadena: Coexistence of desmin and the fibroblastic intermediate filament subunit in muscle and nonmuscle cells: Identification and comparative peptide analysis.

Tubulin

- M. M. BLACK and L. A. GREENE, Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts: Tubulin accumulation and organization in chick sympathetic neurons during nerve-growth-factor-stimulated neurite outgrowth.
- K. A. ROGERS, C. R. RUDD, J. G. KAPLAN, and D. L. BROWN, Department of Biology, University of Ottawa, Canada: Colchicine effects on microtubules and commitment of lymphocytes.
- I. LI and L. I. REBHUN, Department of Biology, University of Virginia, Charlottesville: Regulation of polymerization of tubulin via a mixed disulfide of tubulin-SH with glutathione.
- L. I. REBHUN and R. WANG, Department of Biology, University of Virginia, Charlottesville: Reversible inhibition of tubulin polymerization by formation of intrasubunit disulfides.
- H. KIM and L. I. REBHUN, Department of Biology, University of Virginia, Charlottesville: Further analysis of MAP2:tubulin interaction in brain microtubule assembly in vitro.
- M. CAPLOW, B. ZEEBERG, and L. JAMESON, Department of Biochemistry, University of North Carolina, Chapel Hill: Quantitative analysis of GDP and GTP involvement in microtubule assembly and disassembly.
- R. A. B. KEATES and G. B. MASON, Department of Chemistry, University of Guelph, Ontario, Canada: Analysis of the kinetics of inhibition of microtubule polymerization by colchicine and griseofulvin.
- E. MANDELKOW and E. MANDELKOW, Max-Planck-Institute for Medical Research, Heidelberg, Germany: Correlation between structural polarity and polarity of assembly of tubulin.
- F. GASKIN, Department of Pathology, Albert Einstein College of Medicine, Bronx, New York: In vitro tubulin assembly regulation by divalent cations and nucleotides.
- J. MOLE-BAJER and A. S. BAJER, Department of Biology, University of Oregon, Eugene: Experimental modification of microtubule assembly-disassembly as reflected in chromosome movements and spindle fine structure.
- B. S. ECKERT, S. J. KOONS,* and A. W. SCHANTZ, Department of Anatomical Sciences and *Department of Biophysical Sciences, School of Medicine, State University of New York, Buffalo: Cytoskeleton-associated proteins in cultured mammalian cells.
- W. D. COHEN, Department of Biological Sciences, Hunter College, New York, New York: Cytoskeletal structure in anucleate nonmammalian erythrocytes.
- R. B. LUFTIG, B. MILES, D. LEAFFER, J. A. WEATHERBEE, and R. R. WEIHING, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Adenovirus and reovirus associations with microtubules inside infected cells.
- K. R. HINDS and N. W. SEEDS, Department of Biochemistry, Biophysics and Genetics, University of Colorado Medical Center, Denver: Characterization of the microtubule assembly-promoting factor in differentiated neuroblastoma cells.

- R. WEISENBERG, Department of Biology, Temple University, Philadelphia, Pennsylvania: Cooperative oligomer addition model for microtubule assembly.
- D. A. PEPPER and B. R. BRINKLEY, Department of Cell Biology, Baylor College of Medicine, Houston, Texas: Site-specific microtubule nucleation in lysed mitotic cells—Inhibition by tubulin antibody.

Session 7: Cell Surface Dynamics

Chairperson: J. CONDEELIS, Albert Einstein College of Medicine, New York, New York

- R. G. W. ANDERSON,* J. L. GOLDSTEIN,† and M. S. BROWN,† *Department of Cell Biology, and †Department of Molecular Genetics, University of Texas Health Science Center, Dallas: Relationship of low density lipoprotein binding sites to the distribution of coated surface regions of membrane in human fibroblasts.
- W. S. BLOOM,* K. L. FIELDS,† S. H. YEN,† W. SCHOOK,* and S. PUSZKIN,* *Department of Pathology, Mount Sinai School of Medicine, City University of New York; †Department of Pathology, Albert Einstein College of Medicine, Bronx, New York: Brain clathrin—Immunofluorescent studies on fibroblasts, brain tissue sections and brain cell cultures.
- P. DAVIES, D. DAVIES, F. MAXFIELD, A. LEVITZKI, P. MILHAUD, M. WILLINGHAM, and I. PASTAN, NCI, National Institutes of Health, Bethesda, Maryland: Receptor-mediated endocytosis, transglutaminase and the microfilament mat.
- J. SALISBURY, J. CONDEELIS, and P. SATIR, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Isolation of structures involved in capping and endocytosis of Ig-IgM complexes in cultured human B lymphocytes.
- K. BURRIDGE, J. FERAMISCO, and S. BLOSE, Cold Spring Harbor Laboratory, New York: Association of α -actinin and clathrin with the plasma membrane.
- T. F. ROTH, M. P. WOODWARD, and C. LINDEN, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville: Coated vesicles—Subcellular organelles involved in vectorial movement of sequestered proteins.
- W. SCHOOK, E. FEAGESON, W. S. BLOOM, C. ORES, and S. PUSZKIN, Department of Pathology, Mount Sinai School of Medicine, City University of New York: Properties of the clathrin complex that forms in vitro ultrastructural assemblies of baskets and filamentous bundles.
- B. M. JOCKUSCH, European Molecular Biological Laboratories, Heidelberg, Germany: Association of α -actinin with cell membranes.
- G. GABBIANI,* D. HOESSLI,* E. RUNGGER-BRÄNDLE,* and B. M. JOCKUSCH,† *Department of Pathology, University of Geneva, Switzerland; †European Molecular Biology Laboratories, Heidelberg, Germany: Lymphocyte α -actinin—Relationship with cell membrane and co-capping with surface receptors.
- K. L. CARRAWAY, R. C. DOSS, J. W. HUGGINS, and C. A. CAROTHERS, Department of Biochemistry, Oklahoma State University, Stillwater: Effects of cytoskeletal perturbants on the cooperativity of the inhibition of ecto 5'-nucleotidase by concanavalin A in 13762 ascites rat mammary adenocarcinoma cells.
- R. M. EVANS,* D. C. WARD,* and L. M. FINK,† *Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; †Department of Pathology, University of Colorado Medical School, Denver: Characterization of transmembrane proteins in phagolysosomes prepared from cultured mouse L-929 cells.
- D. A. M. MESLAND and U. W. GOODENOUGH, Department of Biology, Washington University, St. Louis, Missouri: Flagellar tip activation during *Chlamydomonas* mating.
- R. A. BLOODGOOD, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Effect of inhibition of protein synthesis on *Chlamydomonas* flagellar adhesiveness and flagellar surface motility.

Session 8: Intermediate Filaments

Chairperson: R. D. GOLDMAN, Carnegie-Mellon University, Pittsburgh, Pennsylvania

- W. W. FRANKE,* E. SCHMID,* M. OSBORN,† K. WEBER,† C. FREUDENSTEIN,* and B. APPELHANS,* *Division of Membrane Biology and Biochemistry, Experimental Pathology, German Cancer Research Center, Heidelberg; †Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany: Occurrence and modes of arrangement of different types of intermediate-sized filaments in tissues and cells.
- T.-T. SUN,* A. VIDRICH,* C. SHIH,† and H. GREEN,† *Departments of Dermatology and of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland; †Department of Biology, Massachusetts Institute of Technology, Cambridge: Keratin cytoskeletons in epithelial cells of internal organs.

- R. K. H. LIEM, J. F. LETERRIER, and M. L. SHELANSKI, Department of Pharmacology, New York University School of Medicine, New York: Interactions between neurofilaments, microtubules and microtubule-associated proteins.
- B. H. ANDERTON,* R. THORPE,* J. COHEN,† and P. WOODHAMS,‡ *Chelsea College, University of London; †University College London; ‡MRC Developmental Neurobiology Unit, Institute of Neurology, London, England: Identity of neurofilament polypeptides.
- J. R. MORRIS and R. J. LASEK, Department of Anatomy and Neurobiology Center, Case Western Reserve University, Cleveland, Ohio, and Marine Biological Laboratory, Woods Hole, Massachusetts: Differential solubilities of cytoskeletal proteins in squid axoplasm.
- E. D. FRANK,* G. P. TUSZYNSKI,† and L. WARREN,* *Wistar Institute, Philadelphia; †Thrombosis Center, Temple University, Philadelphia, Pennsylvania: Classes of 10 nm filament proteins.
- K. L. FIELDS* and S.-H. YEN,† *Departments of Neurology, Neuroscience and †Pathology, Albert Einstein College of Medicine, Bronx, New York: Antibodies to neurofilament, glial filament and fibroblast intermediate filament proteins bind to different cell types of the nervous system.
- V. I. KALNINS, L. SUBRAHMANYAN, A. PORIETIS, and A. O. JORGENSEN, Department of Anatomy, University of Toronto, Canada: Distribution of 54,000 m.w. protein of 10 nm filaments isolated from calf brain.
- S. H. BLOSE, Cold Spring Harbor Laboratory, New York: 10 nm filaments and mitosis—Maintenance of structural continuity in dividing endothelial cells.
- H. HOLTZER, Y. TOYAMA, G. BENNETT, S. FELLINI and C. WEST, Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia: Changes in kind and distribution of the 100 Å filaments in developing muscle.
- I. K. BUCKLEY,* M. STEWART,** D. O. IRVING,* T. R. RAJU,* and W. E. GORDON,† *John Curtin School of Medical Research, Australian National University; †Division of Computing Research, CSIRO, Canberra, Australia; **Cold Spring Harbor Laboratory, New York: Organelle movements, intermediate filaments and actin.
- R. V. ZACKROFF and R. D. GOLDMAN, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: In vitro assembly of intermediate filaments from BHK-21 cells.
- L. M. MILSTONE, Department of Dermatology, Yale University, New Haven, Connecticut: Calf esophagus contains two abundant proteins which form 10 nm filaments.
- T. W. HUIATT, R. M. ROBSON, J. M. O'SHEA, M. K. HARTZER, and M. H. STROMER, Muscle Biology Group, Iowa State University, Ames: Self-assembly of 10 nm filaments from highly purified desmin.

Session 9: Cell Shape and Migration

Chairperson: R. D. ALLEN, Dartmouth College, Hanover, New Hampshire

- L. MAREK and E. BELL, Department of Biology, Massachusetts Institute of Technology, Cambridge: Time-lapse films of fibroblasts in the polarized light microscope.
- G. ALBRECHT-BUEHLER, Cold Spring Harbor Laboratory, New York: Group migration of the PTK1 cells.
- E. B. CRAMER, L. C. MILKS, and G. K. OJAKIAN, Department of Anatomy and Cell Biology, State University of New York Downstate Medical Center, Brooklyn: An in vitro model system of transepithelial migration of human polymorphonuclear leukocytes (PMN's).
- C. A. KING, R. H. MILLER, P. DONOVAN, and T. M. PRESTON, Zoology Department, University College, London, England: Reflexion interference microscopy studies on the loss of membrane material (=trailing) during amoeboid locomotion of free living amoebae and human neutrophil leucocytes.
- C. H. DAMSKY, R. DORIO, and C. A. BUCK, Wistar Institute, Philadelphia, Pennsylvania: Manipulation of cell-cell and cell-substrate interactions in mammary epithelial cell lines by antisera against surface glycoproteins.
- T. P. FITZHARRIS* and R. R. MARKWALD,† *Department of Anatomy, Medical University of South Carolina, Charleston; †Texas Tech University School of Medicine, Lubbock: Modification of matrix ordering during in vivo cell movement.
- A. ZURN and F. SOLOMON, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Cellular specification of morphology.
- E. REPASKY and B. S. ECKERT, Department of Anatomical Sciences, State University of New York School of Medicine, Buffalo: Cytoskeletal and contractile elements during maturation of mammalian erythroid cells.

- V. BENNETT and P. J. STENBUCK, Department of Molecular Biology, Wellcome Research Laboratories, Research Triangle Park, North Carolina: Ankyrin, the membrane attachment protein for spectrin, is linked to band 3 in human erythrocyte membranes.
- M. P. SHEETZ, Department of Physiology, University of Connecticut Health Center, Farmington: Internal membrane protein interactions with triton cytoskeletons of erythrocytes.
- S. L. BRENNER and E. D. KORN, NHLBI, National Institutes of Health, Bethesda, Maryland: The interaction of spectrin with actin.

Session 10: Brush Border and Microfilament Organization

Chairperson: H. HUXLEY, MRC Laboratory of Molecular Biology, Cambridge, England

- M. S. MOOSEKER* and R. E. STEPHENS,† †Marine Biological Laboratory, Woods Hole, Massachusetts; *Department of Biology, Yale University, New Haven, Connecticut: Brush border α -actinin?
- A. BRETSCHER and K. WEBER, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany: Analysis of the major cytoskeletal components of chicken intestinal brush borders and their distribution between the microvilli and terminal web region.
- S. W. CRAIG and C. L. LANCASHIRE, Department of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Evidence for lack of homology between alpha actinin and brush border 100K polypeptide.
- P. MATSUDAIRA and D. BURGESS, Department of Biology, Dartmouth College, Hanover, New Hampshire: Helical arrangement of lateral arm projections and their removal from microvillar core microfilaments.
- D. F. ALBERTINI and K. FUJIWARA, Department of Anatomy and Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts: Membrane-cytoskeletal interactions during hormone and mitogen-induced cellular rounding.
- T. J. BRADLEY and P. SATIR, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Microfilament-associated mitochondrial movement.
- B. GEIGER and S. J. SINGER, Department of Biology, University of California, San Diego: A 130K-protein isolated from chicken gizzard—Its localization at the termini of stress fibers, in areas of cell-substrate and cell-cell contact.
- J. R. FERAMISCO, Cold Spring Harbor Laboratory, New York: Microinjection of fluorescently labeled α -actinin into living fibroblasts.
- A. B. FULTON, J. PRIVES, F. FARMER, and S. PENMAN, Massachusetts Institute of Technology, Cambridge: Transient instability of skeletal framework and its surface lamina during myoblast fusion.
- R. E. POLLACK,* K. SMITH,* N. SPURR,† and Y. ITO,† *Department of Biological Sciences, Columbia University, New York; †Imperial Cancer Research Fund, London, England: A polyoma virus mutant, DL-23, is defective in changing the cytoskeletal actin pattern of infected cells.
- A. M. OTTO, A. ZUMBÉ, L. J. GIBSON, A.-M. KUBLER, and L. JIMENEZ DE ASUA, Friedrich Miescher-Institut, Basel, Switzerland: Cytoskeleton-disrupting drugs enhance the initiation of DNA synthesis stimulated by growth factors.

MEMBRANE BIOGENESIS. May 22 – May 27

arranged by

Günter Blobel, Rockefeller University, New York, New York

David Sabatini, New York University Medical Center, New York

206 participants

Welcoming Remarks: D. D. SABATINI, New York University Medical Center, New York

Session 1: Introductory Session

- G. PALADE, Department of Cell Biology, Yale University, New Haven, Connecticut: Membrane biogenesis—Past and perspectives.
- J. BECKWITH, Harvard Medical School, Boston, Massachusetts: Genetics of protein secretion in bacteria.

- V. MARCHESI, Yale University, New Haven, Connecticut: Proteins that make channels across membranes.
D. D. SABATINI, T. MORIMOTO, M. ADESNIK, and G. KREIBICH, Department of Cell Biology, New York University School of Medicine, New York: Functions of membrane-bound ribosomes in eukaryotic cells.

Session 2A: Synthesis of Secretory, Membrane, and Lysosomal Proteins in the Rough Endoplasmic Reticulum. I.

Chairperson: G. BLOBEL, Rockefeller University, New York, New York

- Y. BURSTEIN, O. WOLF, and I. SCHECHTER, Weizmann Institute of Science, Rehovot, Israel: Structure and function of immunoglobulin precursors.
D. SHIELDS, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: In vitro synthesis, processing and segregation of secretory glycoproteins by heterologous microsomal membranes.
E. C. HEATH and T. H. HAUGEN, Department of Biochemistry, University of Iowa, Iowa City: Cell free synthesis, processing, and glycosylation of an active RNase precursor.
T. MORIMOTO, Department of Cell Biology, New York University School of Medicine, New York: Functional binding of ribosomes to rat liver endoplasmic reticulum membranes in vitro.
G. KREIL, C. MOLLAY, and L. HAIML, Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg: Melittin—A soluble peptide in search of a membrane.
J. R. LINGAPPA, V. R. LINGAPPA, and G. BLOBEL, Department of Cell Biology, Rockefeller University, New York, New York: A competitive inhibitor of nascent chain translocation across microsomal membranes—A tryptic putative signal peptide from chicken ovalbumin.
A. H. ERICKSON and G. BLOBEL, Rockefeller University, New York, New York: In vitro synthesis of β -glucuronidase by rat liver and preputial gland RNA.
M. ROSENFELD, G. KREIBICH, D. POPOV, and D. D. SABATINI, Department of Cell Biology, New York University Medical Center, New York: Site of synthesis and processing of β -glucuronidase in rat liver and preputial gland.
H. F. LODISH, W. BRAELL, M. PORTER, and A. ZILBERSTEIN, Massachusetts Institute of Technology, Cambridge: Biosynthesis of a viral and a cellular glycoprotein.
H. P. GHOSH,* F. TONEGUZZO,* R. IRVING,* J. CAPONE,* and T. HOFMANN,† *Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada; †Department of Biochemistry, University of Toronto, Ontario, Canada: Membrane biogenesis—Synthesis, glycosylation, insertion into membrane and topology of a viral transmembrane glycoprotein.
H. GAROFF, K. SIMONS, and B. DOBBERSTEIN, European Molecular Biology Laboratory, Heidelberg, Germany: Structure and assembly of the Semliki Forest virus membrane.
S. BONATTI and G. BLOBEL, Laboratory of Cell Biology, Rockefeller University, New York, New York: Sindbis virus glycoprotein PE² lacks an amino terminal cleavable signal sequence.

Session 2B: Synthesis of Secretory, Membrane, and Lysosomal Proteins in the Rough Endoplasmic Reticulum. II.

Chairperson: T. OMURA, Kyushu University, Fukuoka, Japan

- H. PLOEGH,* H. ORR,* E. CANNON,† and J. L. STROMINGER,* *The Biological Laboratories, Harvard University, Cambridge; †Department of Biology, Brandeis University, Waltham, Massachusetts: Biosynthesis of HLA-A and HLA-B antigens.
B. DOBBERSTEIN,* H. GAROFF,* G. WARREN,* and P. J. ROBINSON,† *European Molecular Biology Laboratory; †Institute for Immunology and Genetics, German Cancer Research Center, Heidelberg, Germany: Cell-free synthesis and membrane insertion of mouse H-2D_a histocompatibility antigen and β^2 -microglobulin.
I. D. ALGRANATI, A. ZIEGLER, and C. MILSTEIN, MRC Laboratory of Molecular Biology, Cambridge, England: Regulation of biosynthesis and expression of human major histocompatibility antigens.
D. S. PAPERMASTER,*† B. G. SCHNEIDER,* Y. BURSTEIN,‡ and I. SCHECHTER,† *Department of Pathology, Yale Medical School, New Haven, Connecticut; †Departments of *Organic Chemistry and ‡Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: Biosynthesis of rhodopsin—Involvement of Golgi in transport of a membrane protein synthesized without a short-lived NH₂-terminal peptide.

- E. SABBAN, D. SABATINI, M. ADESNIK, and V. MARCHESI,* Department of Cell Biology, New York University School of Medicine, New York; *Department of Pathology, Yale University School of Medicine, New Haven, Connecticut: Biosynthesis of murine erythrocyte membrane protein band 3.

Session 3A: Role of Endoplasmic Reticulum in Protein Biosynthesis and Processing. I.

Chairperson: G. KREIBICH, New York University Medical Center, New York

- G. KREIBICH, New York University Medical Center, New York: Proteins associated with ribosomal binding sites in the rough endoplasmic reticulum.
- R. A. RACHUBINSKI, D. P. S. VERMA,* and J. J. M. BERGERON, Departments of Anatomy and *Biology, McGill University, Montreal, Canada: Synthesis of rat liver microsomal cytochrome b⁵ by free ribosomes.
- Y. OKADA, D. D. SABATINI, and G. KREIBICH, New York University School of Medicine, New York: Sites of synthesis of rat liver cytochrome b⁵ and NADPH cytochrome P-450 reductase.
- T. OMURA, Department of Biology, Kyushu University, Fukuoka, Japan: Role of free polyribosomes in the biosynthesis of membrane proteins in hepatocytes and in adrenocortical cells.

Session 3B: Role of Endoplasmic Reticulum in Protein Biosynthesis and Processing. II.

Chairperson: J. D. JUDAH, University College Hospital, Medical School, London, England

- S. BAR-NUN, G. KREIBICH, M. ADESNIK, L. ALTERMAN, M. NEGISHI, and D. D. SABATINI, New York University School of Medicine, New York: Synthesis and insertion of cytochrome P-450 into endoplasmic reticulum membranes.
- N. BORGESSE and J. MELDOLESI, CNR Center of Cytopharmacology and Department of Pharmacology, University of Milan, Italy: Biogenesis and turnover of NADH cytochrome b⁵ reductase, a membrane protein with multiple localization in rat hepatocytes.
- A. AMAR-COSTESE, H. BEAUFAY, and C. DE DUVE, Université de Louvain and International Institute of Cellular and Molecular Pathology, Brussels, Belgium: The distribution of enzymes in subcellular membranes from rat liver.
- R. C. JACKSON* and G. LOBEL†, *Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire; †Department of Cell Biology, Rockefeller University, New York, New York: Post-translational processing of full-length presecretory proteins with canine pancreatic signal peptidase.
- M. ZIMMERMAN,* R. A. MUMFORD,* G. KREIBICH,† and A. W. STRAUSS,‡ *Merck Institute for Therapeutic Research, Rahway, New Jersey; †Department of Cell Biology, New York University, New York; ‡Department of Biological Chemistry, Washington University, St. Louis, Missouri: Characterization of a membrane endopeptidase possibly involved in pre-protein processing.
- M. CZAKO-GRAHAM, D. D. SABATINI, E. BARD, and G. KREIBICH, New York University School of Medicine, New York: Specific binding of secretory polypeptide precursors to reconstituted rat liver rough microsomal membranes.
- P. WALTER and G. LOBEL, Laboratory of Cell Biology, Rockefeller University, New York, New York: Tryptic dissection and reconstitution of the translocation activity for nascent presecretory proteins across microsomal membranes.
- G. VON HEIJNE and C. BLOMBERG, Department of Theoretical Biophysics, Royal Institute of Technology, Stockholm, Sweden: Extrusion of nascent polypeptides through a lipophilic membrane—An energy analysis.
- J. D. JUDAH, Department of Experimental Pathology, University College Hospital Medical School, London, England: Membrane function in the secretion of albumin.
- C. M. REDMAN, D. BANERIEE, and S. YU, Lindsley F. Kimball Research Institute of the New York Blood Center, New York: Precursor processing of rat serum albumin in liver and Morris hepatomas 5123C and 7800.
- L. GERACE and G. LOBEL, Department of Cell Biology, Rockefeller University, New York, New York: A model for the reversible mitotic disassembly of the higher eukaryotic nuclear envelope.
- M. BUDARF, P. ROJA, and E. HERBERT, Chemistry Department, University of Oregon, Eugene: Effect of tunicamycin on the synthesis and processing of pro-ACTH-endorphin.

Session 4: Synthesis of Secretory and Membrane Proteins in Prokaryotic Cells. I.

Chairperson: J. BECKWITH, Harvard Medical School, Boston, Massachusetts

- J. BECKWITH, P. BASSFORD, A. SARTHY, B. WANNER, T. SILHAVY, and H. INOUE, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Genetics of secretion of periplasmic proteins in *E. coli*.
- C. N. CHANG and G. BLOBEL, Department of Cell Biology, Rockefeller University, New York, New York: Processing and segregation of *E. coli* periplasmic enzyme alkaline phosphatase.
- W. P. SMITH, Vick Divisions Research and Development, Mt. Vernon, New York: Co-translational secretion of diphtheria toxin and alkaline phosphatase in vitro—Involvement of membrane protein(s).
- B. D. DAVIS, W. P. SMITH, and P.-C. TAI, Harvard Medical School, Boston, Massachusetts: Protein secretion in bacteria.
- M. SARVAS,* K. P. HIRTH,† E. FUCHS,† and K. SIMONS,* *European Molecular Biology Laboratory, Heidelberg; †Institut für Molekulare Genetik, Universität Heidelberg, Germany: Secretion of the penicillinase of *Bacillus licheniformis*.
- J. B. K. NIELSEN,* C. N. CHANG,† K. IZUI,* G. BLOBEL,† and J. O. LAMPEN,* *Waxman Institute of Microbiology, Rutgers University, Piscataway, New Jersey; †Rockefeller University, New York, New York: Stages in the biosynthesis of penicillinase in *Bacillus licheniformis* 749.
- V. G. WILSON and R. W. HOGG, Department of Microbiology, Case Western Reserve University, Cleveland, Ohio: Sequence studies of the precursor form of the arabinose binding protein.
- D. L. OXENDER, C. J. DANIELS, R. LANDICK, and J. J. ANDERSON, Department of Biological Chemistry, University of Michigan, Ann Arbor: Precursor forms of the periplasmic leucine binding proteins in *E. coli* K12.
- K. ITO, G. MANDEL, P. HEARNE, and W. WICKNER, Department of Biological Chemistry and Molecular Biology Institute, University of California, Los Angeles: Synthesis, assembly and processing of a precursor of the M13 coat protein, an integral membrane protein.
- C. N. CHANG, P. MODEL, and G. BLOBEL, Rockefeller University, New York, New York: Asymmetric insertion of an integral transmembrane protein into the membrane is coupled to translation.
- B. L. WANNER,* A. SARTHY,† and J. R. BECKWITH,† *Department of Biology, Massachusetts Institute of Technology, Cambridge; †Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: An *E. coli* pleiotropic mutant defective in the synthesis of periplasmic and outer membrane proteins.
- J. J. C. LIN and H. C. WU, Department of Microbiology, University of Connecticut Health Center, Farmington: Biosynthesis and assembly of murein lipoprotein in *E. coli*.
- S. D. EMR and T. J. SILHAVY, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: An analysis of signal sequence mutations in the gene coding for the λ receptor, an outer membrane protein of *E. coli*.

Session 5: Synthesis of Secretory and Membrane Proteins in Prokaryotic Cells. II

Chairperson: T. SILHAVY, Harvard Medical School, Boston, Massachusetts

- L. MINDICH, R. HUANG, and B. STITT, Public Health Research Institute of the City of New York, New York: $\phi 6$ mutants that synthesize temperature sensitive P12—A protein necessary for phage membrane assembly.
- G. BREWER, Department of Microbiology, University of Southern California School of Medicine, Los Angeles: Assembly of the membrane of bacteriophage PM2.
- M. INOUE, J. DIRIENZO, T. MAEDA, K. NAKAMURA, R. PIRTLE, I. PIRTLE, R. MOVVA, and E. WURTZEL, Department of Biochemistry, State University of New York, Stony Brook: Biosynthesis and assembly of outer membrane proteins of *E. coli*.
- G. FERRO-LUZZI AMES, Department of Biochemistry, University of California, Berkeley: Interaction between membrane and periplasmic proteins is a requisite for transport.
- D. J. WAXMAN and J. L. STROMINGER, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Structure of the COOH-terminal hydrophobic region of a penicillin-sensitive bacterial D-alanine carboxypeptidase.
- M. ACITMAN, P. A. MANNING, R. THOMPSON, C. EDELBLUTH, and P. HERRLICH, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: *E. coli* cell envelope proteins encoded by the F sex factor.

- U. HENNING and C. P. HOLLENBERG, Max-Planck-Institute für Biologie, Tübingen, Germany: Cloning of structural genes for integral *E. coli* membrane proteins.
- C. LAZDUNSKI, J. M. PAGES, M. PIOVANT, S. VARENNE and D. BATY, Centre de Biochimie et Biologie Moléculaire du CNRS, Marseille, France: Synthesis and export of alkaline phosphatase across the cytoplasmic membrane of *E. coli*—Importance of the membrane fluidity, inhibition by procaine, effect of antibiotics of low polarity.
- V. ZOGBI and D. P. S. VERMA, Department of Biology, McGill University, Montreal, Canada: Homologies between the plasma membrane and the membrane envelope enclosing the bacteroids in soybean root nodules.
- J. P. ROSEBUSCH, Department of Microbiology, University of Basel, Switzerland: Reconstitution of matrix protein (porin) in single bilayer lipid membranes.

Session 6A: Glycosylation and Synthesis of Membrane Proteins of Enveloped Viruses. I.

Chairperson: S. KORNFELD, Washington University, St. Louis, Missouri

- S. KORNFELD, I. TABAS, M. MICHAEL, and A. CHAPMAN, Washington University School of Medicine, St. Louis, Missouri: Oligosaccharide processing during glycoprotein biosynthesis.
- P. ROBBINS, Massachusetts Institute of Technology, Cambridge: Synthesis and processing of cell surface glycoproteins.
- J. R. ETCHISON and D. F. SUMMERS, University of Utah Medical Center, Salt Lake City: Structure and processing of glycoprotein oligosaccharides in two lectin-resistant CHO cell lines.
- R. G. SPIRO and M. J. SPIRO, Harvard Medical School, Boston, Massachusetts: Role of glucose-containing oligosaccharide-lipids in glycoprotein biosynthesis.
- S. SCHLESINGER, R. GIBSON, and S. KORNFELD, Washington University Medical School, St. Louis, Missouri: The role of glycosylation of G protein in the replication of VSV.
- G. HART, J. HANOVER, U. CZICHI, and W. J. LENNARZ, Department of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Glycosylation of asparagine-linked glycoproteins—Regulation, role, and subcellular localization.
- D. S. BAILEY, Biology Department, McGill University, Montreal, Canada: Early events in membrane glycoprotein assembly—In vitro and in vivo synthesis of lipid intermediates.
- I. BOIME and M. BIELINSKA, Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri: Processing of the mannose-rich glycosylated α subunit of human chorionic gonadotropin in ascites tumor cell-free lysates.
- H. BEAUFAY, D. GODELAINE, M. WIBO, and J. PAIEMENT, Université de Louvain and International Institute of Cellular and Molecular Pathology, Brussels, Belgium: Functional and structural changes induced by GTP in stripped rough microsomes from rat liver.
- R. W. COMPANS, J. A. GRIFFIN, M. G. ROTH, and J. P. FITZPATRICK, University of Alabama Medical Center, Birmingham: Studies on the assembly of enveloped viruses.
- A. SCHEID, M.-C. HSU, C. D. RICHARDSON, and P. W. CHOPPIN, Rockefeller University, New York, New York: Studies on the structure and function of paramyxovirus membrane glycoproteins.
- L. KÄÄRIÄINEN, C.-H. VON BONSDORFF, K. HASHIMOTO, S. KERÄNEN, and J. SARASTE, Department of Virology, University of Helsinki, Finland: Transport of Semliki Forest virus envelope glycoproteins in infected cells.

Session 6B: Glycosylation and Synthesis of Membrane Proteins of Enveloped Viruses. II.

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

- R. ROTT, Institut für Virologie, Justus-Liebig-Universität, Giessen, Germany: Biological significance of proteolytic cleavage of the HA glycoprotein of influenza viruses.
- H.-D. KLENK, Institut für Virologie, Justus-Liebig Universität, Geissen, Germany: Defective processing of a viral membrane glycoprotein.
- M. J. SCHLESINGER and M. F. G. SCHMIDT, Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Evidence for covalent binding of fatty acids to viral membrane glycoproteins.

R. GREEN and E. RODRIGUEZ BOULAN, New York University School of Medicine, New York: The role of glycosylation in the biogenesis of epithelial polarity.

Session 7A: Golgi Apparatus—Role in Secretion and Membrane Dynamics. I.

Chairperson: M. FARQUHAR, Yale University, New Haven, Connecticut

M. G. FARQUHAR, Section of Cell Biology, Yale University School of Medicine, New Haven, Connecticut: Traffic flow and membrane specialization in the Golgi complex.

J. J. M. BERGERON* and B. I. POSNER,† Departments of *Anatomy and †Medicine, McGill University, Montreal, Canada: Role of the Golgi apparatus in the elaboration and recycling of the insulin and prolactin receptors of hepatocytes.

D. J. MORRÉ, Department of Medicinal Chemistry and Pharmacognosy and Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Sites of synthesis of membrane constituents within the endomembrane system of rat liver.

C. C. WIDNELL,** Y.-J. SCHNEIDER,† and A. TROUET,† †Université Catholique de Louvain and ICP, Bruxelles, Belgium; *University of Pittsburgh School of Medicine, Pennsylvania: Evidence for an internal pool of membrane in rat fibroblasts which exchanges with the cell surface.

Session 7B: Golgi Apparatus—Role in Secretion and Membrane Dynamics. II.

Chairperson: G. DALLNER, University of Stockholm, Sweden

G. DALLNER, Institute of Biochemistry, University of Stockholm, Sweden: Transport and glycosylation of microsomal membrane proteins in rat liver.

B. FLEISCHER, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Orientation and distribution of some components of rat liver Golgi membranes.

L. H. ROME, E. F. NEUFELD, and A. HASLIK, Genetics and Biochemistry Branch, NIAMDD, National Institutes of Health, Bethesda, Maryland: Biosynthesis and transport of lysosomal enzymes in cultured human fibroblasts.

M. GRATZL,* R. EKERDT,* and G. DAHL,† *Department of Physiological Chemistry, University of Saarland, Homburg, Germany; †Department of Physiology and Biophysics, University of Miami, Florida: Membrane fusion studied with isolated secretory vesicles.

E. J. PATZER and J. E. ROTHMAN, Department of Biochemistry, Stanford University Medical Center, California: Reversible dissociation of polypeptides from coated vesicles.

R. S. GAROFALO, J. K. C. KNOWLES, and B. H. SATIR, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Calmodulin and secretory mutants.

P. NOVICK and R. SCHEKMAN, Department of Biochemistry, University of California, Berkeley: Secretion and cell surface growth are blocked in a temperature sensitive mutant of *S. cerevisiae*.

D. DOYLE, H. BAUMANN, and R. WARREN, Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York: Metabolism of surface membrane proteins of rat hepatoma and primary liver cells in culture.

D. BOK, B. MATSUMOTO, and D. DEFOE, Department of Anatomy and Jules Stein Eye Institute, University of California, Los Angeles: Cytological events in the renewal of vertebrate rhodopsin.

A. MERCURIO and E. HOLTZMAN, Department of Biological Sciences, Columbia University, New York, New York: Agranular reticulum and membrane genesis in frog retinal photoreceptors.

J. H. KEEN, I. PASTAN, and M. C. WILLINGHAM, NCI, National Institutes of Health, Bethesda, Maryland: Clathrin-coated vesicles—Dissociation and reconstitution of the basket structure.

Session 8: Plasma Membranes—Structure and Biosynthesis

Chairperson: J. SINGER, University of California, San Diego

M.S. KRANGEL,* D. PIOUS,† and J. L. STROMINGER,* *The Biological Laboratories, Harvard University, Cambridge, Massachusetts; †Departments of Pediatrics and Genetics, University of Washington, Seattle: Assembly of HLA-A and HLA-B antigens in vivo.

- L.-N. L. CHAN * and J. DELENICK,† *Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston; †Department of Biology, Yale University, New Haven, Connecticut: Plasma membrane biogenesis in embryonic chick red blood cells.
- F. R. LANDSBERGER and L. D. ALTSTIEL, Rockefeller University, New York, New York: Structural changes in plasma membranes induced by enveloped viruses.
- J. D. JAMIESON,* M. P. SARRAS, JR.,* and M. F. MAYLIÉ-PFENNINGER,† *Yale University Medical School, New Haven, Connecticut; †Columbia University, New York, New York: Developmental regulation of expression of pancreatic cell surface glycoconjugates.
- F. RIEGER, Département de Biologie et Pathologie Neuromusculaires, INSERM, Paris, France: Regulation of biosynthesis and cellular localization of the multiple molecular forms of acetylcholinesterase—In vivo and in vitro synapse formation.
- R. B. KELLY, S. S. CARLSON, J. A. WAGNER, J. W. DEUTSCH, and R. VON WEDEL, Department of Biochemistry and Biophysics, University of California, San Francisco: The membranes of purified synaptic vesicles.
- K. H. PFENNINGER, R. SMALL, and M.-F. MAYLIÉ-PFENNINGER, Department of Anatomy, Columbia University, New York, New York: Insertion of membrane components during plasmalemmal growth in neurons.
- C. PERACCHIA, Department of Physiology, University of Rochester Medical Center, New York: Gap junction crystallinity and cell coupling.
- M. FINBOW, B. YANCEY, R. JOHNSON, and J. P. REVEL, Division of Biology, California Institute of Technology, Pasadena: Identification of the protein component of mammalian gap junctions.
- S. RAZIN, A. AMAR and S. ROTTEM, Biomembrane Research Laboratory, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Is the vertical disposition of mycoplasma membrane proteins affected by membrane fluidity?
- W. FRANKE,* J. KARTENBECK,* G. KROHNE,* J. STADLER,* C. FREUDENSTEIN,* and T. W. KEENAN,† *Division of Membrane Biology and Biochemistry, Experimental Pathology, German Cancer Research Center, Heidelberg; †Department of Animal Sciences, Purdue University, Lafayette, Indiana: Membrane-associated insoluble plaque structures.

Session 9: Plasma Membrane Differentiation

Chairperson: J. JAMIESON, Yale University, New Haven, Connecticut

- E. B. GRIEPP, W. J. DOLAN, E. S. ROBBINS, and D. D. SABATINI, Department of Cell Biology, New York University School of Medicine, New York: Studies on the development of tight junctions in epithelial cells.
- E. B. GRIEPP, N. L. SHINOWARA, A. S. HUDSPETH, and J.-P. REVEL, Division of Biology, California Institute of Technology, Pasadena: Gap junction formation in beating embryonic heart cells.
- E. RODRIGUEZ BOULAN and M. PENDERGAST, New York University School of Medicine, New York: Polarized distribution of viral envelope proteins in infected epithelial cells.
- L. LOUVAR, European Molecular Biology Laboratory, Heidelberg, Germany: Immunofluorescent studies on the polarity of the epithelial cells.
- J. BRUNNER,* H.-P. HAURI,† and G. SEMENZA,* *Department of Biochemistry, Swiss Institute of Technology, Zurich; †Massachusetts General Hospital, Boston: Sucrase-isomaltase (SI), a "stalked" intrinsic plasma membrane protein of intestinal brush borders (BB), is anchored to the membrane via a hydrophobic segment of the N-terminal region of the I-subunit—Biosynthetic implications.

Session 10: Biogenesis of Chloroplasts

Chairperson: N. H. CHUA, Rockefeller University, New York, New York

- A. GROSSMAN, S. BARTLETT, G. SCHMIDT, and N.-H. CHUA, Rockefeller University, New York, New York: Post-translational transport of a cytoplasmically-synthesized protein into chloroplasts.
- G. W. SCHMIDT,* A. R. CASHMORE,* †M. K. BROADHURST,† S. BARTLETT,* and N.-H. CHUA,* *Rockefeller University, New York, New York; †Applied Biochemistry Division, DSIR, Palmerston North, New Zealand: Biosynthetic precursors of integral chloroplast membrane proteins.
- M. EDELMAN, A. REISELD, H. HOFFMAN, and Z. E. KAHANA, Department of Plant Genetics, Weizmann Institute of Science, Rehovot, Israel: Synthesis, processing and control of P-32000, the major membrane protein translated within the chloroplast.

- C. A. PRICE, A. WATANABE, and R. E. ZIELINSKI, Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Synthesis and assembly of chloroplast membrane proteins—Cytochrome *b559* and coupling factor 1.
- L. BOGORAD, A. GREBANIER, G. LINK, L. MCINTOSH, and K. STEINBACK, Department of Biology, Harvard University, Cambridge, Massachusetts: The photogene for a maize chloroplast membrane protein.
- L. A. STAHELIN, D. P. CARTER, A. McDONNELL, and S. SPATH, Department of Molecular Cellular, and Developmental Biology, University of Colorado, Boulder: In vivo and in vitro manipulation of the supramolecular organization of chloroplast membranes.
- K. R. MILLER, J. R. ELLENSON, R. A. CUSHMAN, and G. F. COX, Harvard University, Cambridge, Massachusetts: A study of individual components of the photosynthetic membrane in model systems.
- J. K. HOOBER, Department of Biochemistry, Temple University Medical School, Philadelphia, Pennsylvania: The major polypeptides of *Chlamydomonas reinhardtii* thylakoid membranes—Properties and control of synthesis.
- C. J. ARNTZEN, K. LETO, J. MULLEN, and K. STEINBACK, Department of Botany, University of Illinois, Urbana: Regulation of stoichiometry of chloroplast membrane constituents.
- R. K. TOGASAKI and M. O. HUDOCK, Department of Biology, Indiana University, Bloomington: Analysis of photosynthetic competence during the early zygote maturation period in *Chlamydomonas reinhardtii*.
- M. M. MARGULIES and J. W. WEISTROP, Radiation Biology Laboratory, Smithsonian Institution, Rockville, Maryland: A sub-thylakoid fraction containing ribosomes.
- I. OHAD,* J. GERSHONI,* S. SHOCHAT,* and S. MALKIN,† *Department of Biological Chemistry, Hebrew University of Jerusalem: †Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel: Stepwise synthesis and assembly of photosynthetic reaction centers and chlorophyll-protein complexes in algae.

Session 11: Biogenesis of Mitochondria and Peroxisomes

Chairperson: R. O. POYTON, University of Connecticut Health Center, Farmington

- R. O. POYTON, C. GEORGE-NASCIMENTO, K. SEVARINO, AND S. POWER, University of Connecticut Health Center, Farmington: Synthesis and assembly of yeast cytochrome *c* oxidase, an oligomeric membrane protein.
- M.-L. MACCECCHINI, University of Basel Biocenter, Switzerland: Energy-dependent import of proteins into mitochondria.
- W. NEUPERT, M. A. HARMEY, E. M. NEHER, and R. ZIMMERMANN, Physiologisch-Chemisches Institut der Universität, Göttingen, Germany: Precursors of mitochondrial proteins.
- S. MATSUURA,* T. MORIMOTO,* D. SABATINI,* and E. MARGOLIASH,† *Department of Cell Biology, New York University School of Medicine, New York, †Department of Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois: Synthesis of cytochrome *c* in rat liver free polysomes and in vitro transfer into mitochondria.
- I. Z. ADES and R. A. BUTOW, Biochemistry Department, University of Texas Health Science Center, Dallas: Fate of products synthesized by the mitochondria-bound cytoplasmic polysomes in yeast.
- H. R. MAHLER,* D. HANSON,* J. JOHNSON,* P. S. PERLMAN,† and N. ALEXANDER,† *Department of Chemistry, Indiana University, Bloomington; †Department of Genetics, Ohio State University, Columbus: Mitochondrial biogenesis—Organization, expression and regulation of mitochondrial genes.
- B. M. GOLDMAN and G. BLOBEL, Rockefeller University, New York, New York: Biogenesis of peroxisomes—Intracellular site of synthesis of catalase and uricase.
- P. B. LAZAROW, Rockefeller University, New York, New York: Biogenesis of peroxisomes in rat liver.

Summary: G. BLOBEL, Rockefeller University, New York, New York

THE MOLECULAR BIOLOGY OF YEAST, August 14 – August 19

arranged by

James Broach, James B. Hicks, Amar J. S. Klar, and Jeffrey N. Strathern, Cold Spring Harbor Laboratory

Session 1: Mating Type

Chairperson: I. HERSKOWITZ, University of Oregon, Eugene

- H. LIAO and J. THORNER, Department of Microbiology and Immunology, University of California, Berkeley: The mating pheromone α -factor inhibits membrane-bound adenylyl cyclase in *S. cerevisiae*.
- R. BETZ and W. DUNTZE, Institut für Physiologische Chemie, Ruhr-Universität Bochum, Germany: Isolation of mating hormones produced by *S. cerevisiae* strain X2180-1A.
- G. F. SPRAGUE, JR., J. RINE, and I. HERSKOWITZ, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: Structure and function of the mating type locus.
- J. RINE and I. HERSKOWITZ, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: Regulation of cryptic mating type loci expression.
- A. J. S. KLAR, J. HICKS, J. STRATHERN, and J. BROACH, Cold Spring Harbor Laboratory, New York: Interconversion of yeast mating types by mobile genes.
- J. E. HABER, D. W. MASIOLI, W. T. SAVAGE, and L. B. ROWE, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Mechanism of mating type gene transpositions in yeast.
- R. E. MALONE and R. E. ESPOSITO, Chairman of Biology and Committee on Genetics, University of Chicago, Illinois: The *rad52* gene product is required for mating type switching in *S. cerevisiae*.
- R. EGEL, Institute of Genetics, Copenhagen, Denmark: Reduced rates of mating-type switching and site-specific mitotic recombination in homothallic fission yeast.
- M. CRANDALL, School of Biological Sciences, University of Kentucky, Lexington: Induction of ascosporeogenesis in the yeast *Hansenula wingei* by maltose and starvation for nitrogen.

Session 2: Cell Cycle, Sporulation, and Morphogenesis

Chairperson: H. O. HALVORSON, Brandeis University, Waltham, Massachusetts

- B. SLOAT, R. WATER, A. ADAMS, K. COLEMAN, and J. PRINGLE, Division of Biological Sciences, University of Michigan, Ann Arbor: Cellular morphogenesis in the cell cycle of *S. cerevisiae*.
- R. SCHEKMAN, P. NOVICK, C. FIELD, S. FRIED, and S. FERRO, Department of Biochemistry, University of California, Berkeley: Secretion and cell surface growth are blocked in a temperature-sensitive mutant of *S. cerevisiae*.
- G. P. SAMOKHIN, L. V. LIZLOVA, M. I. TITOV, and V. N. SMIRNOV, USSR Research Center of Cardiology, Moscow: The effect of α -factor on transition probability in yeast.
- A. KLAR, Cold Spring Harbor Laboratory, New York: Mating type functions for meiosis and sporulation act through cytoplasm.
- S. DUTCHER and L. HARTWELL, Department of Genetics, University of Washington, Seattle: Cytoplasmic location of *kar1* gene product.
- D. SCHILD* and B. BYERS,† *Department of Biophysics and Medical Physics, University of California, Berkeley; †Department of Genetics, University of Washington, Seattle: Diploid spore formation and other meiotic effects of *cdc5* and *cdc14* in *S. cerevisiae*.
- D. SCHILD,* H. N. ANANTHASWAMY,† and R. K. MORTIMER,* *Department of Biophysics and Medical Physics, University of California, Berkeley; †Cancer Biology Program, Frederick Cancer Research Center, Maryland: An endomitotic effect of *cdc31-2*.
- S. KLAPHOLZ,* and R. E. ESPOSITO,** *Committee on Genetics and †Department of Biology, University of Chicago, Illinois: Genetic analysis of two meiotic mutants that undergo a single nuclear division and produce asci containing two diploid spores.
- G. S. ZUBENKO and E. W. JONES, Carnegie-Mellon University, Mellon Institute, Pittsburgh, Pennsylvania: Protein degradation and sporulation in proteinase deficient mutants of *S. cerevisiae*.
- C. FIELD and R. SCHEKMAN, Department of Biochemistry, University of California, Berkeley: Localized secretion of acid phosphatase reflects the pattern of yeast cell surface growth.

Session 3: Poster Session

- F. HILGER,* R. CONTOPOULOU,† and R. MORTIMER,† *Faculté des Sciences Agronomiques de L'Etat, Gembloux, Belgium; †Department of Biophysics and Medical Physics, University of California, Berkeley:

- S. cerevisiae* has seventeen chromosomes again — Mapping of *arg1* and *arg8* on the left arm of chromosome XV.
- D. CAMPBELL, J. DOCTOR, J. FEUERSANGER, and M. DOOLITTLE, Department of Biology, Holy Cross College, Worcester, Massachusetts: Aneuploid tolerance and stability in yeast.
- R. K. CHAN and C. A. OTTE, Department of Microbiology, University of Cincinnati College of Medicine, Ohio: Isolation and characterization of yeast mutants supersensitive to α factor.
- E. CIEJEK and J. THORNER, Department of Microbiology and Immunology, University of California, Berkeley: Recovery of *S. cerevisiae* a cells from G1 arrest by α -factor pheromone requires endopeptidase action.
- Y. JONES-BROWN and J. THORNER, Department of Microbiology and Immunology, University of California, Berkeley: α -Factor biosynthesis—Immunological detection of mutants deficient in the elaboration of extracellular pheromone.
- L. M. MELNICK and J. BLAMIRE, Department of Biology, Brooklyn College, New York: A gene involved in mating type regulated sporulation in *S. cerevisiae*.
- I. EDELMAN,* M. CULBERTSON,* and J. B. HICKS,† *Laboratories of Molecular Biology and Genetics, University of Wisconsin, Madison; †Cold Spring Harbor Laboratory, New York: Heterothallic mating type interconversion.
- J. BLAMIRE and L. M. MELNICK, Department of Biology, Brooklyn College, New York: Analysis of the results of homozygous *MAT α* rare mating events that produce modifications of chromosome III.
- J. H. MCCUSKER and J. E. HABER, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Unstable intermediates of chromosome loss induced in heterothallic and homothallic strains.
- J. E. HABER, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Control of the direction of mating type conversions by mating phenotype.
- L. C. BLAIR and I. HERSKOWITZ, Institute of Molecular Biology, University of Oregon, Eugene: Isolation of *hma* and *hm α* alleles from *HO HMa HM α* homothallic spores.
- P. KUSHNER, L. BLAIR, and I. HERSKOWITZ, Institute of Molecular Biology, University of Oregon, Eugene: Mutant alleles of *MAT α* produced by switching of *HM α* mutants.
- G. KAWASAKI and D. HAWTHORNE, Department of Genetics, University of Washington, Seattle: Mapping *HO* and a translocation of mating type by chromosome loss induced in yeast cell cycle mutants.
- W. L. WHELAN, R. M. PARTRIDGE, and P. T. MAGEE, Michigan State University, East Lansing: Evidence for heterozygosity and mitotic segregation in *Candida albicans*.
- P. L. SKATRUD, E. J. KOT, and J. R. HELBERT, Miller Brewing Company, Milwaukee, Wisconsin: Fusion of brewer's yeast.
- G. KAWASAKI, Department of Genetics, University of Washington, Seattle: The *cdc19* mutation of *S. cerevisiae* is a temperature-sensitive defect for the glycolytic enzyme, pyruvate kinase.
- J. PRINGLE, S. PARIS, A. TSCHUNKO, and A. ADAMS, Division of Biological Sciences, University of Michigan, Ann Arbor: Isolation and characterization of temperature-sensitive cell cycle mutants of *S. cerevisiae*.
- R. A. SINGER, D. BEDARD, and G. C. JOHNSTON, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada: Nalidixic acid causes a transient G1 arrest in the yeast *S. cerevisiae*.
- G. C. JOHNSTON and R. A. SINGER, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada: Ribosomal RNA transcription and progression through G1 in the yeast *S. cerevisiae*.
- K. NASMYTH, S. REED, and B. HALL, Department of Genetics, University of Washington, Seattle: The molecular cloning of a yeast cell cycle "start" gene by transformation—Isolation by genetic complementation and identification by genetic mapping.
- J. BOSSINGER,* N. -H. XUONG,† ‡ and E. P. GEIDUSCHEK,* Departments of *Biology, †Chemistry, and ‡Physics, University of California, San Diego: Two dimensional protein patterns of yeast cell division cycle mutants.
- D. G. KAY, R. A. SINGER, and G. C. JOHNSTON, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada: Ornithine decarboxylase activity as a marker of cell cycle arrest in the yeast *S. cerevisiae*.
- E. KRAIG and J. E. HABER, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: A comparison of protein synthesis in vegetative and sporulating yeast.
- N. J. PEARSON and J. E. HABER, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Regulation of ribosomal protein synthesis during sporulation.
- M. PASTORCIC, J. E. GOLIN, and M. S. ESPOSITO, Department of Biology and Committee on Genetics, University of Chicago, Illinois: Spontaneous mitotic recombination in *cdc9-1/cdc9-1* diploids of *S. cerevisiae*.

- K. WOLF, Genetisches Institut der Universität München, Germany: Uniparental inheritance is promoted by delayed cell division of the zygote in the fission yeast *Schizosaccharomyces pombe*.
- E. R. UNGER,* B. TUNG,* B. LEVIN,[†] and G. S. GETZ,* Department of *Pathology and [†]Medicine, University of Chicago, Illinois: Influence of fatty acyl chain stereoisomerism on the respiratory activity of an unsaturated fatty acid auxotroph of *S. cerevisiae*.
- N. NELSON and T. MASON, Biozentrum, University of Basel, Switzerland: The cytoplasmically-made cytochrome c oxidase subunits V and VI each accumulate as larger forms under conditions of mitochondrial ATP-depletion.
- R. MOROSOLI and C. V. LUSENA, Division of Biological Sciences, National Research Council of Canada, Ottawa: Endonuclease M from yeast mitochondria.
- R. MATNER and F. SHERMAN, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: The presence of an apo-protein of cytochrome c in a cytochrome c deficient mutant of yeast.
- B. C. HYMAN and T. W. JAMES, Department of Biology, University of California, Los Angeles: Visualization and analysis of mitochondrial DNA in *Wickerhamia iluorescens*.
- S. F. COTTRELL, Department of Biology, Brooklyn College, New York: Variations in mitochondrial DNA concentration as a function of different growth conditions in *S. cerevisiae*.
- S. ULASZEWSKI, J. KOŁODYŃSKI, T. M. LACHOWICZ, and Z. KOTYLAK, Department of Genetics, Institute of Microbiology, Wrocław, Poland: Attempts of localization mit⁻ mutations responsible for resistance to visible light on mitochondrial DNA in baker's yeast.
- M. MISIEWICZ, T. M. LACHOWICZ, and Z. KOTYLAK, Department of Genetics, Institute of Microbiology, Wrocław, Poland: The map of deletion mutants in the OX13 region.
- K. WAKABAYASHI, Faculty of Medicine, University of Tokyo, Japan: Inversions in mitochondrial erythromycin resistance gene.
- G. GROSCH, A. HAID, C. SCHMELZER, R. J. SCHWEYEN, and F. KAUDEWITZ, Genetisches Institut der Universität München, Germany: The COB/BOX region on mit DNA. II. Genetic/physical map and transcripts.
- D. K. HANSON,* H. R. MAHLER,* J. A. JOHNSON,* and M. CLAISSE,[†] *Department of Chemistry, and Molecular, Cellular and Developmental Biology Program, Indiana University, Bloomington; [†]Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Nature and origin of novel polypeptides accumulating in intron mutants in the COB-BOX region of the mitochondrial genome.
- C. JACQ, J. LAZOWSKA, L. GIRALDINO-ROBAINA, and P. P. SLONIMSKI, Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: Physical studies of the mutations in the yeast mosaic cytochrome *b* gene.
- A. HALBREICH, P. PAJOT, M. FOUCHER, C. GRANDCHAMP, and P. P. SLONIMSKI, Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: An analysis of mtRNA species from yeast mitochondrial petite mutants which complement *box3* mutants.
- G. DUJARDIN, O. GROUDINSKY, P. PAJOT, and P. P. SLONIMSKI, Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: Specific control circuits within the mitochondrial genome and between the mitochondrial and nuclear genomes.
- H. BLANC, B. DUJON, C. GRANDCHAMP, and P. P. SLONIMSKI, Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: Hyper-suppressive "petites" replicate preferentially their mit DNA and share a common sequence.
- C. JACQ,* F. CARON,* and J. ROUVIERE-YANIV,[†] *Centre de Genetique Moleculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette; [†]Institut Pasteur, Paris, France: Characterization of a histone-like protein extracted from yeast mitochondria.
- J. KREIKE,* H. BECHMANN,[†] F. J. VAN HEMERT,* P. H. BOER,* R. J. SCHWEYEN,[†] F. KAUDEWITZ,[†] and G. S. P. GROOT,* *Department of Molecular Biology, University of Amsterdam, The Netherlands; [†]Department of Genetics, University of Munich, Germany: The identification of cytochrome *b* as a mitochondrial gene product in yeast.
- P. H. BOER, E. ROSENDAAL, G. J. B. VAN OMMEN, M. DE HAAN, and L. A. GRIVELL, Laboratory of Biochemistry, University of Amsterdam, The Netherlands: The gene for cytochrome *b* on yeast mtDNA.

Session 4: Mitochondria. I.

Chairperson: P. PERLMAN, Ohio State University, Columbus

- N. J. ALEXANDER,* D. K. HANSON,[†] H. R. MAHLER,[†] and P. S. PERLMAN,* *Ohio State University, Columbus;

- [†]Indiana University, Bloomington: Further studies of the mosaic structure of the mitochondrial *cob-box* gene in *S. cerevisiae*.
- G. J. B. VAN OMMEN, L. A. GRIVELL, and G. S. P. GROOT, Laboratory of Biochemistry, University of Amsterdam, The Netherlands: Mitochondrial transcription and splicing in different wild-type and mutant yeast strains.
- M. SOLIOZ, Biocenter, University of Basel, Switzerland: Effect of intron and exon mutations in the mitochondrial cytochrome *b* gene on the cytochrome *b* apoprotein.
- A. LAMOUREUX,* A. DE KOCHKO,[†] P. BAJOT,* A. M. COLSON,[†] and P. P. SLONIMSKI,* *Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France; [†]Laboratoire d'Enzymologie, Université de Louvain, Belgium: Complementation between exons and introns within a mosaic mitochondrial gene—A tool to investigate the mechanism of splicing.
- R. J. SCHWEYEN, H. BECHMANN, A. HAID, G. GROSCH, and F. KAUEWITZ, Genetisches Institut der Universität München, Germany: The COB/BOX region on mitDNA—Mutants and gene products.
- T. D. FOX, Biozentrum, University of Basel, Switzerland: DNA sequence analysis of mitochondrial mutations at the *oxi 1* locus.
- R. M. SYNENKI, S. MERTEN, T. CHRISTIANSEN, J. LOCKER, and M. RABINOWITZ, Franklin McClean Memorial Research Institute, University of Chicago, Illinois: Processing and mapping of mitochondrial transcripts in yeast.
- R. D. VINCENT,* P. S. PERLMAN,* R. L. STRAUSBERG,[†] and R. A. BUTOW,[†] *Developmental Biology Program and Department of Genetics, Ohio State University, Columbus; [†]Biochemistry Department, University of Texas Health Science Center, Dallas: Physical mapping of determinants affecting the size of the *var1* protein.
- H. P. ZASSENHAUS and P. S. PERLMAN, Developmental Biology Program and Department of Genetics, Ohio State University, Columbus: Respiration deficient mutants in the *var1* region.
- L. A. M. HENSGENS,* A. C. ARNBERG,[†] L. A. GRIVELL,* A. SONNENBERG,* and G. J. B. VAN OMMEN,* *Laboratory of Biochemistry, University of Amsterdam; [†]Biochemical Laboratory, State University at Groningen, The Netherlands: The expression of the mitochondrial gene for the ATPase subunit 9.
- L. A. GRIVELL,* A. C. ARNBERG,[†] G. J. B. VAN OMMEN,* E. F. J. VAN BRUGGEN,[†] and P. BORST,* *Laboratory of Biochemistry, University of Amsterdam; [†]Biochemical Laboratory, State University at Groningen, The Netherlands: Some yeast mitochondrial RNAs are circular.
- E. P. SENA, Case Western Reserve University, Cleveland, Ohio; Molecular basis of mitochondrial transmissional types.

Session 5: Mitochondria. II.

Chairperson: P. PERLMAN, Ohio State University, Columbus

- B. DUJON, The Biological Laboratories, Cambridge, Massachusetts: DNA sequence of the ω and ribosomal region of the yeast mitochondrial genome.
- H. F. TABAK, J. L. BOS, and G. J. B. VAN OMMEN, Laboratory of Biochemistry, University of Amsterdam, The Netherlands: Studies on the yeast mitochondrial rRNA genes.
- N. C. MARTIN and C. SIGURDSON, Department of Biochemistry, University of Minnesota, Minneapolis: Studies on yeast mitochondrial tRNA genes and transcripts.
- M. MONNEROT* and M. WESOLOWSKI,[†] *Laboratoire de Biologie Générale, Université d'Orsay; [†]Institut Curie, Orsay, France: Genetic and physical maps of tRNA genes of yeast mitochondria.
- R. D. TODD, P. C. MCADA, and M. G. DOUGLAS, Department of Biochemistry, University of Texas Health Science Center, San Antonio: A nuclear mutation altering the assembly of the yeast mitochondrial inner membrane.

Session 6: Extrachromosomal Elements

Chairperson: W. FANGMAN, University of Seattle, Washington

- R. B. WICKNER, NIAMDD, National Institutes of Health, Bethesda, Maryland: Control of exclusion of the K² killer plasmid.
- J. R. BROACH, J. N. STRATHERN, and J. B. HICKS, Cold Spring Harbor Laboratory, New York: Analysis of 2 μ circle replication by transformation in yeast.

- J. B. MCNEIL, R. K. STORMS, P. S. KHANDEKAR, and J. D. FRIESEN, Department of Biology, York University, Downsview, Ontario, Canada: Transformation of yeast with chimeric plasmids containing different portions of the yeast plasmid 2 micron circle.
- D. M. LIVINGSTON, Department of Biochemistry, University of Minnesota, Minneapolis: Isolation of a condensed, intracellular form of the 2 μ m DNA plasmid.
- S. M. JAZWINSKI, M. TAKETO, and G. M. EELMAN, Department of Developmental and Molecular Biology, Rockefeller University, New York, New York: Replication in vitro of the 2 μ m DNA plasmid of yeast.
- L. BELL and B. BYERS, Department of Genetics, University of Washington, Seattle: Detection of crossed-strand exchange forms in yeast 2 μ m DNA during meiosis.

Session 7: Poster Session

- H. BUSSEY and D. SAVILLE, Department of Biology, McGill University, Montreal, Quebec: Binding of radioactive killer toxin to cell wall receptors of sensitive yeast.
- K. A. BOSTIAN, J. A. STURGEON, and D. J. TIPPER, Brandeis University, Waltham, Massachusetts, and University of Massachusetts Medical Center, Worcester: Establishment of a functional relationship within the segmented dsRNA virus-like-particle genome of *S. cerevisiae*.
- J. D. WELSH and M. J. LEIBOWITZ, Department of Microbiology, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway: Transcription of yeast double-stranded RNA in vitro.
- W. P. KANF and J. BRUENNI, Division of Cell and Molecular Biology, State University of New York, Buffalo: Structure and evolution of defective interfering double stranded RNAs of virus-like particles of yeast.
- S. G. OLIVER and J. J. CLARE, Biological Laboratory, University of Kent at Canterbury, United Kingdom: The effect of starvation on the synthesis and maintenance of yeast double-stranded RNAs.
- L. DEL GIUDICE* and K. WOLF† *International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, Naples, Italy; †Genetisches Institut der Universität München, Germany: 2 μ m Covalently closed non-mitochondrial circular DNA in the petite-negative yeast *Schizosaccharomyces pombe*.
- H. GOLD and C. SHALITIN, Technion-Israel Institute of Technology, Haifa: Transcription of 2 μ m circular DNA from *S. cerevisiae*.
- A. BRUNNER-L., Centro de Investigaciones Universidad Nacional Autonoma de Mexico, en Fisiologia Celular, Mexico City: Chromosomal and extrachromosomal inheritance to antimycin resistance in the petite-negative yeast *Kluyveromyces lactis*.
- M. F. TUTE, B. S. COX, and M. J. DOBSON, Botany School, Oxford, United Kingdom: Induced mutation of the cytoplasmically-inherited [psi+] factor and other plasmids of *S. cerevisiae*.
- R. G. BUCKHOLZ and B. G. ADAMS, Department of Microbiology, University of Hawaii, Honolulu: Control and processing of yeast α -galactosidase.
- P. ALEXANDER and B. G. ADAMS, Department of Microbiology, University of Hawaii, Honolulu: Isolation and characterization of α -galactosidase mutants of *S. cerevisiae*.
- H. FEDEROFF,* R. ECCLESHALL,* R. NEEDLEMAN,† and J. MARMUR,* *Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York; †Department of Biochemistry, Wayne State University, Detroit, Michigan: Synthesis of maltase by *S. carlsbergensis*.
- A. P. BOLLON, Department of Biochemistry, University of Texas Health Science Center, Dallas: Analysis of the *ilv 1-OP* control region.
- M. L. GREENBERG and S. A. HENRY, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Isolation of a regulatory mutant constitutive for inositol-1-phosphate synthase.
- B. SHICKER and S. A. HENRY, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Yeast mutant defective in the conversion of phosphatidylethanolamine to phosphatylcholine.
- K. D. ATKINSON,* B. JENSEN,* A. I. KOLAT,† E. M. STORM,† S. A. HENRY,† and S. FOGEL,* *Department of Genetics, University of California, Berkeley; †Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Yeast mutants defective in phosphatidylserine synthesis.
- D. GRIMAL and R. LABBE-BOIS, Laboratoire Biochimie des Porphyrines, Université Paris VII, France: Mutants of *S. cerevisiae* deficient in heme synthesis.
- A. P. MITCHELL and B. MAGASANIK, Department of Biology, Massachusetts Institute of Technology, Cambridge: Mutations affecting glutamine synthetase regulation.
- A. PIÉRARD, F. MESSENGUY, A. FELLER, and F. HILGER, Laboratoire de Microbiologie, Université Libre de Bruxelles; Institut de Recherches du CERIA, Brussels; Chaire de Microbiologie, Faculté des Sciences Agronomiques de l'Etat, Gembloux, Belgium: Regulation of the synthesis of the arginine pathway carbamoylphosphate synthase of *S. cerevisiae*—General and specific control mechanisms.

- J. MCKELVEY and T. G. COOPER, University of Pittsburgh, Pennsylvania: Oxalurate transport and its relation to nitrogen repression.
- V. TUROSCY and T. G. COOPER, University of Pittsburgh, Pennsylvania: Allantoic acid transport in *S. cerevisiae*.
- C. J. PANCHAL and G. G. STEWART, Labatt Brewing Company, London, Ontario, Canada: Uptake of monosaccharides by a brewing yeast strain of *Saccharomyces uvarum* (*carlsbergensis*).
- M. BORBOLLA and A. PEÑA, Departamento de Microbiología, Centro de Investigaciones en Fisiología Celular and Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, México City: Calcium uptake and binding by *S. cerevisiae*.
- E. DUBOIS and M. GRENSON, Laboratoire de Microbiologie, Université Libre de Bruxelles and Institut de Recherches du CERIA, Belgium: Multiplicity and regulation of methylamine/ammonia permeases in yeast.
- C. GAILLARDIN and H. HESLOT, Institut National de la Recherche Agronomique, Paris: Mutations affecting lysine specific or general control of saccharopine dehydrogenase in *Saccharomycopsis lipolytica*.
- T. G. COOPER and C. LAM, University of Pittsburgh, Pennsylvania: Structural organization of the *dur1,2* gene in yeast.
- R. SUMRADA, C. LAM, and T. G. COOPER, University of Pittsburgh, Pennsylvania: An inducible multifunctional protein degrades urea in yeast.
- T. ACHSTETTER and W. BUSCH, Institut für Biologie, Universität Tübingen, Germany: Multiple forms of two different esterases in laboratory strains of *S. cerevisiae*.
- W. A. FONZI and D. J. OPHEIM, Texas A & M University, College Station: Inducible alkaline phosphatase from *S. cerevisiae*.
- A. M. SCHWEINGRUBER and M. E. SCHWEINGRUBER, Institute for General Microbiology, Altenbergrain, Bern, Switzerland: Posttranslational control of acid phosphatase in *S. cerevisiae*.
- J. S. MKACZ, H. R. ONISHI, T. MIZUNAGA, and J. O. LAMPEN, Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Glycosylated enzymes of yeast—Continued synthesis of the protein moieties when glycosylation is blocked by tunicamycin.
- J. H. SCOTT and R. SCHEKMAN, Department of Biochemistry, University of California, Berkeley: Lyticase—Endoglucanase and protease activities that act together in yeast cell lysis.
- L.-L. S. CHIA and C. S. MCLAUGHLIN, Department of Biological Chemistry, University of California, Irvine: Effect of translation inhibitors on the synthesis of individual proteins—Discriminatory synthesis of proteins in the presence of initiation inhibitors as potential evidence for translational control in yeast.
- L. MCALLISTER and D. B. FINKELSTEIN, Department of Biochemistry, University of Texas Health Science Center, Dallas: Transient changes in translational patterns induced by heat shock of *S. cerevisiae*.
- M. J. MILLER,* N.-H. XUONG,†‡ and E. P. GEIDUSCHEK,* Departments of *Biology, †Physics, and ‡Chemistry, University of California, San Diego: Analysis of the response of protein synthesis to temperature shift in *S. cerevisiae*.
- P. FARABAUGH, C. ILGEN, and G. R. FINK, Department of Biochemistry, Cellular and Molecular Biology, Cornell University, Ithaca, New York: The structure of the *his4* region.
- S. HOLMBERG,* M. C. KIELLAND-BRANDT,* T. NILSSON-TILLGREN,† and J. G. L. PETERSEN,* *Department of Physiology, Carlsberg Laboratory, Copenhagen;† Institute of Genetics, University of Copenhagen, Denmark: Genetic transformation of *his4* deletion mutants in yeast without the involvement of bacterial plasmids.
- J. SCHWARTZ, W. LOOS, and H. GREER, Biology Department, Harvard University, Cambridge, Massachusetts: Regulatory mutations of the *his4* promoter and of genes involved in general control of amino acid biosynthesis.
- T. NILSSON-TILLGREN,* J. G. L. PETERSEN,† S. HOLMBERG,† and M. C. KIELLAND-BRANDT,† *Institute of Genetics, University of Copenhagen; †Department of Physiology, Carlsberg Laboratory, Copenhagen, Denmark: Stabilization by chromosomal integration of *his4* from an unstable yeast transformant.
- G. MCKNIGHT and F. SHERMAN, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: A deletion causing overproduction of yeast iso-2-cytochrome c.
- G. FAYE, K. TATCHELL, K. NASMYTH, and B. HALL, Department of Genetics, University of Washington, Seattle: Transformation of yeast by plasmids containing the iso-1-cytochrome c gene.
- R. S. ZITOMER,* D. L. MONTGOMERY,† D. L. NICHOLS,* and B. D. HALL,† *Department of Biological Sciences, State University at Albany, New York; †Genetics Department, University of Washington, Seattle: Transcriptional regulation of the yeast iso-1-cytochrome c gene.

- M. CRABEEL,* F. MESSENGUY,† F. LACROUTE,‡ and N. GLANSORFF,* *Vrije Universiteit Brussels, Belgium; †CERIA, Institut de Recherches, Brussels; ‡IBMC, Centre National de la Recherche Scientifique, Strasbourg, France: Cloning of *ar3*, the structural gene for ornithine carbamoyltransferase (OTCase) in *S. cerevisiae*.
- D. GALLWITZ, Institut für Physiologische Chemie, Universität Marburg, Germany: Molecular cloning of the actin gene from yeast (*S. cerevisiae*).
- V. M. WILLIAMSON, K. NASMYTH, J. BENNETZEN, B. D. HALL, and E. T. YOUNG, Department of Biochemistry and Department of Genetics, University of Washington, Seattle: Cloning the constitute alcohol dehydrogenase gene by transformation of yeast.
- P. SHALIT, K. LOUGHNEY, M. V. OLSON, and B. D. HALL, Department of Genetics, University of Washington, Seattle: Isolation and characterization of the DNA corresponding to the *CYC1-SUP4* interval in *S. cerevisiae*.

Session 8: Regulation. I.

Chairperson: F. LACROUTE, Centre National de la Recherche Scientifique, Strasbourg, France

- L. RODRIGUEZ, T. MIZUNAGA, V. L. MACKAY, J. S. TKACZ, and J. O. LAMPEN, Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: *SUC1* is a structural gene for invertase.
- D. PERLMAN and H. O. HALVORSON, Brandeis University, Waltham, Massachusetts: Regulation of the *SUC* genes in *S. cerevisiae*.
- M. CARLSON and D. BOTSTEIN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Genetic studies of sucrose utilization in yeast.
- K. A. BOSTIAN, R. C. LEE, and H. O. HALVORSON, Brandeis University, Waltham, Massachusetts: Genetic regulation of multiple mRNAs coding for repressible yeast acid phosphatase polypeptide.
- M. E. SCHWEINGRUBER and A. M. SCHWEINGRUBER, Institute for General Microbiology, Bern, Switzerland: Repressible acid phosphatase is developmentally modulated in *S. cerevisiae*.
- A. P. BOLLON, Department of Biochemistry, University of Texas Health Science Center, Dallas: Analysis of *ilv 1* functional domains.
- B. ERREDE,* F. SHERMAN,* E. DUBOIS, † J. DESCHEMPS,† and J. M. WIAME,† *Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York; †Institut de Recherches du CERIA, Brussels, Belgium: Concomitant control of a class of regulatory mutations and mating functions in *S. cerevisiae*.
- M. CIRIACY, Institut für Mikrobiologie der Technischen Hochschule, Darmstadt, Germany: Cis- and transacting regulatory elements of glucose-repressible alcohol dehydrogenase (ADHII) synthesis.
- L. BISSON and J. THORNER, Department of Microbiology and Immunology, University of California, Berkeley: Thymidylate synthetase from *S. cerevisiae* and its overproduction.

Session 9: Regulation. II.

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

- D. CHALEFF, S. ROEDER, and G. R. FINK, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: An insertion mutation in the *his4* region.
- T. P. ST. JOHN, S. SCHIERER, M. McDONNELL, and R. W. DAVIS, Department of Biochemistry, Stanford University School of Medicine, California: A transcriptional and genetic analysis of galactose inducible yeast genes.
- K. STRUHL and R. W. DAVIS, Department of Biochemistry, Stanford University School of Medicine, California: DNA sequence analysis of *his3* promoter mutants.
- M. R. CHEVALLIER, R. JUND, and F. LACROUTE, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France: Study on specific mRNAs produced in yeast by a chimeric yeast-*E. coli* plasmid.
- J. P. HOLLAND, G. THILL, and M. J. HOLLAND, Department of Biochemistry, University of Connecticut Health Center, Farmington: Structural analysis of repeated yeast glyceraldehyde 3-phosphate dehydrogenase and enolase genes.
- D. L. MONTGOMERY,* D. W. LEUNG,† M. SMITH,† G. FAYE,* P. SHALIT,* and B. D. HALL,* *Department of Genetics, University of Washington, Seattle; †Department of Biochemistry, University of British Columbia, Vancouver, Canada: Cloning and sequencing the iso-2-cytochrome c gene.
- J. I. STILES, C. HELMS, T. S. CARDILLO, and F. SHERMAN, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: A transposition of the *CYC1* gene causing overproduction of iso-1-cytochrome c in yeast.

- J. M. BOSS, M. D. DARROW, and R. S. ZITOMER, Department of Biological Sciences, State University of New York, Albany: Characterization of the iso-1-cytochrome c mRNA.
- T. DONAHUE and S. HENRY, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Biochemical and genetic analysis of inositol-1-phosphate synthase mutants of yeast.

Session: 10 Poster Session

- M. D. MENDENHALL, C. CUMMINS, and M. R. CULBERTSON, Laboratories of Genetics and Molecular Biology, University of Wisconsin, Madison: Properties of yeast cloning vectors.
- K. NASMYTH and B. HALL, Department of Genetics, University of Washington, Seattle: A simple method for constructing random DNA fragment libraries.
- R. NEEDLEMAN, J. COHEN, H. FEDEROFF, and J. MARMUR, Albert Einstein College of Medicine, Bronx, New York: Isolation of yeast genes by shotgun transformation of yeast—Cloning of the genes *ILV1* and *LYS1*.
- B. HOHN and A. HINNEN, Friedrich Miescher-Institut, Basel, Switzerland: Yeast "transfusion" with a cosmid type cloning vector.
- J. F. SCOTT, Molecular Biology Institute, University of California, Los Angeles: Construction and characterization of a high-copy-number circular yeast minichromosome containing the *trp1* gene.
- M. SALVATO, T. ETCHÉVERRY, and C. GUTHRIE, Department of Biochemistry and Biophysics, University of California, San Francisco: Isolation of the gene for the tRNA_{UCC} of *S. cerevisiae*.
- J. R. BROACH, Cold Spring Harbor Laboratory, New York: Identification and recovery of specific alleles by transformation in yeast.
- D. Y. THOMAS and A. P. JAMES, Division of Biological Sciences, National Research Council, Ottawa, Canada: Transformation of yeast with suppressor tRNA genes.
- J. OLAH and H. FELDMAN, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Germany: Analysis of tRNA genes in yeast.
- R. C. DICKSON, Department of Biochemistry, University of Kentucky, Lexington: Expression of a foreign eukaryotic gene in *S. cerevisiae*.
- H. FEDEROFF and H. FRIED, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Behavior of the mouse β -globin gene in *S. cerevisiae*.
- J. VAN DEN BERG,* J. D. BEGGS,[†] A. VAN OUYEN,* and C. WEISSMANN,* *Institut für Molekularbiologie 1, Universität Zürich, Switzerland; [†]Plant Breeding Institute, Cambridge, England: Transcription of a rabbit β -globin gene in yeast cells.
- J. COHEN,* R. NEEDLEMAN,[†] R. ECCLESHELL,* H. FEDEROFF,* and J. MARMUR,* *Albert Einstein College of Medicine, Bronx, New York; [†]Wayne State Medical School, Detroit, Michigan: Functional expression of an *E. coli* plasmid gene, *cam^r* in the yeast *S. cerevisiae*.
- A. KLAR, J. HICKS, M. DUBOW, J. BROACH, and J. STRATHERN, Cold Spring Harbor Laboratory, New York: Expression of bacterial genes in yeast.
- D. KASUNIC and S. R. KUSHNER, Department of Biochemistry, University of Georgia, Athens: Factors affecting the expression of yeast genes in *E. coli* K-12.
- A. STOTZ and P. PHILIPPEN, Biozentrum, University of Basel, Switzerland: Analysis of expression of cloned yeast suppressor tRNA genes in *E. coli* and *S. cerevisiae*.
- M. ROSE and D. BOTSTEIN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Expression of the yeast *ura3* gene on phage λ in *E. coli*.
- K. STRUHL and R. W. DAVIS, Department of Biochemistry, Stanford University School of Medicine, California: Expression and regulation of the *his3* gene in yeast and in *E. coli*.
- J. A. JAEINING, L. POLDER, and R. W. DAVIS, Department of Biochemistry, Stanford University, California: Selective interactions of *E. coli* RNA polymerase with the yeast *his* gene.
- D. COLBY, T. ETCHÉVERRY, and C. GUTHRIE, Department of Biochemistry and Biophysics, University of California, San Francisco: Nucleotide modification in ochre specific suppressor tRNAs.
- T. ETCHÉVERRY and C. GUTHRIE, Department of Biochemistry and Biophysics, University of California, San Francisco: Analysis of recessive lethality caused by an ochre suppressor tRNA in *S. cerevisiae*.
- D. D. WOMBLE, J. H. CRAMER, and R. H. ROWND, Laboratory of Molecular Biology and Department of Biochemistry, University of Wisconsin, Madison: Analysis of variant ribosomal DNA types in yeast.
- T. J. ZAMB and T. D. PETES, Department of Microbiology, University of Chicago, Illinois: Characterization of junction fragments in *Saccharomyces*.

- S. SMOLIK-UTLAUT and T. PETES, Department of Microbiology, University of Chicago, Illinois: A comparison of the restriction patterns for two forms of ribosomal DNA in *S. cerevisiae*.
- R. ROTHSTEIN, Department of Microbiology, New Jersey School of Medicine, Newark: Deletions of *SUP4* are promoted by δ sequences.
- H. FRIED and H. FEDEROFF, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York: The reverse transcription of yeast rRNA.
- G. E. WILLICK,* M. YAGUCHI,* and R. N. MAZAR,† *Division of Biological Sciences, National Research Council, Ottawa, Canada; †Department of Botany and Genetics, University of Guelph, Canada: The 5S RNA protein complex from *S. cerevisiae*.
- A. RUET, F. IBORRA, J. HUET, M. SAWADOGO, A. SENTENAC, and P. FROMAGEOT, Commissariat à l'Énergie Atomique, Centre d'Études Nucléaires de Saclay, Département de Biologie, Service de Biochimie, Gif-sur-Yvette, France: Subunit function in yeast RNA polymerases.
- J. WALLIS,* K. E. DIAMOND,* L. HEREFORD,† and M. GRUNSTEIN,* *Molecular Biology Institute and Department of Biology, University of California, Los Angeles; †Rosenstiel Institute, Brandeis University, Waltham, Massachusetts: Histone gene activity in *S. cerevisiae*.
- A. SINGH, Department of Biochemistry, University of Wisconsin, Madison: Hygromycin B resistant mutants of *S. cerevisiae*.
- J. PLESSET, M. TUIE, C. S. MCLAUGHLIN, and K. MOLDAVE, Department of Biological Chemistry, University of California, Irvine: Characterization of a yeast in vitro protein synthesizing system.
- M. TUIE, J. PLESSET, C. S. MCLAUGHLIN and K. MOLDAVE, Department of Biological Chemistry, University of California, Irvine: Faithful translation of natural mRNAs in a yeast cell-free system.
- P. BAUM, R. KUNISAWA, and J. THORNER, Department of Microbiology and Immunology, University of California, Berkeley: A new method for the preparation of nuclei from *S. cerevisiae*.
- G. IDE and C. SAUNDERS, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: Rapid isolation of yeast nuclei.
- H. KOCH* and J. KIEFER,† *Max-Planck-Institut für Biologie, Tübingen; †Strahlencentrum/Biophysik, Giessen, Germany: Transcript sizes in yeast as determined by UV-induced premature termination of transcription.
- H. DAVIS,* B. CROSBY,† L. PRAKASH,† and D. HINKLE,* Departments of *Biology and †Radiation Biology and Biophysics, University of Rochester, New York: Purification and characterization of a uracil-DNA glycosylase from yeast.
- C. MOORE, Department of Biology and Department of Radiation Biology and Biophysics, University of Rochester, New York: DNA damage and cell-killing by phleomycin and bleomycin.
- B. MONTELOONE,* L. PRAKASH,† and S. PRAKASH,* Departments of *Biology and †Radiation Biology and Biophysics, University of Rochester, New York: Effects of the *mms21* gene on recombination and spontaneous and induced mutations in *S. cerevisiae*.
- D. LOHR,* K. TATCHELL,† and J. MARDIAN,‡ *Department of Chemistry, Arizona State University, Tempe; †Department of Genetics, University of Washington, Seattle; ‡Biology Division, Oak Ridge National Laboratory, Tennessee: The capability of yeast histone to reconstitute a native core particle.
- U. WINTERSBERGER and D. BLUTSCH, Institute for Cancer Research, University of Vienna, Austria: Identification of a multifunctional DNA binding protein from yeast?
- S. G. LABONNE and L. B. DUMAS, Department of Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois: A DNA helix-destabilizing protein from baker's yeast.
- M. F. TUIE, L. DICAPRIO, B. S. COX, and B. FUTCHER, Botany School, Oxford, England: Suppression of alleles of *rad 6*, 5-phase repair—The theory of mutation induction.
- R. J. REYNOLDS and E. C. FRIEDBERG, Department of Pathology, Stanford University, California: UV-induced, endogenous single-strand breaks in excision deficient *rad* mutants of *S. cerevisiae*.

Session 11: Regulation. III.

Chairperson: E. JONES, Carnegie-Mellon University, Pittsburgh, Pennsylvania

- T. G. COOPER, University of Pittsburgh, Pennsylvania: Control and coordination of nitrogen metabolism in yeast—A model.
- B. A. HEMMINGS, NHLBI, National Institutes of Health, Bethesda, Maryland: Regulation of nitrogen metabolism in yeast by proteolysis and protein phosphorylation.
- M. C. BRANDRISS and B. MAGASANIK, Department of Biology, Massachusetts Institute of Technology, Cambridge: One enzyme, two pathways, two inducers.

- M. S. COHN, C. W. TABOR, and H. TABOR, National Institutes of Health, Bethesda, Maryland: Mutations affecting expression of yeast ornithine decarboxylase.
- F. LACROUTE, R. LOSSON, and G. LOISON, Institut de Biologie Moléculaire at Cellulaire, Centre National de la Recherche Scientifique, Strasbourg, France: Regulatory mutations constitutive for OMPdecase, product of the yeast *ura 3* gene.
- G. IDE, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: In vitro initiation of transcription in isolated yeast nuclei.
- S. LILLIE, M. BANUELOS, and J. R. PRINGLE, Division of Biological Sciences, University of Michigan, Ann Arbor: Roles and control of reserve carbohydrate metabolism during the yeast life cycle.
- J. WOOLFORD, L. HERFORD, K. FARHNER, and M. ROSBASH, Department of Biology and Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Ribosomal protein genes of yeast.
- D. R. KIEF and J. R. WARNER, Departments of Cell Biology and Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Coordinate control of synthesis of rRNA and ribosomal protein during a nutritional shift-up.

Session 12: rDNA and tRNA

Chairperson: F. SHERMAN, University of Rochester, New York

- J. W. SZOSTAK, Division of Genetics, Sidney Farber Cancer Institute, Boston, Massachusetts: Replication and sequence rectification of the rDNA of yeast.
- T. D. PETES, Department of Microbiology, University of Chicago, Illinois: Unequal meiotic recombination of yeast ribosomal DNA genes.
- A. K. HOPPER, L. SCHULTZ, and R. SHAPIRO, University of Massachusetts Medical School, Worcester: Yeast mutants defective in tRNA biosynthesis.
- J. KURJAN,* B. D. HALL,* and M. SMITH,† *Department of Genetics, University of Washington, Seattle; †Department of Biochemistry, University of British Columbia, Vancouver, Canada: A fine structure genetic and sequence analysis of the *SUP4* locus.
- C. GUTHRIE, D. COLBY, T. ETCHEVERRY, and M. SALVATO, Department of Biochemistry and Biophysics, University of California, San Francisco: Processing of *S. cerevisiae* tRNA precursors containing intervening sequences.
- J. KOHLI, S. SHARP, and D. SÖLL, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: UGA suppressor tRNAs in *Schizosaccharomyces pombe*.
- C. CUMMINS,* R. GABER,* I. EDELMAN,* M. MENDENHALL,* M. CULBERTSON,* R. MANN,† and G. FINK,† *Laboratories of Genetics and Molecular Biology, University of Wisconsin, Madison; †Department of Botany, Genetics, and Development, Cornell University, Ithaca, New York: Isolation and properties of group III frameshift suppressors.
- M. MASUREKAR,* E. PALMER,† J. M. WILHELM,† and F. SHERMAN,* Departments of *Radiation Biology and Biophysics and †Microbiology, University of Rochester School of Medicine and Dentistry, New York: Altered 40 S ribosomal subunits from an omnipotent suppressor strain.

Session 13: Chromosome Structure

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

- L. HERFORD and K. FAHRNER, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Yeast histone genes.
- D. B. KABACK* and L. HERFORD,† *Department of Chemistry, California Institute of Technology, Pasadena; †Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Organization of yeast histone 2A and 2B genes and other transcribed sequences as investigated by R-loop mapping.
- U. WINTERSBERGER and B. MARIAN, Institute for Cancer Research, University of Vienna, Austria: Uncoupling of histone synthesis from premeiotic DNA replication in sporulating yeast, *S. cerevisiae*.
- D. H. WILLIAMSON and D. J. FENNEL, National Institute for Medical Research, Mill Hill, England: Non-random assortment of sister chromatids in mitotically dividing cells of *S. cerevisiae*.

- D. LOHR* and K. E. VAN HOLDE,† *Department of Chemistry, Arizona State University, Tempe; †Department of Biochemistry/Biophysics, Oregon State University, Corvallis: The arrangement of nucleosomes in yeast chromatin.
- A. J. KINGSMAN,* A. C. CHINAULT,† L. CLARKE,* R. HITZEMAN,* and J. CARBON,* *Department of Biological Sciences, University of California, Santa Barbara; †Department of Medicine, Baylor College, Houston, Texas: Organization of sequences surrounding the centromere of chromosome III in *S. cerevisiae*.
- L. DAVIDOW, L. GOETSCH, and B. BYERS, Department of Genetics, University of Washington, Seattle: Genetic basis and consequences of a reversible thermal arrest of yeast meiosis at pachytene.
- J. GAFNER,* H. EIBEL,* M. BRENNAN,* B. HOHN,† and P. PHILIPPSEN,* *Department of Microbiology, Biozentrum, University of Basel; †Friedrich Miescher Institut, Basel, Switzerland: Analysis of integration sites of mobile elements in yeast.
- H. GREER and D. PHEASANT, Biology Department, Harvard University, Cambridge, Massachusetts: A yeast transposable element which carries the *his4C* gene.
- C. NEWLON, J. N. STRATHERN, and J. B. HICKS, Cold Spring Harbor Laboratory, New York: Isolation of a circular derivative of yeast chromosome III.
- G. P. JANSEN, M. C. BARNEY, and J. R. HELBERT, Miller Brewing Company, Milwaukee, Wisconsin: Nonvector-associated genetic transformation of flocculence in yeast.

Session 14: Recombination, Repair, and DNA Synthesis

Chairperson: S. FOGEL, University of California, Berkeley

- S. GOLTZ,* M. BELFORT,† L. DUMAS,* and D. BENESH,* *Department of Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois; †Division of Laboratories and Research, New York State Department of Health, Albany: Isolation and characterization of λ -yeast recombinant phages able to replicate in DNA primase defective *E. coli*.
- M. N. CONRAD and C. S. NEWLON, Department of Zoology, University of Iowa, Iowa City: Characterization of DNA synthesis in *cdc2*.
- B. K. TYE and C. S. M. CHAN, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Chromosomal replication origins in yeast.
- D. STINCHCOMB, M. THOMAS, and R. DAVIS, Department of Biochemistry, Stanford University School of Medicine, California: Isolation and characterization of yeast chromosomal replicators.
- B. CROSBY,* L. PRAKASH,* and D. HINKLE,† Departments of *Radiation Biology and Biophysics and †Biology, University of Rochester, New York: Isolation of a DNA binding protein from yeast.
- B. MONTELONE,* L. PRAKASH,† and S. PRAKASH,* Departments of *Biology and †Radiation Biology and Biophysics, University of Rochester, New York: Characteristics of spontaneous mitotic recombination in *S. cerevisiae*.
- J. E. WAGSTAFF, J. E. GOLIN, and M. S. ESPOSITO, Department of Biology and Committee on Genetics, University of Chicago, Illinois: Spontaneous mitotic recombination in *a/a* and α/α diploids of *S. cerevisiae*.
- V. L. MACKAY and L. BLISS, Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Mating-type dependent repair of MMS damage is a constitutive function.
- M. FRANKENBERG-SCHWAGER, D. FRANKENBERG, D. BLÜCHER, and C. ADAMCZYK, Gesellschaft für Strahlen und Umweltforschung, München, and Abteilung Biophysikalische Strahlenforschung, Frankfurt, Germany: Repair of DNA double-strand breaks under nongrowth conditions.
- W. J. JACHYMZYK*† and R. C. VON BORSTEL,* *Department of Genetics, University of Alberta, Edmonton, Canada; †Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw: Removal of interstrand cross-links from DNA of *S. cerevisiae*—Comparison of two pathways for DNA repair.

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BACTERIOPHAGE MEETING, August 21 – August 26

arranged by

Ahmad I. Bukhari and Thomas R. Broker, Cold Spring Harbor Laboratory

Session 1: DNA Replication

- M. LUSKY and G. HOBOM, Institut für Biologie III, Universität Freiburg, Germany: Inceptor and origin of DNA replication in bacteriophage lambda.
- K. GEIDER and T. F. MEYER, Abteilung Molekulare Biologie, Max-Planck-Institut, Heidelberg, Germany: Replication of fd RF with purified proteins.
- T. F. MEYER and K. GEIDER, Abteilung Molekulare Biologie, Max-Planck Institut, Heidelberg, Germany: Properties of isolated phage fd gene II-protein.
- R. A. GRANT and R. E. WEBSTER, Department of Biochemistry, Duke University, Durham, North Carolina: Studies on the gene V protein/DNA complex of the filamentous bacteriophage F1.
- S. WICKNER, NCI, National Institutes of Health, Bethesda, Maryland: Phage P22 gene 12 protein functions in vitro in oligonucleotide synthesis catalyzed by dnaG protein.
- R. MAURER and D. BOTSTEIN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Interaction between host- and phage-specified replication proteins during growth of *Salmonella* phage P22.
- L. W. BORQUE and J. R. CHRISTENSEN, Department of Microbiology, University of Rochester, New York: DNA synthesis of bacteriophage T1.
- G. L. STETLER, G. J. KING, and W. M. HUANG, Department of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: T4 DNA-delay proteins, required for specific T4 DNA replication, form a complex which untwists superhelical DNA in an ATP-dependent reaction.
- D. GHOSAL, G. MOSIG, and S. BOCK, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Genetic instability in gene 32 (DNA binding protein, HDP) of phage T4.
- S. BENEDICT and G. MOSIG, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Analysis of replicating T4 DNA by Southern hybridization.
- M. E. HALPERN, Department of Human Genetics, University of Pennsylvania, Philadelphia: The origins of T4 DNA replication—the first two.
- M. J. STEPHEN,* D. VAPNEK,* and J. R. SCOTT,† *Department of Microbiology, University of Georgia, Athens; †Department of Microbiology, Emory University, Atlanta, Georgia: Localization of the origin(s) or replication of bacteriophage P1.
- F. TAMANOI and C. C. RICHARDSON, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Site specific initiation of DNA replication in vitro on a cloned segment of T7 DNA.
- K. J. BARRETT,* A. BLINKOWA,† and G. ARNOLD,* *Max-Planck-Institut für Virusforschung, Tübingen, Germany; †Institute of Microbiology, University of Warsaw, Poland: Isolation and characterization of temperature-sensitive mutants in the α gene of phage P4.

Session 2: Morphogenesis

- R. HAMATAKE,* R. MUKAI,† and M. HAYASHI,* *Department of Biology, University of California, San Diego; †Institute of Basic Medical Science, University of Tsukuba, Nihari-gun, Japan: Morphogenesis of bacteriophage ϕ X174.
- Y. NAKASHIMA,* R. L. WISEMAN,† B. FRANGIONE,‡ and W. H. KONIGSBERG,§ *National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; †Department of Biochemistry, Public Health Research Institute of the City of New York, Inc.; ‡Irvington House Institute, New York University Medical Center, New York; §Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Structure of the major coat protein of the filamentous bacteriophages.
- Y. C. NG and A. K. DUNKER, Chemistry Department, Washington State University, Pullman: Effects of oxidative phosphorylation uncouplers on the assembly of the filamentous phage fd.
- M. F. SHEMYAKIN and S. K. ZAVRIEV, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, and Beritashvili Institute of Physiology, Georgian SSR Academy of Sciences, Tbilisi: Blocking of DNA transfer from T7 virion into host cell by inhibitors of bacterial RNA polymerase.
- M. ONTELL and D. NAKADA, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: Involvement of T7 phage gene 2 DNA packaging.
- B. KEMPER, Institut für Genetik, Universität Köln, Germany: Action of purified gene 49-controlled nuclease on very fast sedimenting DNA and its role in the development of phage T4.

- D. W. BOWDEN and P. MODRICH, Department of Biochemistry, Duke University, Durham, North Carolina: In vitro studies on the P2 *ter* reaction.
- J. SEDIVY, B. WALSH, R. GOLDSTEIN, and J. GEISSELSODER, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Triangulation numbers of the phages P2 and P4.
- M. B. ADAMS and S. CASIENS, Department of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: On the autogenous regulation of P22 scaffolding protein.
- M. FULLER and J. KING, Department of Biology, Massachusetts Institute of Technology, Cambridge: Assembly of active P22 proheads in vitro from purified coat and scaffolding protein.
- D. GOLDENBERG, D. SMITH, and J. KING, Department of Biology, Massachusetts Institute of Technology, Cambridge: Temperature sensitive mutants blocking maturation of the thermostable P22 tailspike protein.
- J. KOCHAN and H. MURIALDO, Department of Medical Genetics, University of Toronto, Canada: Biologically active intermediates in λ prohead assembly.
- M. SUMNER-SMITH, A. BECKER, and H. MURIALDO, Department of Medical Genetics, University of Toronto, Canada: In vitro complementation between λ Nul⁻ and λ Aam extracts.
- J. O. THOMAS and M. E. KERSHAW, Department of Biochemistry, New York University School of Medicine, New York: The in situ arrangement of phage DNA.

Session 3: Bacteriophage Mu

- P. VAN DE PUTTE, Laboratory of Biochemistry, University of Leiden, The Netherlands: The G area of bacteriophage Mu.
- R. CLAYTON, W. SCHUMANN, and E. G. BADE, Universität Konstanz, Germany: In vitro insertions and deletions in the G segment of phage Mu DNA do not abolish the inversion process.
- D. KWOH, T. J. KWOH, and D. ZIPSER, Cold Spring Harbor Laboratory, New York: Analysis of the cloned genome of phage Mu.
- C. CHASE and R. BENZINGER, Department of Biology, University of Virginia, Charlottesville: Identification of a protein which protects bacteriophage Mu DNA against host cell nuclease.
- J. WILLIAMS and C. RADDING, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Partial purification of an exonuclease inhibitor induced by bacteriophage Mu.
- V. N. KRYLOV, V. G. BOGUSH, A. S. YANENKO, and N. B. KIRSANOV, All-Union Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow, USSR: Bacteriophages of *Pseudomonas aeruginosa* with Mu-like DNA structure.

Session 4: Replication and Transposition of Mu

- L. T. CHOW, J. M. SCOTT, and R. LA PORTA, Cold Spring Harbor Laboratory, New York: The relationship between phages Mu and D108.
- D. KAMP and R. KAHMANN, Cold Spring Harbor Laboratory, New York: Structural aspects of bacteriophage Mu integration.
- G. CHACONAS and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: Association of Mu containing plasmids with the *E. coli* chromosome upon prophage induction.
- R. M. HARSHEY and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: Electron microscopic study of Mu DNA transposition.
- J. W. SCHUMM and M. M. HOWE, Department of Bacteriology, University of Wisconsin, Madison: Properties of λ phages containing both ends of Mu.
- R. YOSHIDA and M. M. HOWE, Department of Bacteriology, University of Wisconsin, Madison: Lysogenization and development of Mu and Mu *nu* phages in *E. coli* and *him* mutant strains.
- A. TOUSSAINT, M. FAELEN, L. DESMET, and A. RESIBOIS, Faculté de Médecine, Université Libre de Bruxelles, Belgium: Physiological relationship between mutator phages Mu and D108.
- R. FITTS and A. L. TAYLOR, University of Colorado Health Sciences Center, Denver: Integration of Mu DNA at host chromosomal replication forks.
- B. WAGGONER,* A. TOUSSAINT,† and M. PATO,* *National Jewish Hospital and Research Center, Denver, Colorado; †Free University of Brussels, Belgium: A new function required for normal Mu DNA replication.

- H. BIALY, B. WAGGONER, and M. PATO, National Jewish Hospital and Research Center, Denver, Colorado: The fate of an RP4: μ plasmid after induction of the prophage.
- K. H. WESTPHAL, and E. G. BADE, Fachbereich Biologie, Universität Konstanz, Germany: Early replication of temperate bacteriophage μ .

Session 5: Integrative Recombination

- R. HOESS,* K. BIDWELL,* C. FOELLER,[†] and A. LANDY,[†] *Cancer Biology Program, Frederick Cancer Research Center, Maryland; [†]Division of Biology and Medicine, Brown University, Providence, Rhode Island: DNA sequence analysis of the lambda *int-xis* region.
- M. MIZUUCHI and K. MIZUUCHI, Laboratory of Molecular Biology, National Institutes of Health, Bethesda, Maryland: In vitro study of the integrative recombination of λ .
- K. ABREMSKI and S. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: A comparison between λ integration and excision in vitro.
- C. GRITZMACHER, L. ENQUIST, and R. WEISBERG, National Institutes of Health, Bethesda, Maryland: Xis-independent excision of bacteriophage λ —Isolation and characterization of an *xin* mutant.
- L. W. ENQUIST and M. J. MADDEN, NCI, National Institutes of Health, Bethesda, Maryland: Does λ have a preferred secondary integration site?
- M. L. PEARSON,* C. EPP,* and L. W. ENQUIST,[†] *Department of Medical Genetics, University of Toronto, Canada; [†]NCI, National Institutes of Health, Bethesda, Maryland: Cis-dominant inhibition of λ INT synthesis by the β -region.
- D. MASCARENHAS, M. BENEDIK, and A. CAMPBELL, Department of Biological Sciences, Stanford University, California: DNA sequence of *trp-int* fusions in the integrase regulatory region of bacteriophage lambda.
- M. J. BENEDIK and A. M. CAMPBELL, Department of Biological Sciences, Stanford University, California: Genetic analysis of integrase regulation in bacteriophage λ .
- S. AUSTIN and N. STERNBERG, Cancer Biology Program, Frederick Cancer Research Center, Maryland: A novel role for site-specific recombination in the maintenance of the P1 plasmid prophage.
- T. J. FOSTER, K. TAKESHITA, and N. KLECKNER, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: The role of Tn10-encoded functions and sites in transposon-related recombination events.
- V. LUNDBLAD and N. KLECKNER, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Precise excision and nearly-precise excision of Tn10.
- R. A. FISHER, K. KRIZANOVICH-WILLIAMS, and M. FEISS, Department of Microbiology, University of Iowa, Iowa City: Packaging of the bacteriophage lambda chromosome—Isolation and characterization of the *cos* 1 mutation and the analysis of *cos*⁺ revertants.
- C. PARSONS,* P. TOMICH,* R. DEANS,[†] and D. FRIEDMAN,* Departments of *Microbiology and [†]Biological Chemistry, University of Michigan, Ann Arbor: λ RF—A phage that rescues "silent" copies of P22 genes in an *E. coli* K12 strain.

Session 6: λ Promoters and Operators

- J. ABRAHAM, R. FISCHER, and H. ECHOLS, Department of Molecular Biology, University of California, Berkeley: Sequence analysis of the P₁ promoter region of bacteriophage lambda.
- G. LAUER and R. PASTRANA, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Interactions of the bacteriophage 434 repressor with operator DNA.
- A. JOHNSON, B. J. MEYER, and M. PTASHNE, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Specific cooperative interactions between DNA-bound repressors govern regulation by the λ phage repressor.
- B. J. MEYER, R. MAURER, and M. PTASHNE, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Molecular mechanisms for the regulation of the *cl* and *cro* genes of phage λ mediated by λ repressor and by *cro* product.
- U. SCHMEISSNER, D. COURT, H. SHIMATAKE, and M. ROSENBERG, NCI, National Institutes of Health, Bethesda, Maryland: Promoter for the establishment of repressor synthesis in bacteriophage λ .
- D. HAWLEY and W. MCCURE, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: In vitro comparison of the initiation properties of bacteriophage lambda wild-type P_R and x₃ mutant promoters.

- E. C. ROSENVOLD, E. CALVA, R. R. BURGESS, and W. SZYBALSKI, McArdle Laboratory, University of Wisconsin, Madison: In vitro initiation of decanucleotide and longer transcripts at the leftward promoter p_{BL} in the b_2 region of bacteriophage λ DNA.
- E. CALVA and R. R. BURGESS, McArdle Laboratory, University of Wisconsin, Madison: End-labeled in vitro transcripts of bacteriophage lambda—Characterization of a rho-dependent 5S RNA.
- S. ADHYA and M. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: Promoter competition in bacteriophage λ .
- E. D. SVERDLOV, E. M. HODKOVA, L. I. PATRUSHEV, and M. F. SHEMAKIN, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow: The cloning of fragments of phage λ *imm434* DNA, which contain some regulator sequences.

Session 7: λ —Regulation of Gene Expression

- N. C. FRANKLIN, Department of Biological Sciences, Stanford University, California: The *N* protein of lambda defined by its DNA sequence.
- J. GREENBLATT and J. LI, Banting and Best Department of Medical Research, University of Toronto, Canada: The gene *N* protein of bacteriophage λ —Purification, properties, and specific interactions and host-coded proteins.
- S. GOTTESMAN, P. TRISLER, and M. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: The functional decay of lambda *N* protein.
- J. SHAW,* M. L. PEARSON,* and S. GOTTESMAN,† *Department of Medical Genetics, University of Toronto, Canada; †NCI, National Institutes of Health, Bethesda, Maryland: Stability of bacteriophage lambda *N*, *O*, and *cII* proteins in *lon*₋ and *rho*₋ bacteria.
- H. SHIMATAKE, C. QUEEN, B. PATERSON, and M. ROSENBERG, NCI, National Institutes of Health, Bethesda, Maryland: Multiple forms of λ *cII* protein.
- D. FORBES and I. HERSKOWITZ, Institute of Molecular Biology, University of Oregon, Eugene: Relief of polarity in the *gal* operon by the action of *Q* in trans.
- E. J. GRAYHACK, M. G. SCHECHTMAN, and J. W. ROBERTS, Department of Biochemistry, Cornell University, Ithaca, New York: Late gene regulation in phage lambda.
- J. N. ALEGRE, M. G. SCHECHTMAN, and J. W. ROBERTS, Department of Biochemistry, Cornell University, Ithaca, New York: Phage ϕ X82 late gene regulation.
- M. A. MOZOLA and D. I. FRIEDMAN, Department of Microbiology, University of Michigan, Ann Arbor: A λ mutant displaying a temperature-sensitive growth defect in *himA*₋ strains.
- E. L. FLAMM and D. I. FRIEDMAN, University of Michigan, Ann Arbor: Characterization of a λ *nutR* mutant.
- J. SWINDLE and C. GEORGOPOULOS, Department of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: Studies with the *groNB* *E. coli* mutants.
- K. TILLY and C. GEORGOPOULOS, Department of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: Studies on *groE* mutants of *E. coli*.
- H. MURIALDO, W. FIFE, A. BECKER, M. FEISS, and J. YOCHEN, Department of Medical Genetics, University of Toronto, Canada, and Department of Microbiology, University of Iowa, Iowa City: Isolation and characterization of *Fl*-independent mutants of λ .

Session 8: Poster Session

- M. M. HOWE,* J. W. SCHUMM,* J. A. ENGLER,† W. SCHUMANN,‡ and E. G. BADE,‡ *Department of Bacteriology, University of Wisconsin, Madison; †Cold Spring Harbor Laboratory, New York; ‡Fachbereich Biologie, Universität Konstanz, Germany: Correlation of the genetic and physical maps of bacteriophage μ .
- M. DUBOW, D. EVANS, N. SARVETNICK, and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: Utilization of β -lactamase as a measure of *amp*-encoding DNA replication.
- J. E. MOTT and J. F. SPEYER, Department of Biological Sciences, University of Connecticut, Storrs: Characterization of T^+ mutants able to overcome restriction by P^2 lysogens.
- S. HILLIKER, M. SCHLEIN, and M. DECOU, Department of Biology, Bucknell University, Lewisburg, Pennsylvania: Characterization of *Salmonella* phages P22 and L by means of λ -P22 and λ -L hybrid phages.
- M. KAHN,* D. OW,† B. SAUER,† R. CALENDAR,† and A. RABINOWITZ,* *Department of Biology, University of California at San Diego, La Jolla; †Molecular Biology Department, University of California, Berkeley: Physical genetic map of the P4 phage genome.

- T. C. LIN and W. H. KONIGSBERG, Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Isolation and characterization of a low molecular weight viral coat protein from bacteriophage fd.
- W. GERALD and J. KARAM, Department of Biochemistry, Medical University of South Carolina, Charleston: Separate transcripts for T4 genes 45 and 44.
- B. LABELAN and E. B. GOLDBERG, Tufts University School of Medicine, Boston, Massachusetts: Role for a membrane potential in phage T4 DNA injection (protonmotive force/inhibitors/phage/adsorption).
- A. LUDER and G. MOSIG, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Effects of mutations in gene 58-61 on T4 DNA metabolism.
- L. GOLD, M. A. NELSON, D. PRIBNOW, B. SINGER, G. STORMO, M. COLKITT, and L. HUNTER, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: What's new in rII, and some old stuff too.
- G. H. WEVER and J. S. WIBERG, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, New York: *regA* constraint on expression of recombinant DNA in early genes of bacteriophage T4.
- M. COUTURIER, F. BEX, J. JANSSENS, and A. DESMYTER, Department of Molecular Biology, Universite Libré de Bruxelles, Belgium: Construction in vitro and properties of "phage-plasmid" chimerae.

Session 9: P1, P2, P4, and P22

- P. YOUNDERIAN and R. GOLDSTEIN, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Control of satellite P4 protein synthesis.
- P. YOUNDERIAN,* G. DEHO,* M. CHIDAMBARAM,* G. GROVER,* L. TINELLI,* D. SHORE,* J. GEISSELSODER,* R. GOLDSTEIN,* and E. LJUNGQUIST,† *Department of Microbiology and Molecular Genetics Harvard Medical School, Boston, Massachusetts; †Department of Microbial Genetics, Karolinska Institutet, Stockholm, Sweden: A conditional lethal mutant of satellite bacteriophage P4 unable to derepress its provirus helper.
- B. H. LINDQVIST, Institute of Medical Biology, University of Tromsø, Norway: Isolation of viable recombinants between satellite phage P4 and its helper P2.
- D. OW and F. AUSUBEL, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Coliphage P4 as a cloning vector in *Klebsiella pneumoniae*.
- A. R. POTEETE, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Control of P22 PR and PRM promoters by repressor.
- F. WINSTON and D. BOTSTEIN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Lysogeny and anti-immunity in phage P22.
- M. M. SUSSKIND, Department of Microbiology, University of Massachusetts Medical School, Worcester: Identification of the P22 P_{ANT} in vitro transcript and of P22 P_{ANT} mutations.
- K. R. LEASON, T. F. WEIGHOUS, and E. N. JACKSON, Department of Microbiology, University of Michigan, Ann Arbor: Regulation of transcription of the late gene region of bacteriophage P22.
- E. JACKSON and J. RUTILA, Department of Microbiology, University of Michigan, Ann Arbor: Genetic analysis of cloned fragments of P22 DNA—Definition of a P22 physical gene map.
- B. R. BAUMSTARK and J. R. SCOTT, Department of Microbiology, Emory University, Atlanta, Georgia: c1 repressor of phage P1.
- H. HEILMANN,* A. PÜLLER,* H. J. BURKARDT,* and J. N. REEVE,† *Institut für Mikrobiologie der Universität Erlangen; †Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: Molecular analysis of the restriction gene of phage P1.
- R. J. MURAL, N. STERNBERG, and M. POWERS, Cancer Biology Program, Frederick Cancer Research Center, Maryland: Isolation and characterization of λ -P1 miniplasmids and their use in generating mutations in plasmid maintenance functions.

Session 10: T-phage Chromosome Structure and Function

- M. D. ROBERTS and H. DREXLER, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: A transducing mutant of T1 which cannot degrade *E. coli* DNA.
- G. WILSON,* K. YOUNG,* G. EDLIN,* and W. KONIGSBERG,† *Department of Genetics, University of California, Davis; Department of Molecular Biophysics and Biochemistry Yale University, Connecticut: Generalized transduction by bacteriophage T4.

- G. WILSON,* R. NEVE,* G. EDLIN,* and W. KONIGSBERG,* *Department of Genetics, University of California, Davis; †Department of Molecular Biophysics and Biochemistry, Yale University, Connecticut: The genetic location of the *Bam*HI restriction site in the chromosome of bacteriophage T4.
- K. CARLSON, Institute of Medical Biology, University of Tromsø, Norway: Mapping of T4 DNA with restriction endonucleases.
- V. I. TANYASHIN, L. I. LI, A. I. KRUTILINA, G. G. PRYTISIUK, V. F. ZINKEVICH, V. N. BOLEZNINA, and A. A. BAYEV, Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow: T4 DNA structure and function—Genes 42–46 and 50–48 regions.
- R. MARSH, M. MAHER, and J. YEE, Biology Program, University of Texas, Dallas: Restriction map of bacteriophage T4.
- C. G. GOFF, Biology Department, Haverford College, Pennsylvania: Cloning T4 genes which affect *E. coli* RNA polymerase.
- K. JACOBS, D. SHIBATA, and E. P. GEIDUSCHEK, Department of Biology, University of California, San Diego: Regulation of expression of cloned T4 late genes by infecting T4 bacteriophage.
- D. H. HALL, C. E. SMITH, and A. J. DERSHOWITZ, School of Biology, Georgia Institute of Technology, Atlanta: Gene expression by bacteriophage T4 on mutants of *E. coli* with defective ribosomes.
- B. GOMEZ, L. NUALART, and J. DEL BOSQUE, Departamento de Ecología Humana, Facultad de Medicina, Universidad Nacional Autónoma de México: Abortive infection by bacteriophages T7 of an *E. coli* strain in which no viral proteins are synthesized.
- E. P. AMANN and J. N. REEVE, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: The expression of *B. subtilis* phage SPP1 genes in *E. coli*.
- F. BRUNEL, J. DAVISON, and M. MERCHEZ, Unit of Molecular Biology, International Institute of Cellular Pathology, Brussels, Belgium: Partial correlation of the physical and genetical maps of bacteriophage T5.
- A. ZACHARY* and L. A. MCNICOL† *Department of Biological Chemistry, University of Maryland Medical School, Baltimore; †Department of Microbiology, University of Maryland, College Park: T-even bacteriophage from the marine environment.
- K. HABERER*† and J. MANILOFF,* *Department of Microbiology, University of Rochester Medical Center, New York; †Abteilung Mikrobiologie, Universität Ulm, Germany: Structure and replication of mycoplasma virus MVL3—A unique cytocidal virus infecting prokaryotes.

Session 11: Recombination; cloning

- W. BRADLEY, L. BEATTY, D. VETTER, and P. D. SADOWSKI, Department of Medical Genetics, University of Toronto, Ontario, Canada: Further characterization of a biological assay for recombination of bacteriophage T7 DNA in vitro.
- D. K. CHATTORAJ, Institute of Molecular Biology, University of Oregon, Eugene: Recombinational intermediates of phage lambda.
- R. ZAGURSKY, T. JORDAN, and J. HAYS, Department of Chemistry, University of Maryland Baltimore County, Catonsville: Recombination of lambda tandem duplication phages—Intramolecular recombination and interclonal mixing.
- J. S. SALSTROM, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: New *tii*-like mutants of the *recA* gene of *E. coli*.
- R. BRENT, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: The *lexA* gene and its protein product.
- C. DAMBLY, Z. TOMAN, and M. RADMAN, Department of Molecular Biology, Free University of Brussels, Belgium: A lambda test system detecting mutational and epigenetic changes.
- L. D. VALES, J. B. MURPHY, and J. W. CIASE, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Studies of lambda DNA prophage induction in *E. coli* K-12 strains containing defects in the single-strand DNA binding protein.
- N. E. MELECHEN and G. GO, Department of Microbiology, St. Louis University School of Medicine, Missouri: Induction of lambdaoid phages by amino acid deprivation—Questions about the role of *RecA*.
- B. E. KORBA and J. B. HAYS, Department of Chemistry, University of Maryland Baltimore County, Catonsville: *arl* mutants of *E. coli* generate recombinogenic lambda phages.
- N. L. CRAIG, E. M. PHIZICKY, C. W. ROBERTS, and J. W. ROBERTS, Department of Biochemistry, Cornell University, Ithaca, New York: Function of the *E. coli RecA* protein in phage induction.
- E. JEDLICKI, J. P. KUSNIERZ, and O. REYES, Institut Pasteur, Lille, France: Inhibition of P2 interference.

- J. DAVISON, F. BRUNEL, and M. MERCHIEZ, Unit of Molecular Biology, International Institute of Cellular Pathology, Brussels, Belgium: λ gtWES.T5-622 an improved vector allowing positive selection for recombinants having foreign DNA inserts.
- K. MCKENNY, H. SHIMATAKE, D. COURT, and M. ROSENBERG, NCI, National Institutes of Health, Bethesda, Maryland: A plasmid vector to examine transcription regulatory signals.

Session 12: RNA Polymerases and Transcription

- H. STRAUSS, R. BURGESS, and M. T. RECORD, JR. McArdle Laboratories, University of Wisconsin, Madison: The selective binding of *E. coli* RNA polymerase to promoters on phage T7 DNA.
- J. S. HEILIG, J. G. SCAIFE, and R. CALENDAR, Molecular Biology Department, University of California, Berkeley: Genetic studies of the sigma subunit of *E. coli* RNA polymerase—Suppressor mutations and cloned genes.
- U. HANSEN and W. McCLURE, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Release of sigma from *E. coli* RNA polymerase after initiation of transcription.
- G. LEE, C. TALKINGTON, and J. PERO, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Distinctive nucleotide sequences of promoters recognized by RNA polymerase containing a phage-coded "sigma-like" protein.
- J. ROMEO, B. CHELM, S. BRENNAN, and E. P. GEIDUSCHEK, Department of Biology, University of California, San Diego: (At least) 25 early SPO1 promoters.
- S. BRENNAN, P. U. GIACOMONI, B. K. CHELM, and J. ROMEO, Department of Biology, University of California, San Diego: In vitro transcription of SPO1 early RNA.
- B. BROWNSTEIN, S. USALA, H. AGRAWAL, and R. HASELKORN, Department of Biophysics and Theoretical Biology, University of Chicago, Illinois: In vitro transcription by virion RNA polymerase of bacteriophage $\phi 6$.
- R. ZIVIN, W. ZHRING, K. VANDER LAAN, and L. B. ROTHMAN-DENES, Departments of Biophysics and Theoretical Biology, and Microbiology, University of Chicago, Illinois: Involvement of *E. coli* RNA polymerase in coliphage N4 transcription.
- B. SAUER, D. OW, L. LING, and R. CALENDAR, Department of Molecular Biology, University of California, Berkeley: Mutants of satellite phage P4 which are defective in the suppression of transcriptional polarity.
- J. S. FASSLER and I. TESSMAN, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Parallel effects of ultraviolet irradiation and rho deficiency in relieving polarity in $\phi X174$.
- J. S. CASHMAN, R. E. WEBSTER, and D. A. STEEGE, Department of Biochemistry, Duke University Medical Center, Durham, North Carolina: In vivo transcription of bacteriophage f1.
- B. STITT* and H. R. REVEL,† *Public Health Research Institute of the City of New York, Inc., New York; †Department of Microbiology and Molecular Biology, Tufts University Medical School, Boston, Massachusetts: The role of the host in phage T4 development—*E. coli* mutants with a pleiotropic effect.

HERPESVIRUSES, August 28 – September 2

arranged by

Elliott Kieff, University of Chicago, Illinois

Priscilla A. Schaffer, Harvard Medical School, Boston, Massachusetts

335 participants

Session 1: DNA Structure

Chairpersons: B. FLECKENSTEIN, Universität Erlangen, Germany

W. SUMMERS, Yale University, New Haven, Connecticut

- J. CÉBRIAN,* C. KASCHKA-DIERICH,† N. BERTHELOT* and P. SHELDRIK,* *Institut de Recherches Scientifiques sur le Cancer, Villejuif, France; †Institut für Klinische Virologie der Universität Erlangen, Germany: Inverted repeats in the genomes of Marek's disease virus and turkey herpesvirus.

- J. SKARE, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Restriction mapping of infectious bovine rhinotracheitis virus DNA.
- G. S. HAYWARD and R. LAFEMINA, Department of Pharmacology, Johns Hopkins Medical School, Baltimore, Maryland: Comparison of the genome structure in two subgroups of primate cytomegalovirus.
- J. L. M. C. GEELLEN and M. W. WESTSTRATE, Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, The Netherlands: Structure of human cytomegalovirus DNA—Analysis of strain variation by “cross-blot” hybridization and RNA transcription.
- M. W. WESTRATE and J. L. M. C. GEELLEN, Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, The Netherlands: Human cytomegalovirus DNA—Mapping of restriction endonucleases sites for *Bgl*II, *Hind*III, and *Xba*I.
- E. HUANG,* S. STAGNO,† R. PASS,† and C. ALFORD,† *Department of Medicine, University of North Carolina School of Medicine, Chapel Hill; †Department of Pediatrics, University of Alabama Medical School, Birmingham: Molecular epidemiology of perinatal cytomegalovirus infection in man.
- B. E. HENRY, S. A. DAUENHAUER, J. H. WHARTON, and D. J. O’CALLAGHAN, University of Mississippi Medical Center, Jackson: Molecular structure of the genomes of standard virus and DI particles of equine herpesvirus type 1 (equine abortion) and type 2 (equine cytomegalovirus).
- H. LOCKER, B. BURCKART, and N. FRENKEL, Department of Biology, University of Chicago, Illinois: Structure and expression of defective herpes simplex virus genomes.
- K. DENNISTON-THOMPSON, L. W. ENQUIST, and G. F. VANDE WOUDE, Laboratory of Molecular Virology, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of *Eco*R1 fragments from defective HSV-1 (Patton) cloned in phage λ .
- J. M. KOOMEY,* C. MULDER,* M. D. DANIEL,† L. A. FALK,† R. C. DESROSIERS,† and B. FLECKENSTEIN,‡ *University of Massachusetts Medical School, Worcester; †New England Regional Primate Research Center, Harvard Medical School, Southboro, Massachusetts; ‡Universität Erlangen-Nuernberg, Germany: Genome alterations of two attenuated strains of *herpesvirus saimiri*.
- Y. S. LEE, A. TANAKA, R. Y. LAU, M. NONOYAMA, and H. RABIN, Laboratory of Molecular Virology, Life Sciences Biomedical Research Institute, St. Petersburg, Florida, and Biological Carcinogenesis Program, Frederick Cancer Research Center, Maryland: The DNA of herpesvirus papio (baboon herpesvirus) induced by the tumor promoter (12-*O*-tetradecanoylphorbol-13-acetate).

Session 2: DNA Structure and Replication

Chairpersons: B. ROIZMAN, University of Chicago, Illinois
T. BEN-PORAT, Vanderbilt University, Nashville, Tennessee

- N. RAAB-TRAUB, T. DAMBAUGH, W. KING, D. GIVEN, M. HUMMEL, C. BEISEL, and E. KIEFF, University of Chicago, Illinois: DNA of Epstein-Barr virus.
- C. KINTNER and B. SUGDEN, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The structure of the terminal repeats of Epstein-Barr viral DNA in the virion and the arrangement of these sequences in the transformed cell.
- L. NOGEE, C. ANDREWS, G. S. HAYWARD, and S. D. HAYWARD, Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore Maryland: Organization of repeated regions within the Epstein-Barr virus genome.
- J. R. ARRAND,* L. RYMO,† J. WALSH,* E. BJÖRCK,† T. LINDAHL,† and B. GRIFFIN,* *Imperial Cancer Research Fund, Lincoln’s Inn Fields, London, England; †Departments of Medical Chemistry and Clinical Chemistry, University of Gothenburg, Sweden: Cloning of EBV DNA fragments in the *E. coli* plasmid pBR322.
- J. SKARE, C. EDSON, and J. STRÖMINGER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Cloning of EBV *Bam* HI restriction fragments.
- M.-S. CHO, K.-O. FRESEN, and H. ZUR HAUSEN, Institut für Virologie, Zentrum für Hygiene der Universität, Freiburg, Germany: Characterization of the DNA and of biological functions of EBV synthesized after superinfection.
- K.-O. FRESEN and M.-S. CHO, Institut für Virologie, Zentrum für Hygiene der Universität, Freiburg, Germany: Rescue of recombinant Epstein-Barr virus DNA from superinfected NC37 cells.
- J. E. SHAW, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Analysis of EBV DNA in a somatic cell hybrid line.

- B. M. COLBY, J. E. SHAW, and J. S. PAGANO, Department of Bacteriology and Immunology, and Department of Medicine, University of North Carolina School of Medicine, Chapel Hill: Comparative studies of the EBV episomes from producer and nonproducer lymphoblastoid cell lines.
- B. F. LADIN, M. L. BLANKENSHIP, and T. BEN-PORAT, Department of Microbiology, Vanderbilt University, Nashville, Tennessee: Maturation of concatemeric DNA of pseudorabies virus to genome length is related to capsid formation.
- C. V. JONGENEEL and S. L. BACHENHEIMER, Department of Bacteriology and Immunology, University of North Carolina, Chapel Hill: Molecular anatomy of the replicating DNA of HSV-1.
- R. DESROSIERS,* C. MULDER,[†] and B. FLECKENSTEIN,[‡] *New England Regional Primate Research Center, Harvard Medical School, Southboro, Massachusetts; [†]Departments of Pharmacology and Microbiology, University of Massachusetts Medical School, Worcester; [‡]Institut für Klinische Virologie, Erlangen, Germany: Methylation of *Herpesvirus saimiri* DNA in lymphoid tumor cell lines.
- M. WU,* R. W. HYMAN,[†] and N. Davidson,* *Department of Chemistry, California Institute of Technology, Pasadena; [†]Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Electron microscopic mapping of proteins bound to herpes simplex virus DNA.
- Y. BECKER, S. RABKIN, and Y. SHTRAN, Laboratory for Molecular Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Synthesis of wild type and defective herpes simplex virus (HSV) DNA by a subnuclear fraction extracted from infected cells.

Session 3: Poster Session—DNA Structure and Replication: RNA

- D. A. GALLOWAY, Fred Hutchinson Cancer Research Center, Seattle, Washington: Cloning of herpes simplex virus type 2 DNA fragments in a plasmid vector.
- G. J. RUSSELL, N. M. WILKIE, and J. H. SUBAK-SHARPE, MRC Virology Unit, Institute of Virology, Glasgow, Scotland: Doublet frequency analysis of DNA restriction fragments of herpes simplex virus.
- A. M. DUMAS,* J. L. M. C. GEELLEN,[†] and W. MARIS,[†] *Municipal Laboratory for Virology, Rotterdam, The Netherlands; [†]Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, The Netherlands: Isolation and characterization of varicella-zoster virus DNA.
- R. W. HYMAN, J. C. RICHARDS, and F. RAPP, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Analysis of varicella-zoster virus DNA.
- A. TANAKA, Y. S. LEE, and M. NONOYAMA, Life Science Research Laboratories, St. Petersburg, Florida: Presence of defective virus particle in Marek's disease virus.
- N. HORAIST,* S. CHOUSTERMANN,[†] N. BERTHELOT,[†] P. SHEDRICK,[†] and J.-C. GUILLON,* *Institut Pasteur, Paris, France; [†]Institut de Recherches Scientifique sur le Cancer, Villejuif, France; [‡]Characterization of herpesvirus eidolon (HVE), a new virus isolated from African bats and study of its genome.
- S. S. LEINBACH and W. C. SUMMERS, Radiobiology Laboratories, Yale University School of Medicine, New Haven, Connecticut: DNA-protein interactions during replication of herpes simplex virus type 1.
- M. E. MOUTTET and J. M. BÉCHET, Institut Pasteur, Paris, France: Structure of intranuclear viral chromatin late in infection with herpes simplex virus type 1.
- D. S. PARRIS* and P. A. SCHAFER,[†] *Department of Medical Microbiology, Ohio State University College of Medicine, Columbus; [†]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Dependence of herpes simplex virus type 1 recombination on viral DNA replication.
- C. MOORE, J. E. SHAW, and J. GRIFFITH, Cancer Research Center, University of North Carolina, Chapel Hill: Electron microscope analysis of chromatin structural changes in superinfected Raji cells.
- I. W. HALLIBURTON, K. E. QUINN, S. C. SMITH, and L. FRASER, Department of Microbiology, University of Leeds, England: Mapping of herpes simplex virus glycoproteins and antigenic sites involved in neutralization.
- J. K. MCDUGALL, E. J. TOLLENTINO, and T. H. MASSEY, Fred Hutchinson Cancer Research Center, Seattle, Washington: Location of the HSV-2 thymidine kinase gene and its expression in transformed cells.
- D. J. BZIK and S. PERSON, Biophysics Program, Pennsylvania State University, University Park: The capacity of different cells and viruses to promote and inhibit HSV-1 induced cell fusion.
- L. RYMO, Sahlgren's Hospital, University of Gothenburg, Sweden: Identification of transcribed regions of Epstein-Barr virus DNA in Burkitt lymphoma-derived cells.
- R. M. STENBERG,* D. J. SPECTOR,[†] and L. I. PIZER,* *Department of Microbiology/Immunology, University of Colorado Medical Center, Boulder; [†]Washington University, St. Louis: HSV infection alters RNA metabolism in adenovirus transformed cells.

- F. BIA, W. C. SUMMERS, C. K. Y. FONG, and G. D. HSIUNG, Yale University School of Medicine, New Haven, Connecticut: Biological and molecular characterization of a new herpesvirus of guinea pigs.
- G. DARAI, L. ZÖLLNER, U. GRAMLICH, B. MATZ, R. M. FLÜGEL, U. BERGER, C. KUHN, S. MODROW, H. WOLF, H. GELDERBLUM, A. KRIEG, J. M. CAMERON, and H. DELIUS, Institut für Medizinische Virologie, Universität Heidelberg; Institut für Virusforschung, Heidelberg; Fachschule Biologie, Universität Konstanz; Robert-Koch-Institut, Berlin; Biologische Bundesanstalt, Land- und Forstwirtschaft, Darmstadt; Max-von-Pettenkofer-Institut, Universität München; Department of Virology, University of Glasgow, Scotland; European Molecular Biology Laboratory, Heidelberg, Germany: Characterization of a new DNA virus detected in various human cytomegalo- and herpesvirus virus stocks.
- R. DESROSIERS and L. FALK, New England Regional Primate Research Center, Harvard Medical School, Southboro, Massachusetts: Characterization of herpesvirus platyrrhinae, a herpes simplex related virus of new world primates.

Session 4: Genetics

Chairpersons: P. SCHAEFFER, Sidney Farber Cancer Institute, Boston, Massachusetts
N. M. WILKIE, Institute of Virology, Glasgow, Scotland

- R. A. F. DIXON and P. A. SCHAEFFER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: The relationship of genome structure to the genetic map of HSV-1.
- A. J. DAVISON and N. M. WILKIE, MRC, Institute of Virology, Glasgow, Scotland: Fine analysis of HSV intertypic recombinants—Homology for "a" sequences suffices for normal inversion of the long region.
- A. J. DAVISON, H. S. MARSDEN, and N. M. WILKIE, MRC Virology Unit, Institute of Virology, Glasgow, Scotland: Expression of only one copy of the genes coding for the immediate-early polypeptide I.E. 110 is sufficient for a productive infection of BHK cells by HSV.
- D. KNIPE,* W. RUYECHAN,† R. HONESS,‡ and B. ROIZMAN,§ *Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; †Department of Biochemistry, Uniformed Services University, Bethesda, Maryland; ‡National Institute of Medical Research, London, England; §University of Chicago, Illinois: A sequence repeated at the ends of the L and S components of HSV-1 DNA is part of a gene mapping in the S component.
- J. R. SMILEY, M. WAGNER, J. A. SHARP, W. P. SUMMERS, and W. C. SUMMERS, Yale University School of Medicine, New Haven, Connecticut: Genetic and physical fine structure of the thymidine kinase gene of herpes simplex virus 1.
- U. B. DASGUPTA and W. C. SUMMERS, Yale Medical School, New Haven, Connecticut: Study of recombination in mammalian cells using herpes simplex virus type 1.
- F. COLBÈRE-GARAPIN,* S. CHOUSTERMAN,† F. HORODNICEANU,* P. KOURILSKY,* and A. GARAPIN,* *Institut Pasteur, Paris; †Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Cloning of the active thymidine kinase gene of herpes simplex virus type 1 in *E. coli* K12.
- P. CHARTRAND,* C. CRUMPACKER,* P. SCHAEFFER,† and N. M. WILKIE,* *Institute of Virology, Glasgow, Scotland; †Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: A physical and genetic analysis of markers associated with the DNA polymerase activity of herpes simplex virus.
- D. M. COEN and P. A. SCHAEFFER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Genetics of acycloguanosine resistance in HSV-1—Evidence for two distinct loci.
- C. CRUMPACKER,*† L. SCHNIPPER,* P. CHARTRAND,† *Harvard Medical School, Boston, Massachusetts; †Institute of Virology, Glasgow, Scotland: Resistance of herpes simplex virus to acycloguanosine—Role of viral thymidine kinase and viral DNA polymerase.
- S. P. LITTLE and P. A. SCHAEFFER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Two loci control the expression of the syncytial (syn) phenotype in HSV-1, strain KOS.
- V. C. BOND and S. PERSON, Biophysics Program, Pennsylvania State University, University Park: Isolation and partial characterization of mutants of HSV-1 which cause cell fusion.
- R. M. SANDRI, M. LEVINE, and J. C. GLORIOSO, University of Michigan, Ann Arbor: A method for the isolation of mutations in physically defined regions of the herpes simplex virus genome.

Session 5: RNA

Chairpersons: E. K. WAGNER, University of California, Irvine
B. CLEMENTS, University of Glasgow, Scotland

- K. P. ANDERSON, L. E. HOLLAND, and E. K. WAGNER, Department of Molecular Biology and Biochemistry, University of California, Irvine: Characterization of messenger RNA encoded by specific regions of the HSV-1 genome.
- L. E. HOLLAND,* K. P. ANDERSON,* C. SHIPMAN, Jr.,† and E. K. WAGNER,* *Department of Molecular Biology and Biochemistry, University of California, Irvine; †Dental Research Institute, University of Michigan, Ann Arbor: Viral DNA synthesis is required for the expression of specific herpes simplex virus type 1 mRNA species on polyribosomes.
- J. B. CLEMENTS, F. J. RIXON, A. EASTON, and R. J. WATSON, Institute of Virology, Glasgow, Scotland: Immediate early transcription of herpes simplex virus types 1 and 2.
- S. TALLEY-BROWN and R. MILLETTE, Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan: Characterization of the immediate early mRNA of herpes simplex virus 1.
- T. BECK and R. MILLETTE, Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan: In vitro transcription of HSV-1 DNA by RNA polymerase from Hep-2 cells.
- B. JACQUEMONT, R. DANTE, A. GARCIA, and J. HUPPERT, Unité de Virologie, INSERM, Lyon, France: Selective nuclear cleavage of viral high molecular weight RNA in cells infected by herpes simplex type 1.
- L. FELDMAN, A. CHEN, T. BEN-PORAT, and A. S. KAPLAN, Department of Microbiology, Vanderbilt University, Nashville, Tennessee: Fine mapping of the immediate-early transcripts of pseudorabies (Pr) virus.
- M. F. STINSKI, D. R. THOMSEN, M. W. WATHEN, and T. R. LOEPFLE, Department of Microbiology, University of Iowa, Iowa City: Transcription of the human cytomegalovirus genome.
- C. C. CHUA and S. C. ST. JEOR, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Synthesis of virus specific messenger RNA in cells productively infected with human cytomegalovirus.
- W. KING, A. POWELL, N. RAAB-TRAUB, T. DAMBAUGH, and E. KIEFF, University of Chicago, Illinois: Epstein-Barr virus RNA in growth transformed (restringently infected) cells.
- R. Y. LAU, A. TANAKA, and M. NONOYAMA, Laboratory of Molecular Virology, Life Sciences Biomedical Research Institute, St. Petersburg, Florida: Transcription and replication of viral genome in lymphoblastoid cells (super)infected with Epstein-Barr virus.

Session 6: Poster Session—Enzymes, Proteins, and Immunology

- G. PAULI, B. NORRILD, and H. LUDWIG, Institut für Virologie der Freien Universität, Berlin, Germany: Expression of common antigenic determinants on HSV-1 and BHM virus infected cells.
- S. PERSON, K. G. KOUSOULAS, and S. C. WARNER, Biophysics Program, Pennsylvania State University, University Park: The synthesis and processing of glycoproteins specified by syncytial mutants of HSV-1.
- S. WELLING-WESTER, Laboratorium voor Medische Microbiologie Rijksuniversiteit Groningen, The Netherlands: Herpesvirus induced proteins obtained after limited proteolysis of infected Vero cells.
- H. A. BLOUGH, C. B. BARON, and J. IVANOFF, University of Pennsylvania, Philadelphia: The fusion factor of herpesvirus.
- T. BLOCK and R. HUGHES, Department of Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York: Possible co-transfer of unlinked markers to Ltk⁻apt⁻ cells biochemically transformed by HSV.
- A. MINSON, K. BASTOW G. DARBY, and P. WILDY, Department of Pathology, University of Cambridge, England: Properties of cells which carry the HSV-2 thymidine kinase gene.
- M. HELLER and E. KIEFF, Kovler Viral Oncology Laboratory, University of Chicago, Illinois: The transfer of portions of the EBV genome into L TK⁻ cells.
- R. J. FEIGHNY and J. S. PAGANO, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Sequential synthesis of virus-induced proteins after superinfection of Raji cells with EBV.
- D. L. WERNER and W. GIBSON, Johns Hopkins University School of Medicine, Baltimore, Maryland: Identification and characterization of a matrix-like protein in cells infected with human cytomegalovirus.
- M. P. FARRELL, C. MOORE, J. D. GRIFFITH, and E.-S. HUANG, Cancer Research Center, University of North Carolina, Chapel Hill: Subviral nucleoprotein complex of human cytomegalovirus.
- B. WAHREN* and B. ÖBERG† *Department of Virology, Karolinska Institute, Stockholm; †Astra Läkemedel AB, Södertälje, Sweden: Inhibition and induction of CMV late antigens during and after PFA.
- M. I. SIQUEIRA-LINHARES, N. FAUCON-BIGUET, Y. CHARDONNET, and J. P. REVILLARD, INSERM, Lyon, France: Comparative polypeptides of cytomegalovirus isolated from renal transplanted patients and from young children.

- A. E. BOURKAS and J. MENEZES, University of Montreal, Ste. Justine Hospital, Canada: Comparative studies on the immunobiology of Fc receptors induced by human herpesviruses in different lymphoid cell types.
- M. BARTKOSKI, Department of Microbiology, Uniformed Services University School of Medicine, Bethesda, Maryland: The effect of actinomycin D and cordycepin on the pattern(s) of protein synthesis in herpes simplex virus type 1 (HSV-1) infected cells.
- Y. LANGELIER,* S. QUALIZZA,* and †G. BUTTIN, *Institut du Cancer, Montreal, Canada; †Unité de Génétique Cellulaire, Institut de Recherche en Biologie Moléculaire, Paris, France: Characterization of ribonucleotide reductase induction in BHK-21/C13 syrian hamster cell line upon infection by herpes simplex virus (HSV).
- B. ERIKSSON, E. HELGSTRAND, and B. ÖBERG, Astra Läkemedel AB, Research and Development Laboratories, Södertälje, Sweden: Pyrophosphate analogs as inhibitors of herpesvirus DNA polymerase.
- J. J. MCGOWAN and G. A. GENTRY, Department of Microbiology, University of Mississippi Medical Center, Jackson: Differences in the evolutionary status of deoxythymidine kinases of herpesviruses and host cells.
- G. FLAMINIO, G. TARRO, G. GAIA TRINCHERA, W. FOSTER, and G. PAPA, University of Naples, Cotugno Hospital, Italy: HSV-induced nonvirion antigen in guinea pig cells multiplying in spinner flasks.
- D. GROSSBERGER and W. CLOUGH, Molecular Biology Department, University of Southern California, Los Angeles: Epstein-Barr virus induced DNA polymerase.
- W. CLOUGH, Molecular Biology Department, University of Southern California, Los Angeles: Epstein-Barr virus-associated nuclease activity.
- J. E. OAKES, W. B. DAVIS, and W. A. WEPPIER, Department of Microbiology and Immunology, University of South Alabama College of Medicine, Mobile: Role of antibody as a host defense mechanism against primary herpes simplex virus type 1 infection of the cornea.
- J. HILFENHAUS and H. MOSER, Behringwerke Research Laboratories, Marburg, Germany: Comparison of various methods of determining antibodies against herpes simplex virus type 1 proved ELISA as the only assay for selecting seronegative patients.
- H. MOSER, E. WEINMANN, and J. HILFENHAUS, Behringwerke Research Laboratories, Marburg, Germany: Protection of mice infected with herpes simplex virus type 1 or type 2 by immunization with a herpes simplex virus type 1 particle or envelope antigen vaccine.
- S. S. MORSE and P. S. MORAHAN, Department of Microbiology, Medical College of Virginia, Richmond: Inhibition of herpes simplex virus (HSV) by macrophages.
- D. WESTMORELAND, Department of Medical Microbiology, Welsh National School of Medicine, Cardiff, S. Wales, United Kingdom: Lymphoblastoid response to herpes simplex virus type II by human fetal lymphocytes.
- J. SCHIRM and T. H. THE, Clinical Immunology Division, University Hospital, Gronigen, The Netherlands: In vitro lymphocyte transformation induced by cytomegalovirus infected human fibroblasts.
- K. S. ROSENTHAL and J. L. STROMINGER, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: An EBV induced antigenic determinant on EHRB-RAMOS which cross reacts with T cell antigens.
- R. VELTRI, J. MCCLUNG, A. HENDRICKS, J. HICKS, and P. SPRINKLE, Departments of Otolaryngology and Medicine, West Virginia University, Morgantown: Consequences of activation of Epstein-Barr virus in lymphoproliferative diseases.

Session 7: Structural Proteins

Chairpersons: P. SPEAR, University of Chicago, Illinois
M. STINSKI, University of Iowa, Iowa City

- M. PARA, R. BAUCKE, and P. G. SPEAR, University of Chicago, Illinois: An Fc-binding glycoprotein induced by herpes simplex virus (HSV)—Its presence in the virion envelope and introduction into cells by input virus.
- R. COURTNEY, R. EBERLE, E. WENSKE, and R. DIX, Department of Microbiology, University of Tennessee, Knoxville: The major glycoprotein region of herpes simplex virus (HSV), types 1 and 2—Biochemical and immunological studies.
- R. J. EISENBERG* and G. H. COHEN,† *School of Veterinary Medicine and †School of Dental Medicine, University of Pennsylvania, Philadelphia: Structural studies of glycoproteins A, B, and D of herpes simplex virus type 1.

- G. H. COHEN,* M. PONCE DELEON,* and R. J. EISENBERG,† *School of Dental Medicine and †School of Veterinary Medicine, University of Pennsylvania, Philadelphia: Tryptic peptide analysis of the type common glycoprotein D of HSV-1 and HSV-2.
- D. H. WATSON, R. A. KILLINGTON, E. J. OULDRIDGE, P. R. KINCHINGTON, and B. E. DUNCAN, Department of Microbiology, University of Leeds, England: Strain variation in particle surface antigens of herpes simplex virus type 1.
- D. A. THORLEY-LAWSON and D. E. SHALLOWAY, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: The membrane/neutralizing antigen complex of Epstein-Barr virus—I. Identification of the components and their immunogenicity.
- C. M. EDSON and D. A. THORLEY-LAWSON, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: The membrane/neutralizing antigen complex of Epstein-Barr virus—II. Characterization of the components and their assembly.
- B. C. STRNAD, R. H. NEUBAUER, and H. RABIN, Biological Carcinogenesis Program, Frederick Cancer Research Center, Maryland: Three high molecular weight surface glycoproteins associated with Epstein-Barr virus membrane antigen.
- K. GEILINGER and D. A. THORLEY-LAWSON, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Monoclonal antibodies against Epstein-Barr virus membrane antigens.
- G. J. HOFFMAN* and S. D. HAYWARD,† Departments of *Pathology and †Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Monoclonal antibodies against Epstein-Barr virus (EBV).
- N. MUELLER-LANTZSCH, N. YAMAMOTO, B. GEORG, and H. ZUR HAUSEN, Institut für Virologie der Universität Freiburg, Germany: Analysis of early and late EBV associated polypeptides by immunoprecipitation.
- H. WOLF,* G. BORNKAMM,† and A. GRAESSMANN,‡ *Max von Pettenkofer-Institut, Munich; †Institut für Hygiene, Freiburg; ‡Institut für Molekularbiologie und Biochemie, Berlin, Germany: Expression of Epstein-Barr virus genes in different cell types after microinjection of viral DNA.
- P. O'HARE and R. W. HONESS, Division of Virology, National Institute for Medical Research, Mill Hill, London, England: Structural and nonstructural polypeptides of a simian oncogenic herpesvirus—*Herpesvirus saimiri*.

Session 8: Enzymes and Nonstructural Proteins

Chairpersons: S. SILVERSTEIN, Columbia University, New York, New York
J. HAY, Uniformed Services University of Health Sciences, Bethesda, Maryland

- L. HAARR and H. MARSDEN, MRC Virology Unit, Institute of Virology, Glasgow, Scotland: Separation of HSV-1 induced polypeptides by 2-dimensional gel electrophoresis.
- K. WILCOX,* A. KOHN,† E. SKLYANSKAYA,† and B. ROIZMAN,† *Department of Microbiology, Medical College of Wisconsin, Milwaukee; †Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Evidence that phosphorylation regulates the function of virus-specific proteins in cells infected by herpes simplex virus.
- D. MACDONALD, M. SUH, and H. S. MARSDEN, MRC Virology Unit, Institute of Virology, Glasgow, Scotland: Polypeptide processing in HSV-infected cells.
- H. C. KAERNER, K.-W. KNOPF, and H. FISCHER, Institut für Virusforschung, Heidelberg, Germany: Virus specific basic phosphoproteins associated with herpes simplex virus type 1 (HSV-1) infected cells
- W.-C. LEUNG, Department of Pathology, McMaster University, Hamilton, Ontario, Canada: Control of repression of HSV alpha polypeptide synthesis.
- S. LEMASTER and B. ROIZMAN, Department of Microbiology, University of Chicago, Illinois: Some properties of the protein kinase and the ATPase associated with HSV-1 and HSV-2.
- F. WOHLRAB and B. FRANCKE, Department of Human Genetics, Yale University Medical School, New Haven, Connecticut: A deoxyprimidine triphosphatase present in HSV-1 infected but absent from uninfected BHK cells.
- K.-W. KNOPF, Institut für Virusforschung, Heidelberg, Germany: Herpes simplex virus type 1 DNA polymerase exhibits a proof-reading exonucleolytic activity.
- W. GIBSON, T. MALONE, and K.-T. JEANG, Johns Hopkins University School of Medicine, Baltimore, Maryland: An "early" human cytomegalovirus protein binds to DNA.

- S. MICHELSON,* F. HORODNICEANU,† M. TARDY-PANIT,† and M. KRESS,‡ *Laboratoire de Medecine Experimentale, College de France, Paris; †Unité de Virologie Médicale, Institut Pasteur, Paris; ‡Institut de Recherches sur le Cancer, Villejuif, France: Characterization of human cytomegalovirus (HCMV)-induced immediate early antigen.
- Y. C. CHENG,* J. Y. CHEN,* P. J. HOFFMAN,† and R. GLASER,† *Pharmacology Department, University of North Carolina School of Medicine, Chapel Hill; †Department of Medical Microbiology, College of Medicine and Comprehensive Cancer Center, Ohio State University, Columbus: Studies on the activity of DNAse, DNA polymerase, and thymidine kinase associated with EBV replication.
- R. J. FEIGHNY, A. K. DATTA, B. COLBY, and J. S. PAGANO, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Acycloguanosine and Epstein-Barr virus associated DNA polymerase.

Session 9: Poster Session—Latency, Transformation, and Biology

- J. RAJCANI,* J. MATIS,* and H. FIELD,† *Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia; †Department of Pathology, Cambridge University, England: Immediate early and early herpesvirus antigens were not seen in serial sections of ganglia with established latent infection.
- R. W. PRICE, Sloan-Kettering Institute, New York, New York: Outcome of acute ganglionic HSV infection in vivo—Modification by systemic resistance and axonal injury.
- H. J. ZWEERINK,* D. MARTINEX,* and L. COREY,† *Merck Institute for Therapeutic Research, West Point, Pennsylvania; †University of Washington School of Medicine, Seattle: Immunological and virological studies of recurrent herpetic infections.
- G. DARAI, U. GRAMLICH, L. ZÖLLER, B. MATZ, A. SCHWAIER, R. M. FLUGEL, and K. MUNK, Institut für Medizinische Virologie der Universität Heidelberg; Battelle-Institut Frankfurt am Main; Institut für Virusforschung am Deutschen Krebsforschungszentrum Heidelberg, Germany: Experimental infection and the state of viral latency in adult tupaia with herpes simplex virus type 1 and 2—Infection of juvenile tupaia with temperature sensitive mutants of HSV type 2.
- M. SUH, N. POIRIER, A. KESSOUS, and R. SIMARD, Institut du Cancer de Montreal, Centre Hospitalier Notre-Dame, Canada: Immunoprecipitation of polypeptides from hamster cells transformed by herpes simplex virus type 2.
- E. J. SHILLITOE, J. S. GREENSPAN, L. S. HANSEN, and S. SILVERMAN, Department of Oral Medicine, University of California, San Francisco: Herpes simplex virus neutralization by serum from oral cancer patients.
- L. KUCERA,* A. HALE,* L. DANIEL,† and M. WAITE,† Departments of *Microbiology and Immunology, and †Biochemistry, Bowman Gray School of Medicine, Winston-Salem, North Carolina: Parameters distinguishing herpes simplex virus type 2 transformed tumorigenic and nontumorigenic rat cells.
- D. SCHNEIDER and D. FALKE, Division of Experimental Virology, Institute for Medical Microbiology, University of Mainz, Germany: Investigations on the mechanism of induction of the alkaline phosphatase by BdU and hydrocortisone in HSV-transformed cells.
- R. J. REISS-GUTFREUND, V. DOSTAL, and K. LETNANSKY, Institute for Cancer Research, University of Vienna, Austria: HSV infection as a co-factor in tumor formation.
- C. H. SCHRÖDER and G. URBACZKA, Deutsches Krebsforschungszentrum Institut für Virusforschung, Heidelberg, Germany: Homologous interference in serial high multiplicity passages of herpes simplex virus (HSV).
- P. A. FURMAN, P. V. MCGUIRT, and G. B. ELION, Wellcome Research Laboratories, Research Triangle Park, North Carolina: Effect of acyclovir on the DNA synthesis of cells transformed with herpes virus genetic information.
- V. DOSTAL, E. TRAVNICZEK, and R. REISS-GUTFREUND, Institute for Cancer Research, University of Vienna, Austria: Enhanced DNA repair after UV irradiation in human lymphocytes infected with HSV-1 and HSV-2.
- H. HAMPL, J. R. SCHLEHOFER, and K. -O. HABERMEHL, Institut für Klinische und Experimentelle Virologie der Freien Universität Berlin, Hindenburgdamm, Germany: Type specific differences in the release of newly synthesized herpes simplex viruses from HEP-2 cells.
- J. -R. SCHLEHOFER, H. HAMPL, and K. -O. HABERMEHL, Institut für Klinische und Experimentelle Virologie der Freien Universität Berlin, Hindenburgdamm, Germany: Cell-line and virus-strain depending differences in the morphology and permeability of herpesvirus-infected cells.

- S. ALENIUS,* and B. ÖBERG,† *Department of Virology, College of Veterinary Medicine, Uppsala, Sweden; †Astra Läkemedel AB, Research and Development Laboratories, Södertälje, Sweden: Mechanism and therapeutic effect of trisodium phosphonoformate on experimental herpes simplex virus infections.
- M. J. TOCCI and S. C. ST. JEOR, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: The replication of human cytomegalovirus in lymphoblastoid cells.
- L. L. WILLIAMS,* and J. R. BLAKESLEE, JR.,† *Department of Pediatrics and †Department of Veterinary Pathobiology, Ohio State University, Columbus: New human cytomegalovirus isolated from cultured skin fibroblasts of a familial neuropathy.
- J. F. BASKAR and E. -S. HUANG, Cancer Research Center and Department of Medicine, University of North Carolina School of Medicine, Chapel Hill: Effect of murine cytomegalovirus on pre-and post-implantation mouse embryos.
- J. NEDRUD, J. S. PAGANO, and A. M. COLLIER, Cancer Research Center and Departments of Pediatrics, Bacteriology and Immunology, and Medicine, University of North Carolina School of Medicine, Chapel Hill: Persistent murine cytomegalovirus infections in epithelial cells of tracheal organ culture—Possible mechanism of chronicity.
- H. GADLER,* and C.-G. GROTH,† *Department of Virology, National Bacteriological Laboratory, Stockholm; †Transplantation Unit, Huddinge Hospital, Sweden: In vitro activation of human cytomegalovirus.
- J. JONCAS, M. LEYRITZ, J. GAGNON, P. BROCHU, and P. PAYER, University of Montreal, Ste.-Justine Hospital, Canada: Dual congenital infection with the Epstein-Barr virus (EBV) and the cytomegalovirus (CMV).
- T. GOTLIEB-STEMATSKY,* J. ZONIS,† A. ARLOZOROFF,** T. MOSES,‡ and M. SIGAL,‡ *Central Virus Laboratory, Ministry of Health, Tel-Aviv-Yafo; †Department of Neurology, Assaf Harofeh Government Hospital, Zerifin; ‡Ness-Ziona Government Hospital, and *Tel-Aviv University Sackler School of Medicine, Ramat-Aviv, Israel: Association between psychotic disorders and antibody titers to Epstein-Barr virus (EBV) and herpes simplex type 1 (HSV-1).
- L. A. PAQUIN,* H. S. MAURER,† D. T. PURTILLO,* *University of Massachusetts Medical Center, Worcester; †Northwestern University Medical School, Chicago, Illinois: Cytogenetic abnormalities in the X-linked lymphoproliferative syndrome.
- W. MARK,* M. PHELPS,* J. DOMORADZKI,† and B. SUGDEN,* *McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; †Dow Chemical Corporation, Midland, Michigan: The DNA of Epstein-Barr virus is amplified in transformed lymphocytes.
- Y. YAJIMA and R. GLASER, Department of Medical Microbiology and Comprehensive Cancer Center, Ohio State University College of Medicine, Columbus: The interaction of nontransforming Epstein-Barr virus (HR-1 EBV) with EBV positive and negative lymphoblastoid cell lines.
- B. KAYIBANDA and C. ROSENFELD, Institute of Cancer and Immunogenetics, Villejuif, France: Presence of Epstein-Barr virus DNA and nuclear antigen in cell lines spontaneously established from peripheral blood leukocytes from normal and leukemic donors.
- N. YAMAMOTO and H. ZUR HAUSEN, Institut für Virologie der Universität, Freiburg, Germany: Induction of EBV early antigens by TPA is not affected by inhibition of DNA synthesis.
- T. GOTLIEB-STEMATSKY,** A. VONSOVER,* K. PERK,† and A. YANIV‡ *Central Virus Laboratory, Ministry of Health, Tel-Aviv-Yafo; †Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv; ‡Department of Animal Science, Hebrew University of Jerusalem, Rehovot, Israel: Characterization of a type C virus derived from Burkitt's lymphoma cell line P₄HR₁.
- H. K. ADLINDER and D. A. BENFIELD, Department of Microbiology, University of Missouri, College of Veterinary Medicine, Columbia: Latent herpesvirus of turkeys and fertility.
- M. GALLATIN and B. M. LONGENECKER, Department of Immunology, University of Alberta, Edmonton, Canada: Expression of Genetic resistance to Marek's disease at the target cell level.
- E. C. HAHN and A. J. KENYON, Walker Laboratory, Memorial Sloan-Kettering Cancer Center, Rye, New York: Antibody activity to tumor cell antigens in sera from Marek's disease virus-infected chickens.
- M. JERKÓFSKY, Department of Microbiology, University of Maine, Orono: Replication of Marek's disease virus and herpesvirus of turkeys in a newly established stable line of chick cells.
- K. HIRAI,* N. KITAMOTO,† K. IKUTA,‡ and S. KATO,† *Department of Molecular Biology, Tokai University School of Medicine, Isehara, Japan; †Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Suita, Japan: Persistence of both herpesvirus of turkey and Marek's disease virus genomes in a chicken lymphoblastoid cell line.
- R. A. ROBINSON, B. E. HENRY, and D. J. O'CALLAGHAN, Department of Microbiology, University of Mississippi Medical Center, Jackson: Quantitation and identification of equine herpesvirus type 1 (EHV-1).

- D. R. JOHNSON and G. KLEIN, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Herpesvirus ateles (HVA) infection of marmoset (*saguinus*) lymphocytes—Evidence for viurs receptors and stimulation of DNA synthesis after infection.
- L. GEDER, M. DAWSON, R. HYMAN, and F. RAPP, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Properties of mouse cell lines transformed in vitro by the HMC-strain of infectious bovine rhinotracheitis virus.
- G. DARAI, L. ZÖLLER, U. GRAMLICH, B. MATZ, R. M. FLÜGEL, C. SCHRÖDER, K. MUNK, W. HOFFMANN, P. MÖLLER, and H. GELDERBLOM, Institut für Medizinische Virologie, Universität Heidelberg, Germany: Tupaia herpesvirus—Isolation and characterization of a third herpesvirus of tupaia; oncogenic properties of tupaia herpesviruses in rabbits.

Session 10: Immunology

Chairpersons: A. NAHMIAS, Emory University, Atlanta, Georgia
G. MILLER, Yale University, New Haven, Connecticut

- A. M. ARVIN, R. B. POLLARD, L. E. RASMUSSEN, and T. C. MERIGAN, Stanford University Medical Center, California: A prospective study of cell-mediated and humoral immunity to varicella-zoster (VZ), herpes simplex (HSV), and cytomegalymphoma.
- M. D. HILTY, D. R. MANN, and P. J. HOFFMANN, Departments of Pediatrics and Medical Microbiology, Ohio State University, Columbus: Analysis of antibodies to herpes simplex virus (HSV) polypeptides in sera of patients with HSV infection.
- B. T. ROUSE,* A. S. GREWAL,† and L. A. BABIUK,† *Department of Microbiology, College of Veterinary Medicine, University of Tennessee, Knoxville; †Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada: Destruction of herpesvirus infected cells by neutrophils and complement—A new mechanism of antiviral immunity.
- R. N. LAUSCH* and K. A. HAY,† *Department of Microbiology and Immunology, University of South Alabama, Mobile; †Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Cytotoxic activity of lymphoid cells from hamsters sensitized to HSV-1.
- M. COLIN JORDAN, Department of Medicine and Reed Neurological Research Center, University of California Medical School, Los Angeles: Adverse effects of cytomegalovirus vaccination in mice.
- G. QUINNAN and J. MANISCHEWITZ, Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland: Virus specific antibody dependent cell-mediated cytotoxicity (ADCC) during murine cytomegalovirus (MCMV) infection.
- L. M. HUTT-FLETCHER and C. J. GILBERT, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Induction of cytotoxic cells by Epstein-Barr virus antigens.
- C. A. BIRON, L. M. HUTT-FLETCHER, and J. S. PAGANO, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Interferon production and effector cell activation concurrent with lymphoblastoid cell line stimulation in vitro.
- L. E. WALLACE, A. B. RICKINSON, and M. A. EPSTEIN, Department of Pathology, University of Bristol Medical School, England: T cell-mediated immunity to Epstein-Barr virus in virus-infected individuals.
- Y. HINUMA and K. SUGAMURA, Department of Microbiology, Kumamoto University Medical School, Japan: In vitro induction of cytotoxic T cells specific for Epstein-Barr virus (EBV)-transformed cells.
- I. S. MISKO, D. J. MOSS, and J. H. POPE, Queensland Institute of Medical Research, Brisbane, Australia: The specificity of cytotoxic T lymphocytes generated in vitro in response to EB virus.

Session 11: Latency

Chairpersons: D. WATSON, Leeds University, England
J. STEVENS, University of California, Los Angeles

- C. CABRERA, E. CANTIN, A. L. NOTKINS, H. OPENSHAW, A. PUGA, J. D. ROSENTHAL, T. SEKIZAWA, and C. WOHLLENBERG, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland: HSV-1 latency in nervous tissue.
- D. A. GALLOWAY,* C. FENOGLIO,† and J. K. MCDUGALL,* *Fred Hutchinson Cancer Research Center, Seattle, Washington; †Department of Pathology, Columbia University College of Physicians and Surgeons, New York, New York: Detection of herpes simplex RNA in human sensory ganglia.

- R. TENSER, Departments of Medicine and Microbiology, Pennsylvania State University College of Medicine, Hershey: The role of herpes simplex virus (HSV) thymidine kinase (TK) expression in acute and latent infection of the trigeminal ganglion.
- A. L. GOLDIN, M. LEVINE, and J. C. GLORIOSO, University of Michigan, Ann Arbor: Persistence of viral genes in neuronal cells surviving HSV-1 infection.
- A. M. COLBERG-POLEY, H. C. ISOM, and F. RAPP, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Stimulation of herpes simplex virus type 2 replication from a quiescent state by superinfection with human cytomegalovirus.
- J. F. SHERIDAN, A. D. DONNENBERG, R. B. BELL, and L. AURELIAN, Divisions of Comparative Medicine and Biophysics, Johns Hopkins Medical Institutions, Baltimore, Maryland: Mechanism of HSV-induced latency—A transient defect of HSV-specific cell-mediated immunity in recurrent disease.
- J. M. DEMARCHI, C. A. SCHMIDT, and A. S. KAPLAN, Department of Microbiology, Vanderbilt University, Nashville, Tennessee: Analysis of the interactions of various strains of human cytomegalovirus (HCMV) with permissive and nonpermissive cells.
- F. J. DUTKO, W. C. SPEERS, and M. B. A. OLDSTONE, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: Replication of murine cytomegalovirus (MCMV) in differentiated and undifferentiated murine teratocarcinoma cells.
- J. S. PEPOSE, M. L. COOK, and J. G. STEVENS, University of California School of Medicine, Los Angeles: Marek's disease as an animal model for the Guillain-Barré syndrome.
- C. LENOIR,* T. OOKA,† M. TOVEY,‡ M. DE TURENNE,† and J. DAILLIE,† *International Agency for Research on Cancer, Lyon; †Université Claude-Bernard, Villeurbanne; ‡Institut de Recherches sur le Cancer, Villejuif, France: Activation of Epstein-Barr virus in human lymphoid cell lines.
- J.-C. LIN and J. S. PAGANO, Cancer Research Center and Department of Biochemistry and Nutrition, University of North Carolina School of Medicine, Chapel Hill: Chromosomal proteins and the reactivation of latent Epstein-Barr virus genome.
- B. KALLIN, J. LUKA, and G. KLEIN, Department of Tumor Virology, Karolinska Institute, Stockholm, Sweden: Immunochemical analysis of EBV-related polypeptides in latently infected P3H3 cells after induction of the productive cycle by n-butyrate.

Session 12: Transformation and Biology

Chairpersons: S. BACHENHEIMER, University of North Carolina, Chapel Hill
F. RAPP, Pennsylvania State University, Hershey

- S. KIT,* H. QAVI,* M. HAZEN,* D. R. DUBBS,* and S. PATHAK,† *Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas; †Department of Biology, University of Texas System Cancer Center, M.D. Anderson Hospital, Houston: Sites of integration of herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) gene in human chromosomes.
- G. R. REYES and G. S. HAYWARD, Department of Pharmacology, Johns Hopkins Medical School, Baltimore, Maryland: Morphological and biochemical transformation with fragments of HSV-2 DNA.
- A. CAMACHO and P. G. SPEAR, University of Chicago, Illinois: The expression of specific proteins in cells transformed by a fragment of the herpes simplex virus (HSV) genome.
- R. J. JARIWALLA, L. AURELIAN and P.O.P. TS'O, Divisions of Biophysics and Comparative Medicine, Johns Hopkins Medical Institutions, Baltimore, Maryland: Tumorigenic transformation of Syrian hamster embryo (SHE) cells by a specific fragment of herpes simplex virus type 2 DNA.
- F. RAPP and Y. NISHIYAMA, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Inhibition of herpes simplex virus type 2 (HSV-2) induced transformation by interferon.
- J. M. LYDEN and N. FRENKEL, Department of Biology, University of Chicago, Illinois: Mapping of the viral DNA sequences present in herpes simplex virus thymidine kinase transformed cells.
- J. C. M. MACNAB, M. PARK, D. M. LONSDALE, and J. H. SUBAK-SHARPE, Institute of Virology, Glasgow, Scotland: Genetic information retrieval from herpes simplex virus transformed rat embryo cells by recombination with superinfecting herpes simplex virus.
- M. K. HOWETT,* A. E. PEGG,† and F. RAPP,* Departments of *Microbiology and †Physiology, Pennsylvania State University College of Medicine, Hershey: Cocarcinogenesis of viruses and chemicals—Nitrosomethylurea treatment of mouse cells enhances DNA repair.

- M. M. MANAK, L. AURELIAN, and P.O.P. TS'Ō, Divisions of Biophysics and Comparative Medicine, Johns Hopkins University, Baltimore, Maryland: Inactivation of HSV-2 by BUdR and nUV light during the course of the infectious cycle—Time of appearance of transforming activity.
- A. VAHLNE, B. SVENNERHOLM, and E. LYCKE, Department of Virology, Institute of Medical Microbiology, University of Göteborg, Sweden: Evidence for herpes simplex virus type-selective receptors on cellular plasma membranes.
- F. GERVAIS, A. WILLS, M. LEYRITZ, and J. JONCAS, Pediatric Research Center and Department of Microbiology, University of Montreal, Ste. Justine Hospital, Canada: Lack of Epstein-Barr virus (EBV) receptors on B cells from persistently EBV seronegative adults.
- E. VESTERINEN, J. NEDRUD, A. COLLIER, and J. S. PAGANO, Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill: Epstein-Barr virus infection of human cervical epithelial cells in vitro.
- T. L. STANWICK and A. J. NAHMAS, Emory University School of Medicine, Atlanta, Georgia: Inhibition of interferon activity on HSV types 1 and 2 by cyclic GMP enhancing compounds.

Session 13: Transformation

Chairpersons: J. PAGANO, University of North Carolina, Chapel Hill
G. KLEIN, Karolinska Institute, Stockholm, Sweden

- G. MILLER, J. ROBINSON, and E. GROGAN, Yale University School of Medicine, New Haven, Connecticut: Transfection of human lymphoid cells with the DNA of herpes simplex virus.
- D. T. PURTILO, H. J. FREED, K. SAKAMOTO, and L. A. PAQUIN, University of Massachusetts Medical School, Worcester: Immunopathology of the X-linked lymphoproliferative syndrome.
- K. SAKAMOTO, H. J. FREED, and D. T. PURTILO, University of Massachusetts Medical School, Worcester: Epstein-Barr virus antibodies in X-linked lymphoproliferative syndrome.
- J. LUKA and G. KLEIN, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Purification and biochemical studies on Epstein-Barr virus determined nuclear antigen (EBNA).
- D. HENTZEN,* J. DAILLIE,* and G. LENOIR,† *Université Claude-Bernard, Villeurbanne, France; †International Agency for Research on Cancer, Lyon, France: Characterization of the Epstein-Barr virus determined antigen.
- R. H. NEUBAUER, H. RABIN, and B. C. STRNAD, Biological Carcinogenesis Program, Frederick Cancer Research Center, Maryland: Nuclear antigens of Epstein-Barr virus (EBV) and EBV-like simian viruses—Demonstration of multiple antigenic determinants.
- I. ERNBERG,* G. KLEIN,* J. ZEUTHEN,† and B. GIOVANELLA,‡ *Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden; University of Aarhus, Denmark; †Stehlin Foundation for Cancer Research, Houston, Texas: Epstein-Barr virus primary infection of lymphocytes and subsequent selection on soft agarose.
- J. ROBINSON, Departments of Pediatrics and Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut: Kinetics of EBNA expression, DNA synthesis, and cellular proliferation in human lymphocytes infected with Epstein-Barr virus.
- K. TAKADA, K. YAMAMOTO, and T. OSATO, Cancer Institute, Hokkaido University School of Medicine, Sapporo, Japan: Abortive response to the transforming Epstein-Barr virus of leukemic lymphocytes.
- J. S. PAGANO,* J. E. SHAW,* and G. PEARSON,† *Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill; †Department of Microbiology, Mayo Clinic, Rochester, Minnesota: Analysis of nasopharyngeal carcinoma and infectious mononucleosis cell lines of American origin for Epstein-Barr virus DNA content.
- E. F. KALETA,* and K. PRESSLER,† *Klinik für Geflügel, Tierärztliche Hochschule Hannover; †Astawerke AG, Bielefeld, Germany: Prevention of Marek's disease virus induced tumors and nerve lesions by Impacarin R.

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Seventh Cold Spring Harbor Conference on Cell Proliferation:

VIRUSES IN NATURALLY OCCURRING CANCERS, September 4 – September 9

arranged by

Myron Essex, Harvard School of Public Health, Boston, Massachusetts

George Todaro, National Cancer Institute, Bethesda, Maryland

Harald zur Hausen, Albert-Ludwigs-Universität, Freiburg, Germany

200 participants

Introduction: D. BALTIMORE, Massachusetts Institute of Technology, Cambridge

Session 1: DNA Viruses I—Polyoma Viruses

Chairperson: T. BENJAMIN, Harvard Medical School, Boston, Massachusetts

- K. K. TAKEMOTO, NIAID, National Institutes of Health, Bethesda, Maryland: Human polyomaviruses—Evaluation of their possible association with human cancer.
- B. L. PADGET and D. L. WALKER, Department of Medical Microbiology, University of Wisconsin, Madison: Human papovavirus, JC—Natural history, tumorigenicity, and interaction with human cells in culture.
- M. PATER, A. PATER, and G. DI MAYORCA, Department of Microbiology, New Jersey Medical School, Newark: BK virus—A human papovavirus.
- E. GEISSLER,* H.-J. HEROLD,† L. LÜBBE,* M. RUDOLPH,† S. SCHERNECK,* H. WÄHLE,* F. WEICKMANN,‡ and W. ZIMMERMANN,* *Central Institute of Molecular Biology, †Central Institute of Cancer Research, Academy of Sciences of the GDR; ‡Neurosurgical Clinic, Municipal Clinicum, Berlin-Buch, Germany: Further studies on the relation of SV40-like viruses to human tumors.
- S. S. TREVETHIA, Department of Microbiology, Pennsylvania State University College of Medicine, Hershey: Immunological controls in papovavirus SV40 induced neoplasia.
- H. ZUR HAUSEN and L. GISSMANN, Institut für Virologie, Universität Freiburg, Germany: Characterization of a lymphotropic papovavirus.
- M. GREEN, W. S. M. WOLD, K. H. BRACKMANN, M. A. CARTAS, T. MATSUO, Q. KAPOOR, J. MACKAY, K. OLSON, and T. LEE, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Human adenovirus transforming genes—Group relationships, integration into transformed cells and expression of candidate transformation proteins, and analyses of human cancers and tonsils.

Session 2: RNA Viruses I—Murine Retroviruses

Chairperson: D. YOHN, Ohio State University, Columbus

- M. B. GARDNER, S. RASHEED, B. K. PAL, J. SCOTT, and J. D. ESTES, Department of Pathology, University of Southern California School of Medicine, Los Angeles: The natural history of cancer and retroviruses in wild mice.
- P. J. FISCHINGER,* J. N. IHLE,† J. P. LEVY,‡ D. P. BOLOGNESI,§ J. ELDER,** and W. SCHÄFER,†† *NCI, National Institutes of Health, Bethesda, Maryland; †Frederick Cancer Research Center, Frederick, Maryland; ‡Hospital Cochin, Paris, France; §Duke University, Medical Center, Durham, North Carolina; **Scripps Clinic, La Jolla, California; ††Max-Planck Institute, Tübingen, Germany: Recombinant murine leukemia viruses and protective factors in disease.
- R. SCHWARTZ, S. WAKSAL, and P. MORRISSEY, Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts: Abnormal cellular differentiation predisposes to viral leukemia.
- J. N. IHLE, J. C. LEE, and L. ENJUANES, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: Chronic immune stimulation as a possible mechanism in murine C-type virus-induced lymphomas.
- T. MATTHEWS, G. ROLOSON, J. THIEL, J. COLLINS, and D. BOLOGNESI, Department of Surgery, Duke University Medical Center, Durham, North Carolina: Unique properties of protective antibodies against a transplantable murine tumor.
- U. RAPP and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Generation of sarcoma and carcinoma producing viruses by selection from endogenous mouse type C virus stocks.

- R. JAENISCH and D. JAHNER, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Universität Hamburg, Germany: Germ line integration of Moloney leukemia virus—Characterization of independently derived mouse sublines.
- J. HILKENS,* J. HILGERS,* R. MICHALIDES,* and B. VAN DER ZELIJST,† *Divisions of Genetics and Virology, Netherlands Cancer Institute, Amsterdam; †Institute for Virology, Rijksuniversiteit, Utrecht, The Netherlands: Analysis of murine retroviral genes and genes for viral membrane receptors by somatic cell genetics.
- P. J. FIALKOW, Veterans Administration Medical Center and Department of Medicine, University of Washington, Seattle: G6PD studies to determine the uni- or multicellular origin of neoplasms.
- E. KLEIN, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden: Immune response to naturally occurring tumors.

Session 3: DNA Viruses II—EBV and Related Viruses

Chairperson: J. SIMONINGER, Sidney Farber Cancer Institute, Boston, Massachusetts

- W. HENLE and G. HENLE, Division of Virology, Children's Hospital, and University of Pennsylvania School of Medicine, Philadelphia: Epstein-Barr virus in human malignancies.
- G. DE-THÈ, Faculty of Medicine A. Carrel, Lyon, and Cancer Institute, Centre National de la Recherche Scientifique, Villejuif, France: Environment and cancer—The role of the Epstein-Barr virus in initiation, promotion, and onset of malignancies.
- E. GUSSANDER* and A. ADAMS,† *Institute of Medical Microbiology, University of Gothenburg, Sweden; †Wallenberg Laboratory, University of Uppsala, Sweden: The state of the Epstein-Barr virus genome in transformed cells.
- L. FALK, New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts: Lymphotropic herpesviruses of new world monkeys.
- B. FLECKENSTEIN,* G. KEIL,* I. MÜLLER,* M. COOMEY,† and C. MULDER,† *Institute für Klinische Virologie, Universität Erlangen-Nürnberg, Germany; †University of Massachusetts Medical School, Worcester: Generation of recombinants between different strains of *Herpesvirus saimiri*.
- M. A. EPSTEIN,* D. H. CRAWFORD,* G. W. BORNKAMM,† B. G. ACHONG,* S. FINERTY,* and J. L. THOMPSON,* *Department of Pathology, University of Bristol, England; †Institut für Virologie, Albert-Ludwigs-Universität, Freiburg im Breisgau, Germany: Biological and biochemical observations on isolates of EB virus from the malignant epithelial cells of two nasopharyngeal carcinomas.
- R. NATEGH,* A. ATTARZADEH,* K. DOWLATSHAHI,† D. MOJTABAI,‡ SH. AMITI,‡ B. ARAMESH,* J. WANER,§ J. KMET,· and F. MODABBER,** *Department of Pathobiology, Tehran University School of Public Health; †Central Hospital, National Cancer Institute, Tehran; ‡Taj Pahlavi Cancer Institute, Tehran University; §Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts; **International Agency for Research on Cancer, Lyon, France; ††Institute of Biophysics and Biochemistry, Tehran University, Iran: Antibody to viral capsid antigens of Epstein-Barr and cytomegalo viruses in patients with carcinoma of esophagus.
- K.-O. FRISSEN and M.-S. CHO, Institut für Virologie, Zentrum für Hygiene der Universität, Freiburg, Germany: Complementation and recombination between Epstein-Barr virus (EBV) genomes.
- J. ROBINSON, N. BROWN, W. ANDIMAN, M. ROBERT, U. FRANCKE, K. HALLIDAY, D. M. HORSTMANN, and G. MILLER, Yale University School of Medicine, New Haven, Connecticut: Diffuse malignant lymphoma associated with primary Epstein-Barr virus infection.
- G. KLEIN, Karolinska Institute, Stockholm, Sweden: The relative role of EBV transformation and chromosomal changes in the causation of Burkitt's lymphoma.

Session 4: RNA Viruses II—Avian Retroviruses

Chairperson: P. DUESBERG, University of California, Berkeley

- D. P. FRISBY,* R. A. WEISS,* R. MACCORMICK,* D. STEHLIN,† and M. ROUSSEL,† *Imperial Cancer Research Fund Laboratories, London, England; †Institut Pasteur, Lille, France: Distribution of RAV-O related sequences in the DNA of galliform birds.
- R. L. ERIKSON, A. F. PURCHIO, E. ERIKSON, and M. S. COLLETT, Department of Pathology, University of Colorado Health Sciences Center, Denver: Comparative biochemical properties of the avian sarcoma virus transforming gene product and its homolog in normal avian cells.

- P. NEIMAN,** L. N. PAYNE,[†] R. WEISS,* L. JORDAN,* and R. N. EISENMAN,[‡] *Imperial Cancer Research Fund Laboratories, London, England; [†]Houghton Poultry Research Station, Huntingdon, England; [‡]Fred Hutchinson Cancer Research Center, Seattle, Washington: Analysis by restriction endonuclease digestion of viral DNA in bursal lymphomas induced by avian leukosis virus (ALV).
- L. B. CRITTENDEN, Regional Poultry Research Laboratory, US Department of Agriculture, East Lansing, Michigan: Viral induction of lymphoid leukosis in chickens.
- H. L. ROBINSON,* M. N. PEARSON,* P. N. TSICHLIS,[†] and J. M. COFFIN,[‡] *Worcester Foundation for Experimental Biology, Shrewsbury; [†]Tufts University School of Medicine, Boston, Massachusetts: Role of envelope antigens in non-acute avian leukosis virus associated disease.
- J. L. SPENCER* and J. S. GAVORA,[†] *Animal Diseases Research Institute, Agriculture Canada, Nepean, Ontario; [†]Animal Research Institute, Agriculture Canada, Ottawa, Ontario: Lymphoid leukosis—Natural transmission and non-neoplastic effects.
- H. BAUER, Y. YOSHIKAWA, J. IGNIATOVIC, and M. HAYAMI, Institut für Virologie FB Humanmedizin, Giessen, Germany: Onco-fetal antigens as markers for redifferentiation in malignant transformation.
- P. K. VOGT, S. S. F. HU, M. M. C. LAI, and J. NEIL, Department of Microbiology, University of Southern California School of Medicine, Los Angeles: The genetic structure of avian erythroblastosis virus, strain ES-4.
- D. STEHELIN, S. SAULE, M. ROUSSEL, A. SERGEANT, C. LAGROU, M. B. RAES, and C. ROMMENS, INSERM, Pasteur Institute, Lille, France: Three new types of transforming genes in avian acute defective leukemia viruses correlate with distinct phenotypes of virus-transformed cells.

Session 5: DNA Viruses III—Hepatitis B

Chairperson: R. SHEININ, University of Toronto, Canada

- B. S. BLUMBERG, Institute for Cancer Research, Philadelphia, Pennsylvania: Hepatitis B virus and primary cancer of the liver.
- P. L. MARION and W. S. ROBINSON, Department of Medicine, Stanford University School of Medicine, California: Hepatitis B virus in a human hepatocellular carcinoma cell line.
- P. H. HOFSCHEIDER, V. ZASLAVSKY, T.-K. WONG, and O. MARQUARDT, Max-Planck-Institut für Biochemie, Martinsried, Germany: Studies on a DNA-polymerase complex specific for A HBs-antigene producing hepatoma cell-line.
- R. L. SNYDER* and JESSE SUMMERS,[†] *Penrose Research Laboratory, Zoological Society of Philadelphia; [†]Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Strong association between woodchuck hepatitis virus (WHV) and primary liver cancer.
- J. SUMMERS,* J. M. SMOLEC,* B. WERNER,* T. J. KELLY, Jr.,[†] G. TYLER,[‡] and R. SNYDER,[‡] *Institute for Cancer Research, Philadelphia, Pennsylvania; [†]Johns Hopkins University, Baltimore, Maryland; [‡]Penrose Research Laboratory, Philadelphia, Pennsylvania: Hepatitis B— and woodchuck hepatitis virus are members of a novel class of DNA viruses.
- T. T. SUN, JIH T'AN Cancer Hospital, Chinese Academy of Medicine, Peking, China: Immunological investigations on growth pattern—early diagnosis and etiology of human PHC.
- P. MAUPAS,* A. GOUDEAU,* P. COURSAGET,* J. DRUCKER,* F. BARIN,[†] J. PERRIN,[‡] J. P. CHIRON,[†] F. DENIS,[†] I. DIOP MAR,[†] and J. SUMMERS,[‡] *Institut de Virologie, Tours, France; [†]Faculté de Médecine, Dakar, Senegal; [‡]Institute for Cancer Research, Philadelphia, Pennsylvania: HBV infection and hepatoma—Epidemiological, clinical and virology study in Senegal. Perspective of prevention by active immunization.
- K. NISHIOKA, Tokyo Metropolitan Institute of Medical Science, Honkomagome, Bunkyo-ku, Tokyo, Japan: Persistent infection of hepatitis B virus and hepatoma.
- W. SZMUNESS, Lindsley F. Kimball Research Institute, New York Blood Center, New York: The relationship between hepatocellular carcinoma (HCC) and hepatitis B virus (HBV) infections.

Session 6: RNA Viruses III—Feline Retroviruses

Chairperson: A. HURVITZ, Animal Medical Center, New York, New York

- M. ESSEX, A. H. SLISKI, M. WORLEY, C. K. GRANT, H. SNYDER, Jr., W. D. HARDY, Jr., and L. B. CHEN, Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts; and Laboratory of

- Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York: Significance of the feline oncornavirus-associated cell membrane antigen (FOCMA) in the natural history of feline leukemia.
- O. JARRETT, University of Glasgow Veterinary School, Scotland: The natural occurrence of subgroups of feline leukemia virus.
- C. J. SHERR, L. A. FEDELE, L. DONNER, L. TUREK, and G. VANDE WOUDE, NCI, National Institutes of Health, Bethesda, Maryland: Structures of the DNA proviruses of Snyder-Theilen feline leukemia and sarcoma viruses.
- F. WONG-STAAL, R. KOSHY, and R. C. GALLO, NCI, National Institutes of Health, Bethesda, Maryland: Retrovirus genomes associated with the domestic cat—A survey of tissues from leukemic and normal animals.
- E. A. HOOVER,* J. L. ROIKO,* and R. G. OLSEN,*† *Department of Veterinary Pathobiology, Ohio State University, Columbus; †Department of Microbiology, Ohio State University and Comprehensive Cancer Center, Columbus: Host-virus interactions in progressive vs regressive feline leukemia virus infection in cats.
- H. LUTZ,* N. C. PEDERSEN,† J. HIGGINS,* F. A. TROY,‡ and G. H. THEILEN,* *Department of Surgery, University of California School of Veterinary Medicine, Davis; Departments of †Medicine and ‡Biological Chemistry, University of California School of Medicine, San Francisco: Long term immune response to feline leukemia virus components in cats after natural infection.
- R. OLSEN,**† L. MATHES,* M. TARR,* M. STIFF,* S. NICHOLAS,§ S. KRAKOWKA,* and E. HOOVER,* *Department of Veterinary Pathobiology; †Department of Microbiology; ‡Comprehensive Cancer Center; §Department of Pathology, Ohio State University, Columbus: Abrogation of lymphocyte functions by feline retrovirus protein—A model for immunosuppression.
- W. D. HARDY, Jr.,* E. E. ZUCKERMAN,* A. J. MCCLELLAND,* H. W. SNYDER, Jr.,* M. ESSEX,† and D. FRANCIS,† *Memorial Sloan-Kettering Cancer Center, New York, New York; †Harvard School of Public Health, Boston, Massachusetts: The immunology and epidemiology of FeLV non-producer feline lymphosarcomas.
- N. GUTENSOHN, D. P. FRANCIS, W. D. HARDY, Jr., and M. ESSEX, Departments of Epidemiology and Microbiology, Harvard School of Public Health, Boston, Massachusetts, and Laboratory of Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York: Risk to humans from exposure to feline leukemia virus—Epidemiologic considerations.

Session 7: RNA Viruses IV—MTV and Primate Viruses

Chairperson: C. FRIEND, Mount Sinai School of Medicine, New York, New York

- P. BENTVELZEN, Radiobiological Institute TNO, Rijswijk, The Netherlands: Natural history of the murine mammary tumor virus.
- R. D. CARDIFF and L. J. T. YOUNG, Department of Pathology, University of California Medical School, Davis: Mouse mammary tumor biology—A new synthesis.
- J. SCHLOM, J. M. YOUNG, and W. N. DROHAN, NCI, National Institutes of Health, Bethesda, Maryland: The occurrence of spontaneous mammary cancer correlates with the Mendelian segregation of specific murine mammary tumor proviral sequences.
- G. SCHOCHETMAN, L. O. ARTHUR, G. G. LOVINGER, and R. J. MASSEY, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Expression of mouse mammary tumor virus (MMTV) proteins in mammary tumor cells and the development of type-specific cytotoxic and neutralizing antibodies to exogenous and endogenous MMTVs.
- S. SPIEGELMAN,* R. MESA-TEJADA,**† I. KEYDAR,* T. OHNO,* M. RAMANARAYANAN,* R. NAYAK,* J. BAUSCH,* and C. FENOGLIO,† *Institute of Cancer Research; †Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York: The presence and clinical implications of a viral-related protein in human breast cancer.
- T. G. KAWAKAMI, Comparative Oncology Laboratory, University of California, Davis: Infectious primate type-C virus.
- G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Origins and distribution of genetically transmitted retroviruses of primates.
- E. HEFTI, J. T. REYNOLDS, S. PANEM, and W. H. KIRSTEN, Departments of Pathology, Microbiology, and the Committee on Virology, University of Chicago, Illinois: Characterization of unique nucleotide sequences and antigenic determinants of a type C virus isolated from normal human cells (HEL-12 virus).

- R. S. GOODENOW, R. LEVY, and H. S. KAPLAN, Cancer Biology Research Laboratory, Stanford University School of Medicine, California: Partial characterization of the virion proteins of a C-type RNA virus produced by a human histiocytic lymphoma cell line.
- R. GALLO, F. WONG-STAAL, M. REITZ, P. JACQUEMIN, R. RUSCETTI, I. WITZ, C. SAXINGER, A. GANGULY, and R. DALLA FAVERA, NCI, National Institutes of Health, Bethesda, Maryland: Recent studies with retroviruses and primate-human leukemogenesis.

Session 8: DNA Viruses IV—Herpesviruses

Chairperson: G. HENLE, Children's Hospital, Philadelphia, Pennsylvania

- R. RAPP, Department of Microbiology, Pennsylvania State University, College of Medicine, Hershey: Transformation by herpes simplex virus.
- L. AURELIAN, I. I. KESSLER, R. J. JARIWALLA, and M. M. MANAK, Divisions of Biophysics and Comparative Medicine, Johns Hopkins Medical Institutions, and Department of Epidemiology, University of Maryland School of Medicine, Baltimore: Herpes simplex virus type 2 and cervix cancer—Structure/function of transforming gene(s).
- J. K. MCDUGALL,* C. M. FENOGLIO,† and D. A. GALLOWAY,* *Fred Hutchinson Cancer Research Center, Seattle, Washington; †Columbia University College of Physicians and Surgeons, New York, New York: Detection of herpes simplex virus RNA in latently infected ganglion cells and in cervical neoplasia.
- W. E. RAWLS, Department of Pathology, McMaster University, Hamilton, Ontario, Canada: Specific antibodies to HSV-2 among women with cervical cancer.
- T. MACK, Cancer Surveillance Program, University of Southern California, Los Angeles: Epidemiology of young adult Hodgkin's disease—Compatibility with various infectious disease models.
- H. C. HINZE and K. W. LEE, Department of Medical Microbiology, University of Wisconsin, Madison: Secretory antibody and persistent oral shedding of the rabbit mononucleosis agent, *Herpesvirus sylvilagus*.
- K. NAZERIAN, Regional Poultry Research Laboratory, US Department of Agriculture, East Lansing, Michigan: Malignant transformation of lymphoid cells by the herpesvirus of Marek's disease.
- B. W. CALNEK and K. A. SCHAT, New York State College of Veterinary Medicine, Cornell University, Ithaca: Early pathogenesis in Marek's disease.
- T. MIKAMI,* K. SUZUKI,* H. KODAMA,* I. OKADA,† M. ONUMA,* and H. IZAWA,* *Faculty of Veterinary Medicine, Hokkaido University, Sapporo; †Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan: Antigenic difference of Marek's disease tumor-associated surface antigens of MSB-1 and RPL-1 line cells derived from Marek's disease lymphoma.
- C. G. FABRICANT,* J. FABRICANT,* C. R. MINICK,† and M. M. LITRENTA,* *New York State College of Veterinary Medicine, Cornell University, Ithaca; †Cornell University Medical College, New York, New York: Herpesvirus induced atherosclerosis.

Session 9: RNA Viruses V—Bovine and Primate Viruses

Chairperson: R. GILDEN, Frederick Cancer Research Center, Frederick, Maryland

- J. F. FERRER, School of Veterinary Medicine, University of Pennsylvania, Philadelphia: Bovine leukemia—Importance as a model for viral leukemogenesis.
- J. M. MILLER, National Animal Disease Center, US Department of Agriculture, Ames, Iowa: The biology of bovine leukemia virus infection in cattle.
- M. MUSSGAY,* B. FRENZEL,† O.-R. KAADEN,† R. LORENZ,* H. D. MATHEKA,* W. MATTHAEUS,* F. WEILAND,* and J. W. WILESMITH,* *Federal Research Institute for Animal Virus Diseases, Tübingen; †School of Veterinary Medicine, Hannover, Germany: Some properties of bovine leukosis virus and its eradication from infected herds.
- R. KETTMANN,*† M. MEUNIER-ROTIVAL,‡ G. MARBAIX,* J. CORTADAS,‡ Y. CLEUTER,* M. MAMMERICKX,§ A. BURNY,*† and G. BERNARDI,§ *Département de Biologie Moléculaire, Université Libre de Bruxelles, Rhode St.-Genèse, Belgium; †Faculté des Sciences Agronomiques, Gembloux, Belgium; ‡Institut de Recherche en Biologie Moléculaire, Université Paris, France; §Institut National de Recherches Vétérinaires, Uccle, Belgium: Integration of bovine leukemia virus DNA in the bovine genome.
- S. G. DEVARE, NCI, National Institutes of Health, Bethesda, Maryland: Bovine leukemia virus—An etiologic agent associated with lymphosarcoma of domestic cattle.

- J. CLEMENTS,* F. S. PEDERSEN,† and W. A. HASELTINE,† *Johns Hopkins Medical School, Baltimore, Maryland; †Sidney Farber Cancer Institute, Boston, Massachusetts: Antigenic drift of a retrovirus—Analysis of the genome structure of antigenic variants of visna virus isolated from persistently infected sheep.
- P. CHANDRA, L. K. STFEL, H. LAUBF, S. BALIKCIOGLU, B. MILDNER, U. EBENER, K. WELTE, M. REZZONICO, and R. KREINBERG, Center of Biological Chemistry, University Medical School, Frankfurt, Germany: Immunological characterization of reverse transcriptases from human tumors—Evidence for the sub-group-specific interspecies antigenic determinants on the reverse transcriptase molecule.
- D. L. FINE and L. O. ARTHUR, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Prevalence of natural immunity to retroviruses in primates.
- S. O. WARNAAR, P. HERBRINK, G. N. P. VAN MUIJEN, J. E. T. MOËN, and J. BROUWER, Department of Pathology, University of Leiden, The Netherlands: Evidence for type C-viral related antigens and antibodies cross-reactive with type C-viral antigens in human tissues and sera.
- R. KURTH,* R. LÖWER,* J. LÖWER,* H. FRANK,† R. HARZMANN,‡ *Friedrich-Miescher Laboratorium, Max Planck Institut; †Max Planck Institut für Virusforschung, Tübingen, ‡Universitätskliniken, Tübingen, Germany: Three groups of patients with certain malignant diseases react specifically with C-type tumor virus antigens.
- J. THIEL and D. IGLEHART, Department of Surgery, Duke University Medical Center, Durham, North Carolina: Serological reactivities of an antiserum against SSV non-producer cells.
- H. W. SNYDER, JR., M. FOX, and E. FLEISSNER, Laboratory of Viral Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York: Natural human antibodies reactive with retrovirus glycoprotein.

Session 10: DNA Viruses V—Papillomaviruses

Chairperson: C. OLSON, University of Wisconsin, Madison

- W. F. H. JARRETT, University of Glasgow Veterinary School, Scotland: Papillomaviruses and bracken fern in high incidence area of bovine alimentary cancer.
- W. D. LANCASTER* and C. OLSON,† *Department of Surgery, Case Western Reserve University, Cleveland, Ohio; †Department Veterinary Science, University Wisconsin, Madison: Bovine papillomavirus and connective tissue tumors.
- P. M. HOWLEY,* M. F. LAW,* C. A. HEILMAN,* W. D. LANCASTER,‡ D. R. LOWY,* and M. A. ISRAEL,† *NCI and †NIAID, National Institutes of Health, Bethesda, Maryland; ‡ Department of Surgery, Case Western Reserve University, Cleveland, Ohio: Molecular characterization of papillomavirus genomes.
- H. PEISTER, Institut für Virologie, Zentrum für Hygiene, Freiburg, Germany: Characterization of human and bovine papillomaviruses and antibody response of the host.
- G. ORTH,* S. JABLONSKA,† M. FAVRE,* and O. CROISSANT,* *Unité des Papillomavirus, Institut Pasteur, Paris, France; †Department of Dermatology, School of Medicine, Warsaw, Poland: Epidermodysplasia verruciformis—A model for viral carcinogenesis in man.
- J. W. KREIDER, Departments of Pathology and Microbiology, Milton S. Hershey Medical Center, Hershey, Pennsylvania: Factors determining progression of Shope rabbit papilloma-carcinoma complex.
- F. O. WELTSTEIN and J. G. STEVENS, Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles: Distribution and state of viral nucleic acid in Shope papilloma virus induced tumors.

Summary: G. TODARO, National Cancer Institute, Bethesda, Maryland

This meeting was supported in part by the National Cancer Institute, the Federal Drug Administration Bureau of Biologics, and the Fogarty International Center.

POSTGRADUATE TRAINING PROGRAMS

SUMMER 1979

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

ADVANCED BACTERIAL GENETICS, June 10 – June 30

INSTRUCTORS

Davis, Ron, Ph.D., Stanford University, California
Botstein, David, Ph.D., Massachusetts Institute of Technology, Cambridge
Roth, John, Ph.D., University of Utah, Salt Lake City

ASSISTANTS

Koshland, Doug, B.A., Massachusetts Institute of Technology, Cambridge
Shorer, Stewart, B.S., Stanford University, California
Lam, Steve, B.A., University of Utah, Salt Lake City

Starting with a random pool of λ phages carrying cloned inserts of *Salmonella* DNA, students identified phages carrying portions of the histidine 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 r_{fls} (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 Tn10 insertion element. Auxotrophs were made by Tn10 insertion, and Tn10 insertions were selected near particular genes. Tn10 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

Balbinder, Elias, Ph.D., University of Colorado, Denver
Bear, David, Ph.D., University of Oregon, Eugene
Buckel, Peter, Ph.D., Boehringer Mannheim Biochemical Plant, Tutzing, Germany
Chang, Simon H., Ph.D., Louisiana State University, Baton Rouge
Clarke, Catherine M., Ph.D., University of Pittsburgh, Pennsylvania
Dahl, Gary A., B.S., University of Wisconsin, Madison
DeBouck, Christine M., B.S., Université Libre de Bruxelles, Belgium
Gottlieb, Paul D., Massachusetts Institute of Technology, Cambridge
Grana, Dolores, M.S., Centro de Investigacion del IPN, Mexico City, Mexico
Iyer, V.N., Ph.D., Carleton University, Ottawa, Canada
Kilbane, John J., B.S., Tufts University, Boston, Massachusetts
Rutledge, Barbara J., B.A., University of Georgia, Athens
Schild, David, Ph.D., University of California, Berkeley
Skold, Ola E., M.D., University of Uppsala, Sweden
Skolnick, Mark, Ph.D., LDS Hospital, Salt Lake City, Utah
Treisman, Richard H., B.A., Imperial Cancer Research Fund, Cambridge, England

SEMINARS

Silhavy, T., Harvard Medical School. *Signal peptides that position proteins in cell.*
Wensink, P., Brandeis University. *Cloning and analysis of some Drosophila middle repetitive DNA sequences.*
Bukhari, A., Cold Spring Harbor Laboratory. *Phage M: A transposable genetic element.*
Parkinson, S., University of Utah. *Genetic analysis of chemotaxis in E. coli.*
Kleckner, N., Harvard University. *Analysis of the transposable element Tn10.*
Weisberg, R., National Institutes of Health. *Mechanism of λ integration into the E. coli chromosome.*
Ptashne, M., Harvard University. *Activities of λ C1 repressor protein.*
Roberts, J., Cornell University. *The recA protein is too a protease!*
Wigler, M., Cold Spring Harbor Laboratory. *Random joining of DNA fragments in animal cells.*
Zinder, N., Rockefeller University. *My life with single-stranded DNA phages.*
Kaiser, D., Stanford University. *What we've learned about myxo-motility.*

THE TRANSFORMED CELL, June 10 – June 30

INSTRUCTORS

Hynes, Richard, Ph.D., Massachusetts Institute of Technology, Cambridge
Graessmann, Adolph, M.D., Freie Universitet, Berlin, Germany
Risser, Rex, Ph.D., University of Wisconsin, Madison

ASSISTANT

Destree, Antonia, B.S., Massachusetts Institute of Technology, Cambridge

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. The lecture topics covered initiation of transformation by tumor viruses, the parameters of the transformed phenotype, and the nature of the immune response to tumors. Established cell lines and primary cultures were transformed by SV40 virus, by SV40 DNA and 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 and V antigens, cell-surface fibronectin and cytoskeletal proteins; SDS-polyacrylamide gels; immunoprecipitation; plasminogen activator assays; and mutant transport. Mice were injected with an Abelson lymphoma and with Friend virus, and development of tumors and spleen foci were scored. Cytotoxicity tests were performed for a variety of cell-surface antigens.

Additional discussions covered technical aspects of surface labeling, antibody production, characterization and use, and microinjection methods.

PARTICIPANTS

Abrams, Frederick E., B.A., University of California, Berkeley
Armelin, Mari C.S., Ph.D., University of Sao Paulo, Brazil
Fung, Brenda P., B.S., University of California, Los Angeles
Gardner, John M., B.S., Carnegie Institution, Washington, DC
Jonak, Gerald J., Ph.D., Temple University, Philadelphia, Pennsylvania
Kaschka-Dierich, Christine, M.D., University of Göttingen, Germany
Kirschmeier, Paul T., B.S. Douglass College, New Brunswick, New Jersey
Kitagawa, Takayuki, Ph.D., Cornell University, Ithaca, New York
Kline, Stanley A., Ph.D., New York University, New York

Neumann-Haefelin, Dieter, M.D., University of Freiburg, Germany
Petit, Carol Ann, M.S., Institut de Recherches Scientifiques, Villejuif, France
Prasad, Rajendra, Ph.D., New York Medical College, Valhalla
Preston, Christopher M., Ph.D., Medical Research Council Virology Unit, Glasgow, Scotland
Segura, Magdalena, M.S., Instituto Politecnico Nacional, Mexico City, Mexico
Skolnik, Hagit, M.S., Weizmann Institute, Rehovot, Israel
Steck, Peter A., B.A., Michigan State University, East Lansing

SEMINARS

Demars, R., University of Wisconsin. *The transformed phenotype and chemical carcinogens.*
Sambrook, J., Cold Spring Harbor Laboratory. *DNA tumor viruses.*
Topp, W., Cold Spring Harbor Laboratory. *Cellular transformation by DNA tumor viruses.*
Boettiger, D., University of Pennsylvania. *Transformation of differentiated cell types.*
Sugden, W., University of Wisconsin. *Transformation by herpesviruses.*
Graessmann, A., Freie Universitet of Berlin. *Regulation of SV40 gene expression.*
Quigley, J., State University of New York Downstate Medical Center. *Proteases and transformation.*
Burrige, K., Cold Spring Harbor Laboratory. *Antibody and lectin staining of gels.*
Todaro, G., National Institutes of Health. *Growth factors produced by transformed cells.*
Lane, D., Cold Spring Harbor Laboratory. *Hybridomas.*
Hynes, R., Massachusetts Institute of Technology. *Cell surfaces and transformation.*
Feramisco, J., Cold Spring Harbor Laboratory. *Microinjection studies of cytoskeleton.*
Goldman, R., Carnegie-Mellon Institute. *Cytoskeleton, cell structure, and movement.*
Poste, G., Roswell Park Memorial Institute. *Invasion and metastasis.*
Stockert, E. Sloan-Kettering Institute. *Cell surface antigens in mouse leukemia.*
Risser, R., University of Wisconsin. *Abelson and Friend virus-induced antigens.*
Rowe, W., National Institutes of Health. *Viruses and spontaneous leukemia.*
Rosenberg, N., Tufts University. *Transformation in vivo and in vitro by acute defective leukemia viruses.*

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

INSTRUCTORS

Nicholls, John, Ph.D., M.D., Stanford Medical School, California
Muller, Kenneth J., Ph.D., Carnegie Institution of Washington, Baltimore, Maryland
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 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 proteolytic enzymes.

PARTICIPANTS

Boyle, Mary B., B.A., Yale University, New Haven, Connecticut
Fett, Michael J., M.B.B.S., Monash University, Clayton, Australia
Henderson, Leslie P., B.A., Stanford Medical School, California
Houngaard, Jørn, M.D., University of Copenhagen, Denmark
Mason, Adrian J.R., B.A., Portsmouth Polytechnic, England
Nusbaum, Michael P., B.A., University of California, San Diego
Obaid, Ana L., Ph.D., University of Pennsylvania, Philadelphia
Pellegrino, Mario, Ph.D., University of Pisa, Italy
Pine, Jerome, Ph.D., Washington University, St. Louis, Missouri
Zackson, Saul L., B.A., University of California, Berkeley

SEMINARS

- Nicholls, J.G., Stanford Medical School. *Introduction to the leech.*
———. *Sensory and motor cells.*
———. *Pumps and glial cells in the leech.*
———. *Conduction block and potassium accumulation.*
———. *Synaptic transmission—chemical and electrical.*
———. *Quantal analysis of synaptic transmission.*
———. *Cultured ganglia and individual cells.*
- Muller, K.J., Carnegie Institution of Washington. *Circuitry and recording techniques.*
———. *Structure of synapses.*
———. *Abnormal sensory cells.*
———. *The 5 cell.*
———. *Regeneration.*
- Zipser, B., Cold Spring Harbor Laboratory. *Control of sexual function by neurons in the leech CNS.*
- Parnas, I., Hebrew University. *Conduction block.*
———. *Killing single cells.*
- Weisblat, D., University of California, Berkeley. *Biology of leeches.*
———. *Development.*
- Kristan, W., University of California, San Diego. *Swimming.*
———. *Demonstration of swimming.*
- Stent, G., University of California, Berkeley. *Swimming.*
———. *Photoreceptors.*
———. *Control of heartbeat.*
- Stewart, W., National Institutes of Health. *Lucifer yellow for staining leech cells.*

NEUROBIOLOGY OF BEHAVIOR, June 10 — June 23

INSTRUCTORS

- Kandel, Eric R., M.D., Columbia University, New York, New York
Koester, John, Ph.D., Columbia University, New York, New York
Nottebohm, Fernando, Ph.D., Rockefeller University, New York, New York
Pearson, Kier, Ph.D., University of Alberta, Edmonton, Canada

A lecture course on the Neurobiology of Behavior was presented this year for the first time. This course was designed to introduce students to cellular approaches to the study of behavior and learning. Rather than being exhaustive, 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; and 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. This course, designed for advanced graduate and research workers, had an enrollment of 19 students.

PARTICIPANTS

- Anderson, Patricia A., M.A., New York University, New York
Beres, Linda S., B.S., University of Maryland, College Park
Blank, Paul S., B.A., Johns Hopkins University, Baltimore, Maryland
Bricker, Connie S., B.S., Michigan State University, East Lansing
Buchanan, James T., B.A., Washington University, St. Louis, Missouri
Cleary, Len, M.S., Columbia University, New York, New York
Cornejo-Anze, Hector J., Ph.D., University of Chicago, Illinois
Deutsch, Dale G., Ph.D., State University of New York, Stony Brook
Einstein, Jill, B.A., University of Pennsylvania, Philadelphia
Farrow, Simon J., M.A., University of Chicago, Illinois
Fox, Steven E., B.S., Downstate Medical Center, New York, New York
Grega, Debra, M.S., University of Kentucky, Lexington
Irvin, Gregg, B.A., Syracuse University, New York
Lafferman, Jeffrey A., B.S., National Institutes of Health, Bethesda, Maryland
McElligott, Sandra, Ph.D., University of Pennsylvania, Philadelphia
McGlade, Ellen K., B.S., University of Maryland, College Park
Mitchell, Susan J., Ph.D., Johns Hopkins University, Baltimore, Maryland
Niles, Walter D., B.S., University of Wisconsin, Madison
Schmidt, Rupert, Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

- Kandel, E.R., Columbia University. *Introduction to the cellular study of behavior.*
———. *Learning. I. Habituation.*
———. *Learning. II. Sensitization.*
- Koester, J., Columbia University. *Introduction to biophysics of behavior.*
———. *Repetitive firing properties and the control of behavior.*
- Krasne, F., University of Southern California. *Nerve circuitry for simple behavioral acts and their control.*
- Kupfermann, I., Columbia University. *Hormones and behavior. I.*
———. *Motivation.*
- Truman, J., University of Washington. *Hormones and behavior. II.*
- Deadwyler, S., Bowman-Gray Medical School. *Long-term plasticity in the CNS.*
- Urtz, R., National Institutes of Health. *Attention.*
- Pearson, K., University of Alberta. *Introduction to motor sequences.*
———. *Central and reflex control of movements in motor systems.*
———. *Walking the cat.*
- Kristan, W., University of California. *Leech swimming.*
- Ghez, C., Columbia University. *Voluntary movements in mammals.*
- Thach, W. T., Jr., Washington University. *Does the cerebellum learn motor programs?*
- Fuchs, A., University of Washington. *Behavioral modification of vestibulo-ocular reflex.*
- Nottebohm, F., Rockefeller University. *Introduction to communication.*
———. *Communication in birds. I. Predispositions brought to the learning of a complex motor task.*
———. *Communication in birds. II. Brain pathways for vocal learning.*
- Hoy, R., Cornell University. *Communication in crickets.*
- Milner, B., McGill University. *Language and the brain.*
- Geschwind, N., Beth Israel Hospital. *The apraxias: Neural mechanisms of disorders of learned movement.*
- Zigmond, R., Harvard Medical School. *Neurotransmitters: Role in behavioral abnormalities.*

THE MAMMALIAN CENTRAL NERVOUS SYSTEM, June 10 – July 5

INSTRUCTORS

- Hubel, David, Ph.D., Harvard Medical School, Boston, Massachusetts
Kirkwood, Peter, Ph.D., Institute of Neurology, London, England
Malpeli, Joseph, Ph.D., University of Illinois, Champaign
Sherk, Helen, Ph.D., Harvard Medical School, Boston, Massachusetts

This workshop offered laboratories and lectures on the mammalian central nervous system. It began with a week of lectures by David Hubel on general neuroanatomy, the ontogeny of the nervous system, the spinal cord, motor control, and the visual system. These lectures were supplemented by demonstrations of recording from cat thoracic motoneurons, medullary respiratory neurons, and lateral geniculate neurons. The remaining three weeks 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, they learned the appropriate surgical techniques, the preparation of microelectrodes, and histological procedures for reconstructing micro electrode tracks. In one lab participants studied the reflex activity of motoneurons in the spinal cord using both intracellular and extracellular recording techniques. Motoneurons were injected intracellularly with horseradish peroxidase (HRP) to enable their dendritic morphology to be reconstructed. In another lab, the participants recorded extracellularly from neurons in areas S1 and S111 of the somatosensory cortex, observed their response properties to somatic stimuli, and mapped the somatotopic organization of these areas. The other two labs focussed on extracellular recording from visual cortex (area 17 and 18) 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. Retrograde transport of HRP was used in the somatosensory and vision experiments to study the connections between cortical and subcortical structures. The workshop ended with participants applying techniques learned in the spinal cord and vision labs to record intracellularly from neurons in the visual cortex. At intervals throughout the workshop seminars on current research topics were given by five invited speakers and the instructors.

PARTICIPANTS

- Campbell, Norma C., B.S., University of Bristol, England
Cleland, Corey L., B.A., Northwestern University, Evanston, Illinois
Fahle, Manfred W., M.D., Max Planck Institute, Tübingen, Germany

Fischer, K. Ludwig, M.A., University of Konstanz, Germany
Lidov, Hart G.W., B.A., Johns Hopkins School of Medicine, Baltimore, Maryland
Lockton, John W., B.S., University of Glasgow, Scotland
Walsh, Christopher, B.S., University of Chicago, Illinois
Weitzel, Daniel M., B.A., Princeton University, New Jersey

SEMINARS

Gilbert, C.D., Harvard University. *Morphology of physiologically identified cells in the visual cortex.*
Kitai, S.T., Michigan State University. *Connectivity between the substantia nigra and the basal ganglia.*
LeVay, S., Harvard University. *Development and malleability of ocular dominance columns in the visual cortex.*
Moore, G.P., University of Southern California. *Theory and use of cross correlation techniques in the mammalian nervous system.*
Simpson, J.I., New York University. *Physiology and anatomy of the accessory optic system.*

SYNAPTIC STRUCTURE AND FUNCTION, June 25 – July 18

INSTRUCTORS

Rahamimoff, Rami, M.D., Hebrew University Medical School, Jerusalem, Israel
McMahan, U. Jack, Ph.D., Stanford University, California
Purves, Dale, M.D., Washington University, St. Louis, Missouri
Stevens, Charles F., M.D., Ph.D., Yale University, New Haven, Connecticut

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

Anglister, Lili, Ph.D., Weizmann Institute, Rehovot, Israel
Bader, David M., Ph.D., University of Michigan, Ann Arbor
Brett, Roger S., Ph.D., Case Western Reserve University, Cleveland, Ohio
Brown, David L., B.S., University of Virginia, Charlottesville
Eatock, Ruth A., M.S., California Institute of Technology, Pasadena
Feder, Ned, M.D., National Institutes of Health, Bethesda, Maryland
Gordon, Herman, B.A., California Institute of Technology, Pasadena
Hayashi, Jon H., B.A., Bekesy Laboratory of Neurobiology, Honolulu, Hawaii
Heinonen, Erkki A., M.D., University of Helsinki, Finland
Johnson, Jon M., B.S., Stanford University, California
Kauvar, Lawrence M., Ph.D., California Institute of Technology, Pasadena
Laureano, Manuel, B.S., University of Puerto Rico, San Juan
Maehlen, Jan, University of Oslo, Norway
Maron, Ron, M.S., Hebrew University Medical School, Jerusalem, Israel
McManus, Owen B., B.A., University of Utah, Salt Lake City
Rivera, Amelia J., M.S., University of Puerto Rico, San Juan
Stewart, Walter W., B.A., National Institutes of Health, Bethesda, Maryland
Towle, Andrew C., M.S., University of Connecticut, Storrs

SEMINARS

Rahamimoff, R., Hebrew University Medical School. *Principles of signaling in the nervous system.*

- _____ . Forces and fluxes in the generation of membrane potentials.
- _____ . The resting potential.
- _____ . The generation of the action potential.
- _____ . The conduction of the action potential.
- _____ . Ionic basis of the action potential: The currents.
- _____ . The conductances and the action potential: Analysis and reconstruction.
- _____ . The Hodgkin and Huxley model of the action potential.
- _____ . Quantal transmitter release.
- _____ . The role of calcium in transmitter liberation from motor nerve terminals.
- _____ . Frequency modulation of quantal transmitter release.
- _____ . Leakage of neurotransmitter.

McMahan, U. J., Stanford University. *The structure of synapses. I. The neuromuscular junction.*

- _____ . The structure of synapses. II. CNS synapses.
- _____ . Localization of acetylcholine receptors and acetylcholinesterase in muscle.
- _____ . Vesicle recycling at the presynaptic nerve terminal.
- _____ . Reinnervation.
- _____ . Sequence of steps in the development of the neuromuscular junction.

- Purves, D., Washington University. Introduction to excitatory synaptic transmission.
- _____ . *The ionic basis of excitatory synaptic potentials.*
 - _____ . *Synaptic inhibition.*
 - _____ . *Electrical synapses.*
 - _____ . *Synapse box demonstration.*
 - _____ . *Formation and maintenance of synaptic connections: Axon guidance, nerve growth factor, cell death, synapse elimination, selectivity, maintenance.*
 - _____ . *Denervation.*
 - _____ . *Synapse elimination.*
- Stevens, C. F., Yale University. *Steps in the opening of the postsynaptic channel and binding of agonists to receptors.*
- _____ . *Eyring rate theory and voltage dependency of channel opening.*
 - _____ . *Signal channel currents.*
 - _____ . *Fluctuation analysis: Principles and application.*
 - _____ . *Gating currents.*
- Nicholls, J., Stanford University. *Properties, connections, and regeneration of the nervous system of the leech.*
- _____ . *Demonstration of the nervous system of the leech.*
- Goodenough, D., Harvard University. *Structure and function of gap junctions.*
- _____ . *X-ray diffraction and its application to gap-junction structure.*
- Muller, K., Carnegie Institution. *Denervation and reinnervation in the nervous system of the leech.*
- _____ . *Demonstration of the nervous system of the leech.*
- Parnas, I., Hebrew University. *Demonstration of the nervous system of the leech.*
- Hall, Z., University of California, San Francisco. *Structure and activity of cholinesterase in the electric organ and mammalian tissue.*
- _____ . *Properties of the acetylcholine receptor.*
 - _____ . *Regulation of the acetylcholine receptor.*
- Mains, R., University of Colorado, Denver. *An introduction to the role of peptides in neuronal function: Bioassays and radioimmunoassays of peptides.*
- _____ . *Immunostaining and immunoprecipitation of peptides; biosynthesis of peptides; signal peptides.*
 - _____ . *Biosynthesis and localization of peptides.*
 - _____ . *VIP as a tentative inhibitory transmitter.*
 - _____ . *Is substance P a neurotransmitter at the spinal cord?*
- Black, I., Cornell University Medical College. *The catecholamine synapse.*
- _____ . *Growth and development in the sympathetic system.*
 - _____ . *Clinical disorders of the catecholaminergic system: Parkinson's disease, idiopathic hypotension, familial dysautonomia.*
- Patterson, P., Harvard Medical School. *The influence of the environment on neuronal differentiation.*
- Kanner, B., Hebrew University. *Neurotransmitters uptake into isolated presynaptic terminals and membrane vesicles.*
- Yoshikami, D., University of Utah. *Slow synaptic potentials.*
- _____ . *The microphysiology of the postsynaptic membrane.*
- Kehoe, J., Ecole Normale Supérieure. *Transmitter effects in molluscan neurons: Ionic and pharmacological characteristics.*
- LaVail, J., University of California, San Francisco. *Retrograde transport.*
- _____ . *Circadian metabolism in photoreceptors.*
- Stefani, E., Instituto Politecnico Nacional. *Calcium currents in muscle.*
- Zipser, D., Cold Spring Harbor Laboratory. *Computer models in neurobiology.*
- _____ . *Sex neurons in the leech.*
- Albrecht-Buehler, G., Cold Spring Harbor Laboratory. *Cell motility and the centriole.*
- Zigmond, R., Harvard Medical School. *Long-term biochemical changes in neurons as a result of activity.*
- Erulkar, S., University of Pennsylvania. *Functional organization of the spinal cord.*
- _____ . *Neurotransmitters in the CNS.*
 - _____ . *Neurotoxins.*
- Frank, E., Harvard Medical School. *Quantal transmission at the spinal cord.*
- _____ . *Inhibition at the spinal cord and the crayfish neuromuscular junction.*
 - _____ . *Development of the spinal cord.*
- Ascher, P., Ecole Normale Supérieure. *Acetylcholine antagonists.*
- Basbaum, A., University of California, San Francisco. *Pain mechanisms.*
- Raviola, E., Harvard Medical School. *An overview on the structure of the retina.*
- _____ . *Structure of photoreceptors and synapses at the outer and inner layers.*
 - _____ . *Transmitter release and recycling at photoreceptor synapses.*
- Baylor, D., University of California, San Francisco. *Signal flow in the retina.*
- _____ . *Transduction in photoreceptors.*
 - _____ . *Electrical microanalysis of transduction in retinal rods.*
 - _____ . *Cell interactions in the retina.*
- Lass, Y., Tel-Aviv University. *The effect of the microenvironment on the acetylcholine receptor.*
- Hudspeth, J., California Institute of Technology. *Morphological organization and function of acousticolateralis sensory systems.*
- _____ . *The transduction process of vertebrate hair cells.*

ELECTROPHYSIOLOGICAL METHODS FOR CELLULAR NEUROBIOLOGY, July 3 – July 23

INSTRUCTORS

Kehoe, Jacques, Ph.D., Ecole Normale Supérieure, Paris, France
Chiarandini, Dante, M.D., New York University, New York
Stefani, Enrique, M.D., Instituto Politecnico Nacional, 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 iontophoresis 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

Beres, Linda S., B.S., University of Maryland, College Park
Burke, Michael J., B.S., Medical College of Wisconsin, Wauwatosa
Carrow, Grant M., B.A., Harvard University, Cambridge, Massachusetts
Denburg, Jeffrey L., Ph.D., University of Iowa, Iowa City
Eberly, Lewis B., B.A., University of Texas, Galveston
Garb, Frances C., M.S., North Dakota State University, Fargo
Ip, Nancy Y., B.S., Harvard Medical School, Boston, Massachusetts
McDonald, Virginia N., B.A., University of California, Davis
Mellin, Theodore N., Ph.D., Merck Institute, Rahway, New Jersey
Obaid, Ana L., Ph.D., University of Pennsylvania, Philadelphia

THE MOLECULAR BIOLOGY AND DEVELOPMENTAL GENETICS OF *DROSOPHILA*, July 3 – July 23

INSTRUCTORS

Pardue, MaryLou, Ph.D., Massachusetts Institute of Technology, Cambridge
Gelbart, William, Ph.D., Harvard University, Boston, Massachusetts

ASSISTANT

Mohler, James, B.S., Massachusetts Institute of Technology, Cambridge

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 and Italy. 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

Cheney, Clarissa M., Ph.D., University of Pennsylvania, Philadelphia
Clark, Ellen M., B.A., University of Virginia, Charlottesville
Dwyer, Kathleen G., B.S., Princeton University, New Jersey
Kramer, Angela, M.S., University of Heidelberg, Germany
Maine, Eleanor M., B.A., Princeton University, New Jersey
Myers, Paul L., B.A., Harvard University, Cambridge, Massachusetts

O. Cornini, Peter, B.A., Brandeis University, Waltham, Massachusetts
Polito, Catello, M.D., International Institute of Genetics and Biophysics, Naples, Italy
Rosen, Jonathan I., B.A., State University of New York, Stony Brook
Schmidt, Otto, Ph.D., Yale University, New Haven, Connecticut
Spoerel, Nikolaus, Ph.D., Institut für Genetik, Karlsruhe, Germany
Stevens, Bryn, B.A., University of California, Los Angeles
Toole, John J., B.A., Roswell Park Memorial Institute, Buffalo, New York

SEMINARS

Hall, J. C., Brandeis University. *Neurogenetics*.
Strub, S., Stony Brook University. *Pattern formation and regulation*.
Cherbas, P., Harvard University. *Cell culture techniques*.
Ashburner, M., Cambridge University. *Insect endochronology*.
Young, M., Rockefeller University. *Transposable elements*.

RNA TUMOR VIRUSES, July 3 – July 23

INSTRUCTORS

Hopkins, Nancy, Ph.D., Massachusetts Institute of Technology, Cambridge
Weinberg, Robert, Ph.D., Massachusetts Institute of Technology, Cambridge
Villa-Komaroff, Lydia, Ph.D., University of Massachusetts Medical School, Worcester
White, Ray, Ph.D., University of Massachusetts Medical School, Worcester

The course included daily lectures by visitors from across the country. An extensive series of laboratory exercises was performed to develop familiarity with the most recent molecular and biological techniques that are applied to retroviruses. These exercises included titrating of leukemia and sarcoma viruses by plaque and focus titers, transfection of sarcoma and leukemia virus DNAs, Southern gel-filter transfer blotting, and recombinant DNA techniques. Among the most important of the latter were screening of a phage library of mouse cellular DNA clones to detect clones containing endogenomes, genetically-transmitted viral genomes, and subcloning of cloned retrovirus DNAs into plasmids.

PARTICIPANTS

Alkaitis, Saulius A., Ph.D., University of Wisconsin, Madison
Feingold, Jay M., B.S., University of Virginia, Charlottesville
Jaehner, Detlev, M.S., University of Hamburg, Germany
Jongstra, Jan, M.S., Friedrich Meischer Institute, Basel, Switzerland
Khan, Arifa S., Ph.D., Frederick Cancer Research Center, Maryland
Kitamura, Naomi, Ph.D., State University of New York, Stony Brook
Liou, Ruey-Shyan, Ph.D., Oak Ridge National Laboratory, Tennessee
Logan, Jonathan L., Ph.D., Harvard University, Cambridge, Massachusetts
Mol, Joseph N.M., Ph.D., Erasmus University, Rotterdam, The Netherlands
Nerenberg, Michael, I., B.A., Yale University, New Haven, Connecticut
Nissen-Meyer, Jon, Ph.D., Norwegian Radium Hospital, Oslo
Seymore, Beverly D., M.S., University of Texas, Dallas
Triadou, Patrick, M.S., Institut Pasteur, Paris, France
Van der Putten, M.H., Ph.D., University of Nijmegen, Holland
Wang, Tse-Wei, Ph.D., Oak Ridge National Laboratory, Tennessee

SEMINARS

Erikson, R., University of Colorado. *The avian-sarcoma-virus-transforming gene (src) product*.
Todaro, G., National Institutes of Health. *Endogenous viruses and their mode of transmission*.
Rowe, W., National Institutes of Health. *The natural history of murine leukemia viruses*.
Taylor, J., Institute of Cancer Research, Philadelphia. *Synthesis and structure of retrovirus DNAs*.
Hanafusa, H., Rockefeller University. *Origin of the src gene of avian sarcoma viruses*.
Lilly, F., Albert Einstein College of Medicine. *Interaction of host and viral genetic factors in mouse leukemia*.
Fischinger, P., National Institutes of Health. *The transforming genes of murine and feline sarcoma viruses*.
Risser, R., University of Wisconsin. *Viral oncogenesis in hematopoietic tissues*.
Hager, G., National Institutes of Health. *Cloning of retrovirus genomes*.
Robinson, H., Worcester Foundation. *Endogenous viruses and cancer*.
Bishop, M., University of California, San Francisco. *Origins of retrovirus-transforming genes*.
Ihle, J.N., Frederick Cancer Research Center. *Immunobiology of endogenous c-type virus expression*.
O'Donnell, P., Memorial Sloan-Kettering Cancer Center. *MuLV-related cell-surface antigens as serological markers of endogenous AKR viruses*.
Famulari, N., Memorial Sloan-Kettering Cancer Center. *Expression of MuLV env gene products in fibroblasts and lymphoid cells*.

- August, T., Johns Hopkins University. *Derivation and use of monoclonal antibodies against viral antigens.*
 Wigler, M., Cold Spring Harbor Laboratory. *DNA transfection of eukaryotic cells in culture.*
 Hicks, J., Cold Spring Harbor Laboratory. *Development and properties of a yeast cloning vehicle.*
 Gelinas, R., Cold Spring Harbor Laboratory. *DNA sequencing.*
 Gingeras, T., Cold Spring Harbor Laboratory. *Computer analysis of DNA sequences.*
 Fermisco, J., Cold Spring Harbor Laboratory. *Microinjection of cultured mammalian cells.*

BASIC NEUROANATOMICAL METHODS, July 8 – July 28

INSTRUCTORS

- LaVail, Matthew, Ph.D., University of California, San Francisco
 LaVail, Jennifer, Ph.D., University of California, San Francisco
 Basbaum, Allan, Ph.D., University of California, San Francisco

ASSISTANTS

- Sugino, Ilene, M.A., University of California, San Francisco
 Lord, Bonnie, B.A., University of California, San Francisco

This workshop is designed for graduate students and research workers interested in learning basic neuroanatomical techniques. The course is primarily a series of laboratory exercises by the participants, with lectures and demonstrations by the instructors and visiting faculty.

The classic neuroanatomical methods that were covered in this laboratory workshop include: perfusion fixation; embedding in various media; cell-staining methods that highlight neuronal and glial cell nucleic acids; silver-salt-impregnation methods in the intact nervous system and in selectively interrupted nerve-fiber bundles; bright-field, dark-field, and fluorescence light microscopy; electron microscopy; and data gathering with camera lucida and photomicrography.

Among the new methods covered were the following: various histochemical, radiochemical, and immunocytochemical techniques that demonstrate the structure and location of specific neurons or synaptic terminals, based on their characteristic neurotransmitters or state of electrical activity; tracing axonal pathways using the anterograde axoplasmic transport of radioactively labeled protein; and determining the sources of neuronal pathways using the retrograde axonal transport of suitable markers.

PARTICIPANTS

- Aceves-Pina, Efrain O., M.S., Princeton University, New Jersey
 Dennis, Michael J., M.S., Clinical Research Institute, Montreal, Canada
 Gibson, Daniel J., B.S., Johns Hopkins University, Baltimore, Maryland
 Goldman, Steven A., B.A., Rockefeller University, New York, New York
 Pong, Sheng-Shung, Ph.D., Merck Institute, Rahway, New Jersey
 Ranscht, Barbara B., Ph.D., Max-Planck-Institute, Tübingen, Germany
 Tran, Vinh T., B.A., Johns Hopkins University, Baltimore, Maryland
 Tedeschi, Bruce W., M.A., Yale University, New Haven, Connecticut
 Zigmund, Richard E., Ph.D., Harvard Medical School, Boston, Massachusetts
 Zipser, Birgit, Ph.D., Cold Spring Harbor Laboratory, New York

SEMINARS

- Hendrickson, A., University of Washington. *Light and electron microscopy.*
 _____ . *Autoradiographic methods.*
 Raviola, E., Harvard Medical School. *Freeze-fracture methods.*
 Hand, P., University of Pennsylvania. *The 2-deoxyglucose method.*
 Gershon, M., Columbia University. *Fluorescence microscopy and aminergic systems.*
 Glazer, E., University of California, San Francisco. *Immunocytochemical methods.*
 Weiner, R., University of California, San Francisco. *CNS receptors.*

MOLECULAR BIOLOGY AND GENETICS OF YEAST, July 25 – August 14

INSTRUCTORS

- Fink, Gerald R., Ph.D., Cornell University, Ithaca, New York
 Sherman, Fred, Ph.D., University of Rochester, New York
 Hicks, James B. Ph.D., Cold Spring Harbor Laboratory, New York

ASSISTANT

- Zaret, Kenneth, B.A., University of Rochester, New York

This program emphasized the major laboratory techniques used in the genetic analysis of yeast—tetrad analysis, mitotic recombination, and fine-structure mapping. The isolation and characterization of both chromosomal and cytoplasmic mutants were undertaken. Biochemical studies were performed with chromosomal and mitochondrial mutants. Analysis of eukaryotic gene structure used digestion with restriction endonucleases and gel electrophoresis. Several aspects of this yeast transformation system were also investigated.

PARTICIPANTS

Achsteller, Tilman T., M.S., University of Tübingen, Germany
Astell, Caroline, Ph.D., University of British Columbia, Vancouver
Campbell, Judith, M.S., California Institute of Technology, Pasadena
Douglas, Michael G., Ph.D., University of Texas, San Antonio
Dumas, Lawrence B., Ph.D., Northwestern University, Evanston, Illinois
Edwards, John C., B.S., University of Chicago, Illinois
Grimal, Danielle, M.S., University of Paris, France
Grunstein, Michael, Ph.D., University of California, Los Angeles
Johnson, Alexander D., B.A., Harvard University, Cambridge, Massachusetts
Laughton, Allen S., B.S., University of Utah, Salt Lake City
Lobo, Zita, M.S., Tata Institute, Bombay, India
Muller, Heinz K., D.V.M., University Hospital, Zürich, Switzerland
Nall, Barry T., Ph.D., University of Texas, Houston
Philippson, Peter, Ph.D., University of Basel, Switzerland
Procter, Alan R., Ph.D., Pfizer Central Research, Groton, Connecticut
Wills, Norma M., B.A., University of Utah, Salt Lake City

SEMINARS

Byers, B., University of Washington, Seattle. *Cytology of the yeast life cycle.*
Hartwell, L.H., University of Washington, Seattle. *Control of cell division. I.*
———. *Control of cell division. II.*
Lawrence, C., University of Rochester. *Radiation mutagenesis and repair in yeast.*
Petes, T.D., University of Chicago. *Structure and replication of yeast DNA.*
———. *Genetic analysis of repeating ribosomal DNA genes.*
Broach, J., Cold Spring Harbor Laboratory. *The yeast plasmid Scpl.*
Mortimer, R.K., University of California, Berkeley. *Genetic mapping in yeast.*
———. *Gene conversion and post meiotic segregation.*
Tzagloff, A., Columbia University Medical School. *The mitochondrial genetic code.*
Dujon, B., Centre National de la Recherche Scientifique. *Genetics of mitochondrial function.*
Holland, M., University of Connecticut, Farmington. *Isolation and analysis of yeast glycolytic genes.*
Hicks, J., Cold Spring Harbor Laboratory. *Regulation of mating types in yeast.*
Fink, G.R., Cornell University. *Transformation of yeast with hybrid DNA.*
———. *Regulation of HIS4 in yeast.*
Sherman, F., University of Rochester. *Regulation of the iso-cytochromes c.*
———. *Nonsense suppression in yeast.*

IMMUNOGENETICS AND TUMOR IMMUNOLOGY, July 30 – August 12

INSTRUCTORS

Cantor, Harvey, M.D., Harvard Medical School, Boston, Massachusetts
Paul, William, M.D., National Institutes of Health, Bethesda, Maryland
Woodland, Robert, Ph.D., University of Massachusetts Medical Center, Worcester

ASSISTANT

Chun, Linda, Ph.D., Harvard Medical School, Boston, Massachusetts

The course was intended for graduate students and research workers who are interested in studying the lymphocyte population of the mouse as material for analysis of the molecular biology of cell differentiation in higher organisms. Some of the general areas that were considered included (1) studies of lymphocyte development and the molecular basis of lymphocyte differentiation, (2) genetic and biochemical analysis of recognition structures expressed by different sets of lymphocytes, (3) the special requirements for activation of lymphocytes by antigen, (4) the role of interactions between different sets of lymphocytes in regulating the immune response to viruses and other antigens, (5) the use of monoclonal antibodies.

PARTICIPANTS

Basta, Patricia V., A.B., University of Alabama, Birmingham
Bender, Timothy P., M.S., University of Michigan, Ann Arbor

Bottomly, Kim, Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Calman, Françoise, M.D., Institut d'Embryologie, Nogent-sur-Marne, France
Devlin, James J., B.S., University of California, Irvine
DiMarzo, Sheila A., B.A., University of Rochester, New York
Finnegan, Alison, B.S., Tufts University, Boston, Massachusetts
Fleischer, Bernhard, M.D., University Giessen, Germany
Gold, Dan P., B.A., Tufts University, Boston, Massachusetts
Herrlich, Peter A., M.D., Ph.D., Universität Karlsruhe, Germany
Hiernaux, Jacques R., Ph.D., National Institutes of Health, Bethesda, Maryland
Hong, David T., A.B., Texas Technology University, Lubbock
Jakway, James P., B.S., University of Colorado, Denver
Jørgensen, Trond O., M.S., University of Tromsø, Norway
Kaschka, Wolfgang P., M.D., University of Erlangen, Germany
Lugo, James P., B.S., Massachusetts Institute of Technology, Cambridge
Mastro, Andrea M., Ph.D., University of Pennsylvania, Philadelphia
Oppenheim, Stephen, B.A., University of California, Los Angeles
Parnes, Jane R., M.D., Massachusetts Institute of Technology, Cambridge
Quan, Zoe S., B.A., University of Chicago, Illinois
Rock, Kenneth L., M.D., Peter Bent Brigham Hospital, Boston, Massachusetts
Schrier, Rachel D., B.S., University of Colorado, Denver
Zack, Donald J., A.B., Albert Einstein College of Medicine, Bronx, New York
Zlotnik, Albert, B.S., University of Colorado, Denver

SEMINARS

Davie, J., Washington University Medical School, St. Louis. *Organization of immunoglobulin genes.*
Doherty, P., Wistar Institute. *T-cell reactions to viruses.*
Germain, R., Harvard Medical School. *Biochemical analysis of specific suppressive factors.*
Goodman, J., University of California Medical School, San Francisco. *Definition of the T-cell receptor.*
Nathenson, S., Albert Einstein Medical School. *Chemistry of MHC gene products.*
Pernis, B., Columbia University. *Network models of the immune system.*
Raff, M., University College, London. *Definition of the T-cell receptor.*
Rosenthal, A., Merck Institute. *T-cell recognition of antigen.*
Scharff, M., Albert Einstein Medical School. *Cell-cell hybridization techniques—Use of variants of myeloma cells to analyze the immune system.*
Shreffler, D., Washington University Medical School, St. Louis. *Genetics of the major histocompatibility complex.*
Seidman, J., National Institutes of Health. *Arrangement and rearrangement of immunoglobulin genes.*
Sprent, J., University of Pennsylvania Medical School. *Influence of thymus processing on T-cell recognition.*
Weissman, I., Stanford University Medical School. *Development of thymocytes and T cells.*

IN-HOUSE SEMINARS



*Drs. Ian Buckley and John Smart
prepare for 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 postgraduate staff to develop their skills in defending, organizing, and presenting their research. In addition to those listed below, seminars were given by many others involved in research at this laboratory.

1978-1979

October

Ivar Giaever, General Electric Research & Developmental Center, Schenectady, New York: *Protein adsorption to surfaces.*

Francis Galibert, Hospital-St.-Louis, Paris, France: *The sequence of the EcoRI F fragment of Ad2 and its mRNAs.*

Gregory Goldberg, Roche Institute, Nutley, New Jersey: *The regulation of synthesis of ribosomal protein L12 in vitro.*

November

Charles Shoemaker, University of Iowa, Iowa City: *An H3 histone specific protein kinase from bovine thymus chromatin.*

Minoru Hirama, Basel Institute for Immunology, Switzerland: *Do immunoglobulin genes on both chromosomes translocate?*

Jöran Akusjärvi, Uppsala University, Sweden: *Structure of the spliced 5' region of the hexon messenger RNA.*

Andrew Badley, Unilever Research Laboratory, Bedford, England: *Specific fibroblast-substrate adhesions — their formation, structure, and possible role as modulators of cell behavior.*

Pradip Bandyopadhyay, Albert Einstein College of Medicine, Bronx, New York: *Structure and dynamics of DNA binding proteins.*

December

Mark Achtman, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: *Cell-cell interactions between conjugating *Escherichia coli*.*

Eliezer Huberman, Oak Ridge National Laboratory, Tennessee: *Induction of somatic mutations and cell transformation in cultured mammalian cells by chemical carcinogens.*

January

Frank Solomon, Massachusetts Institute of Technology, Cambridge: *Detailed neurite morphologies of sister neuroblastoma cells are related.*

Malcolm J. Casadaban, Stanford University School of Medicine, California: *Gene fusion.*

February

Tauseef R. Butt, Georgetown University School of Medicine, Washington, D.C. *Structure of HeLa cell chromatin as probed by nucleases and nucleoprotein modifications.*

Masayori Inouye, State University of New York, Stony Brook: *Molecular mechanism of biosynthesis and assembly of the outer membrane proteins.*

March

Marilyn Kozak, New York University School of Medicine, New York: *How do eukaryotic ribosomes select initiation regions in messenger RNA?*

Carol Prives, National Institutes of Health, Bethesda, Maryland: DNA binding properties of SV40 T antigen synthesized in vivo and in vitro.

Sherie Morrison, Columbia University, New York: Isolation and characterization of mouse myeloma cells mutant in their production of immunoglobulin.

Philip Musich, Albert Einstein College of Medicine, Bronx, New York: *Resolution of discrete nucleosome subsets in mammalian genomes.*

Edward Wagner, University of California, Irvine: *Pinning down herpes simplex transcripts.*

Sheldon Penman, Massachusetts Institute of Technology, Cambridge: *Cell architecture and macromolecular metabolism.*

April

Martin Gellert, National Institutes of Health, Bethesda, Maryland: *DNA gyrase and the biological significance of DNA supercoiling.*

Gianni Cesareni, MRC Laboratory of Molecular Biology, Cambridge, England: *Plasmid as a tool for DNA cloning and plasmid genetics.*

Rex Risser, University of Wisconsin, Madison: *Cell surface antigens of Abelson or Friend murine leukemia virus.*

Mark Willingham, National Institutes of Health, Bethesda, Maryland: *Cataloging human gene products by two-dimensional gel electrophoresis and computerized data reduction.*

Mark Willingham, National Institutes of Health, Bethesda, Maryland: *Ultrastructural distribution of contractile and cytoskeletal proteins in cultured fibroblasts.*

Leigh Anderson, Argonne National Laboratory, Illinois: *Cataloging human gene products by two-dimensional gel electrophoresis and computerized data reduction.*

May

Mark Ptashne, Harvard University, Cambridge, Massachusetts: *Turning genes on and off with repressors — The role of reiterated binding sites and protein cooperativity.*

Bruce Anderson, University of Miami, Florida: *Biochemistry and genetics of 4-methylphthalic acid utilization in soil pseudomonads.*

Larry Feldman, Vanderbilt University, Nashville, Tennessee: *Transcriptional control of the pseudorabies virus genome.*

June

Dan Skup, McGill University, Montreal, Canada: *Regulation of protein synthesis during reovirus infection.*

July

Richard A.F. Dixon, Sidney Farber Cancer Institute, Boston, Massachusetts: *Correlation of genetic and physical maps of HSV 1.*

UNDERGRADUATE SUMMER RESEARCH PROGRAM

Another aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 205 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.

Martha S. Cyert, Harvard University <i>Research Advisor:</i> B.S. Zain	Nucleotide sequence analysis of the REC/INT sites of Ad ₂ +ND ₁ -dp2 viral DNA.
Samuel M. Kunes, University of Oregon <i>Research Advisor:</i> G.P. Thomas	Studies on the control of adenovirus gene expression.
Joachim Li, University of Chicago <i>Research Advisor:</i> A.I. Bukhari	Sequencing of the products left after excision of Mu DNA from the <i>lacZ</i> gene: Adaptation of Sanger's dideoxy-method and the M13 phage system.
Leona Ling, University of California, Berkeley <i>Research Advisor:</i> Y. Gluzman	Sequence determination of the junctions between Ad ₂ and SV40 genomes in the Ad ₂ -SV40 (HEY and LEY) hybrids.
Suzanne L. Mansour, Radcliffe College <i>Research Advisor:</i> M.B. Mathews	Selection of adenovirus mRNAs using the separated strands of viral DNA fragments.
Mark E. Minie, Wesleyan University <i>Research Advisor:</i> K. Burridge	An attempt to isolate mouse lymphocyte IgG "caps" or "patches."
David Schriger, Amherst College <i>Research Advisor:</i> M. Wigler	Cloning and characterization of deletion mutants of the HSV-1 thymidine kinase gene.
Brook Chase Soltvedt, Wellesley College <i>Research Advisor:</i> Y.-S. E. Cheng	Restriction endonuclease analysis of the cloned <i>lacZ</i> carrying an ochre mutation.
Elizabeth Spatola, Wheaton College <i>Research Advisor:</i> J.B. Hicks	Isolation of mutations in the mating type locus of <i>S. cerevisiae</i> .
Ina Sporecke, Smith College <i>Research Advisor:</i> D.Y. Kwok	Nitrous acid mutagenesis of recombinant plasmids carrying the Mu <i>gin</i> gene.
<i>Olney Fellowship</i> Timothy Mitchison Oxford <i>Research Advisor:</i> M. Botchan	SV40 recombination with chromosomal DNA.



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 408 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 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 biologist. Students participated in biological studies and in the actual sailing of the vessel.

This past Summer, a new course, Nature Bugs, was given, extending the program to five year-olds and offering an early introduction to environmental sciences.

In the Fall, Observational Astronomy, for eight-to-eleven year-olds, was again offered as an introduction to all facets of astronomy. Photography as related to Astronomy was included, with students utilizing our fully equipped darkroom. Telescopes for the observational sessions were loaned by Ehrenreich Photo-Optical, and technical assistance was provided by the Astronomical Society of Long Island and the Long Island Observers Association.

Program Director: Sanford Kaufman, M.S., M.P.A., science teacher, Hewlett High School

INSTRUCTORS

Don Dunn, art and photography teacher, Hewlett High School
Judith Calloway, M.S., math and biology teacher, East Islip High School
Mary Jane Fitzgibbon, M.S., science teacher, East Northport High School
Carolyn Hess, M.A. candidate, 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
Bird Study
They Walk, Swim, and Crawl
Aquatic Biology
Marine Biology
Nature Photography I and II
Fresh Water Life
Observational Astronomy
Adventure Education

BANBURY CENTER

1979 Activities

Somewhat to the surprise of all concerned, the Banbury Center program of conferences and books on biological risk assessment approached its anticipated scope during only the second year of operation. The first two Banbury Reports were published (and the first of these, *Assessing Chemical Mutagens: The Risk to Humans*, sold out in the first 6 months after publication). Four technical conferences drew distinguished specialists to discuss mammalian cell mutagenesis tests, less hazardous cigarettes, health data from human populations at low risk of cancer, and ethylene dichloride. Each meeting disclosed interesting recent findings, such as the rapid maturation of the Chinese hamster ovary test for chemical mutagens; signs of a sharp reduction in lung-cancer risk to smokers, related to progressive changes in composition of cigarettes; progress in codifying genetic information about such well-defined populations as the Mormons; and a fascinating contradiction in the results of different tests designed to discover if ethylene dichloride, a major chemical industry intermediate, is carcinogenic. With the help of professionally prepared transcripts of the technical conferences, the pace of book production effectively doubled. Meanwhile, a beginning was made on a second major program: informational meetings for nontechnical groups having key roles in policy-making, with a session in June for scientists and congressional staff from Washington.

Support

Financial support of the Center's program, so generously begun in 1978 by the Alired P. Sloan Foundation and the Esther A. and Joseph Klingenstein Fund, continued to be encouraging. Payment was received from the Environmental Protection Agency under a contract for its support of our May 1978 conference on assessing chemical mutagens and the publication of its proceedings. After months of uncertainty about the form of support from the National Cancer Institute for the 1979 program of technical conferences, a resolution appeared at year-end in a proposal to purchase volumes of proceedings of the meetings, based on the model of the Institute's purchase of many sets of the now-classic Cold Spring Harbor Laboratory work of 1978, *Origins of Human Cancer*. In November, the Banbury Center informational program received a splendid vote of confidence when the Sloan Foundation trustees appropriated half the projected cost of a 3-year program of meetings designed for groups of journalists and groups of legislative and congressional staff. As 1980 opened, we began planning sessions for the staffs of individual news organizations, inspired in part by the pioneering work of Fred W. Friendly of the Ford Foundation, who has staged a series of meetings for journalists and lawyers to thrash out legal issues that lately have beset the news-gathering profession.



Visitors

With the addition of five Banbury conferences to the 1979 schedule, which also included staging three summer courses in the neurosciences at Banbury and accommodating participants in no less than eight major conferences at the Cold Spring Harbor Laboratory, the use of Banbury facilities was heavier than ever. A roll of Robertson House guests that we sent to Mr. Charles S. Robertson, donor of the Banbury estate, carried the names of 295 scientists, including 60 from 17 foreign countries. As is traditional, the Banbury pool was the site of the annual outing of the Lloyd Harbor Village Police Department. The estate was also the site of two meetings of the Laboratory's Board of Trustees, of a special workshop on virus research organized by Dr. Joseph Sambrook, and dinners bidding farewell to several scientists and a member of the buildings and grounds department. An especially welcome event was the use of the Robertson House living room for a music recital by scientists participating in the summer course on RNA tumor viruses. Of great help to the entire program was the generous loan by the Wood family of the housing facilities of the dramatically sited Fort Hill estate on Lloyd Neck.

Physical Changes

Improvement of the Banbury property proceeded briskly. Two concealed parking areas were created near the new garage and the tennis court, and excavation began for Sammis Hall (scheduled for completion in 1980), which will provide 16 single bedrooms for Banbury guests. The start on Sammis Hall was made possible by generous grants from the Kresge and Fleischmann foundations.

Staff

There was a series of welcome additions to the staff, joining the director, Victor McElheny; the administrative assistant, Beatrice Toliver; the Robertson House housekeeper, Mary Hill; and the grounds keepers, Fred Pfeiffer and Peter Stahl. The new staff included two Banbury editors, Lynda Moran and Judith Cuddihy; an editorial assistant, Kathleen Kennedy; a resident hostess and cook at Robertson House, Katya Davey; and a resident watchman, Chris McEvoy of the Laboratory's buildings and grounds staff. It is a pleasure to record once again the Banbury Center's gratitude for support from every department of the Laboratory and, of particular importance in launching our publications effort, the unstinting help and supervision of Nancy Ford, Director of the Laboratory's Publications Department.



Robertson House

Proceedings published in December 1979 as Banbury Report 2, *Mammalian Cell Mutagenesis: The Maturation of Test Systems*.

The conference heard new evidence concerning such mammalian cell systems as the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovaries (CHO) and the thymidine kinase (TK) locus in mouse lymphoma cells, which revealed that mutations can be experimentally induced and quantitatively analyzed. Unresolved questions were also explored, such as the actual range of mutational events being assayed and whether the events in cell culture reflect the cellular responses in intact animals and humans. Although the conference participants wrestled with a host of questions about means of activating cell systems for screening environmental mutagens, there was conviction that the systems have proved themselves workable, as evidenced by wide use in industrial toxicology and screening programs. The conference brought together leading developers of animal and human cell systems, geneticists using such systems for fundamental biological studies, and participants in industrial and government screening programs. Leading the organization of the meeting were Abraham Hsie and J. Patrick O'Neill of Oak Ridge National Laboratory.

Session 1: Gene Mutation—Exploring the Evidence for Mutation Events in Cultured Mammalian Cells, and the Criteria Utilized to Define Such Events

Chairperson: E. A. ADELBERG, Yale University School of Medicine, New Haven, Connecticut

T. T. PUCK, University of Colorado Medical Center, Denver: Historical perspective of mutation studies with somatic mammalian cells.

L. SIMINOVITCH, University of Toronto, Canada: Studies of mutation in CHO cells.

C. T. CASKEY, Baylor College of Medicine, Houston, Texas: HGPRT mutants in Chinese hamster V79 cells.

Roundtable: Definition of Criteria to Define a Genetic Event

Chairperson: R. L. DAVIDSON, Children's Hospital Medical Center, Boston, Massachusetts

E. A. ADELBERG, Yale University School of Medicine, New Haven, Connecticut

R. J. ALBERTINI, University of Vermont College of Medicine, Burlington

T. T. PUCK, University of Colorado Medical Center, Denver

L. SIMINOVITCH, University of Toronto, Canada

Session 2: Quantitative Mutagenesis with Rodent Cells

Chairperson: L. SIMINOVITCH, University of Toronto, Canada

J. P. O'NEILL, Oak Ridge National Laboratory, Tennessee: CHO/HGPRT mutation assay—Experimental procedure.

M. M. MOORE-BROWN* and D. CLIVE,† *Environmental Protection Agency, Research Triangle Park, North Carolina; †Burroughs Wellcome Co., Research Triangle Park, North Carolina: The L5178Y/TK⁺/⁻ mutagen assay system: *In situ* results.

C. C. CHANG, Michigan State University, East Lansing: The use of Chinese hamster V79 cells for the detection of mutagens and tumor promoters or anti-promoters.

Roundtable: Quantitative Mutational Systems, Evidence for Genetic Events

Chairperson: L. A. CHASIN, Columbia University, New York, New York

A. W. HSIE and J. P. O'NEILL, Oak Ridge National Laboratory, Tennessee

M. M. MOORE-BROWN, Environmental Protection Agency, Research Triangle Park, North Carolina

D. CLIVE, Burroughs Wellcome Co., Research Triangle Park, North Carolina

C. C. CHANG, Michigan State University, East Lansing

Session 3: Roundtable—Criteria for a Mutagen Screening System

Chairperson: D. A. CASCIANO, National Center for Toxicological Research, Jefferson, Arkansas

- J. A. BRADLAW, Food and Drug Administration, Washington, DC
- B. E. BUTTERWORTH, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina
- L. D. KIER, Monsanto Chemical Company, St. Louis, Missouri
- D. F. KRAHN, E. I. du Pont de Nemours & Company, Newark, Delaware
- M. D. WATERS, Environmental Protection Agency, Research Triangle Park, North Carolina
- E. ZEIGER, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Session 4: Genetic, Biochemical and Molecular Analysis of Mutation

Chairperson: P.O.P. TS'O, Johns Hopkins University, Baltimore, Maryland

- E. A. ADELBERG, Yale University School of Medicine, New Haven, Connecticut: Selection methods for membrane transport mutants.
- L. H. THOMPSON, Lawrence Livermore Laboratory, Livermore, California: Analysis of temperature-sensitive mutants and quantitative assay of purine analog resistance in CHO cells.
- L. A. CHASIN, Columbia University, New York, New York: Selection of recessive mutants at diploid loci by titration of the gene product—CHO mutants deficient in dihydrofolate reductase activity.
- M. W. TAYLOR, Indiana University, Bloomington: An analysis of mutation at the APRT locus.
- R. L. DAVIDSON, Children's Hospital Medical Center, Boston, Massachusetts: Mechanisms of resistance to thymidine analogs in mammalian cells.
- R. M. BAKER, Massachusetts Institute of Technology, Cambridge: Nature and use of ouabain resistant mutants.

Session 5: Use of Mutagen Screening Systems

Chairperson: E. ZEIGER, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

- D. F. KRAHN, E. I. du Pont de Nemours & Company, Newark, Delaware: Utilization of the CHO/HGPRT system—Metabolic activation and a method for testing gases.
- J. D. IRR, E. I. du Pont de Nemours & Company, Newark, Delaware: Statistical evaluation of mutagenicity with the CHO/HGPRT system.
- D. E. AMACHER, Pfizer, Inc., Groton, Connecticut: Utility of the mouse lymphoma L5178Y/TK assay for the detection of chemical mutagens.
- J. G. DENT, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: Choice of activating systems for in vitro mutagenesis assays.
- D. B. COUCH, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: The influence of activation systems on the metabolism of 2,4-dinitrotoluene and its mutagenicity to CHO cells.
- J. P. O'NEILL, Oak Ridge National Laboratory, Tennessee: The CHO/HGPRT mutation assay—Adaptation for mutagen screening.

Session 6: Quantitative Mutagenesis of Human Cells

Chairperson: J. P. O'NEILL, Oak Ridge National Laboratory, Tennessee

- R. DEMARS, University of Wisconsin, Madison: Suggestions for increasing the scope of direct testing for mutagens and carcinogens in intact humans and animals.
- W. G. THILLY, Massachusetts Institute of Technology, Cambridge: Study of mutagenesis in diploid human lymphoblasts.
- V. M. MAHER, Michigan State University College of Osteopathic Medicine, East Lansing: Comparing the frequency of mutations induced in strains and diploid human fibroblasts with different capacities for DNA repair.
- R. J. ALBERTINI, University of Vermont College of Medicine, Burlington: Direct mutagenicity testing with peripheral blood lymphocytes.

Session 7: Mutation, Cancer, and Progress with Mutagen Testing

Chairperson: J. A. BRADLAW, Food and Drug Administration, Washington, DC

- P. O. P. T'SO, Johns Hopkins University, Baltimore, Maryland: Current progress in the study of basic mechanisms of neoplastic transformation.
- A. W. HSIE, Oak Ridge National Laboratory, Tennessee: The CHO/HGPRT mutation assay—Progress with quantitative mutagenesis and mutagen screening.
- D. CLIVE* and M. M. MOORE-BROWN,† *Burroughs Wellcome Co., Research Triangle Park, North Carolina; †Environmental Protection Agency, Research Triangle Park, North Carolina: The L5178Y/TK^{+/−} mutagen assay system—Mutant analysis.
- M. HOLLSTEIN, University of California, Berkeley: Evaluation of rapid screening methods that detect carcinogens and mutagens—Collation and comparison of test results.
- M. D. WATERS, Environmental Protection Agency, Research Triangle Park, North Carolina: The Gene-Tox Program.

Conference Summary: L. A. CHASIN, Columbia University, New York, New York

WORKSHOP ON ENVIRONMENTAL HEALTH RISKS, June 8 – June 10

Staff from the offices of individual members of Congress, Congressional committees, research agencies of Congress and the legislative affairs office of an Executive Branch scientific agency heard and questioned intently a distinguished group of scientists who discussed techniques of detecting and assessing risks, the relationship between risk assessment and fundamental biological research, and such specific problems of risk assessment as food safety and the management of wastes from advanced technological industries. Attendees at the meeting, recruited over nearly a year, flew up from Washington during a busy legislative season for the weekend workshop, and promptly sent back comments that were both enthusiastic and helpful for future workshops, planned to be held at least once a year.

Session 1: Introduction

- B. N. AMES, University of California, Berkeley: Identifying chemicals causing cancer and mutations.

Session 2: Risk Assessment in its Scientific Context

- J. HIGHLAND, Environmental Defense Fund, Washington, DC: Neglected risks.
- J. LEDERBERG, Rockefeller University, New York, New York: Intellectual and technical limits of risk assessment.
- D. BALTIMORE, Massachusetts Institute of Technology, Cambridge: The relevance of fundamental biological studies of genes and viruses.

Session 3: Food Safety and Broader Concerns

- P. N. MAGEE, Temple University School of Medicine, Philadelphia, Pennsylvania: Possibilities of cancer prevention.
- S. R. TANNENBAUM, Massachusetts Institute of Technology, Cambridge: Unavoidable exposure to carcinogens—Nitrates, nitrites, pyrolysis products, and carcinogenic spices.



- J. RODRICKS, Food and Drug Administration, Rockville, Maryland: Usefulness and pitfalls of using risk assessment in regulation.

Session 4: The Burden of Wastes

- B. J. PAIGEN, Roswell Park Memorial Institute, Buffalo, New York: Lessons of Love Canal.
H. WHITESIDE, Environmental Protection Agency, Washington, DC: Low-level radiation from the nuclear fuel cycle—A political and scientific problem.
R. WILSON, Harvard University, Cambridge, Massachusetts: Chemical and nuclear wastes; their differences and similarities.

TOWARD A LESS HAZARDOUS CIGARETTE, October 14 – October 16

Proceedings to be published in May 1980 as Banbury Report 3, *A Safer Cigarette?*

The conference heard presentations of evidence, such as declining lung cancer incidence among younger age groups in the United Kingdom, and United States studies reporting lower rates of lung cancer among filter-cigarette smokers and a reduced frequency of bronchial lesions in hospital patients of recent years (with known smoking frequencies) compared with similar groups of patients 15 years earlier. All of this evidence indicates that the risk of smoking-related lung cancer is being reduced by multiple changes in cigarettes whose combined effect has been to reduce the delivery of tar of the average cigarette by more than half in the last 20 years. Among other topics discussed was the recent sharp increase in the market share of cigarettes with less than 15 mg of tar (defined in the United States as low-tar cigarettes) from 24% in the first quarter of 1977 to more than 40% in the second quarter of 1979. Also discussed were studies of such possible continuing smoking risks as carbon monoxide, polonium 210, nitrosamines, and nicotine; and studies indicating that the tendency of smokers to compensate for reduced tar and nicotine by smoking more cigarettes is very moderate. Leading in the organization of the meeting was Gio B. Gori of the National Cancer Institute.

Session 1: Introduction and Epidemiological Trends

Chairperson: E. C. HAMMOND, American Cancer Society, New York, New York

- E. L. WYNDER, American Health Foundation, New York, New York: Epidemiological perspectives.
E. C. HAMMOND, American Cancer Society, New York, New York: The long-term benefits of reducing tar and nicotine in cigarettes.
L. GARFINKEL, American Cancer Society, New York, New York: Changes in the cigarette consumption of smokers in relation to changes in tar and nicotine content of cigarettes smoked.
M. KUNZE, Hygiene-Institut, University of Vienna, Austria: Thresholds of tar exposure—Analysis of smoking histories of male lung cancer cases and controls.
C. LYNCH, Enviro Control, Inc., Rockville, Maryland: Non-detectable risk levels in cigarette smoking.

Session 2: Toxicological Dimensions

Chairperson: F. G. BOCK, Roswell Park Memorial Institute, Orchard Park, New York

- S. P. BATTISTA, Arthur D. Little, Inc., Cambridge, Massachusetts: Inhalation studies of toxicity of tobacco smoke.
M. C. BATTIGELLI, University of North Carolina, Chapel Hill: Reversible versus fixed obstructive disorder of the airways.
L. DIAMOND, University of Kentucky College of Pharmacology, Lexington: Pulmonary toxicity of nitrogen oxides.
P. ASTRUP, Rigshospitalet, Copenhagen, Denmark: Carbon monoxide as a contributor to the health hazards of cigarette smoking.
C. J. SCHWARTZ, University of Texas Health Science Center, San Antonio: Cigarette smoking and cardiovascular diseases.
N. HARLEY, Institute of Environmental Medicine, New York University Medical Center, New York: Polonium 210—A questionable risk factor in smoking-related carcinogenesis.

- B. L. VAN DUUREN, Institute of Environmental Medicine, New York University Medical Center, New York: Co-carcinogens in tobacco smoke.
- D. HOFFMANN, Naylor Dana Institute, American Health Foundation, Valhalla, New York: The role of volatiles and nonvolatiles and nitrosamines in tobacco carcinogenesis.
- F. G. BOCK, Roswell Park Memorial Institute, Orchard Park, New York: Co-carcinogenic properties of nicotine.
- L. GARFINKEL, American Cancer Society, New York, New York: Changes in bronchial epithelium in relation to cigarette smoking: 1955-60 versus 1970-77.

Session 3: Cigarette Engineering

Chairperson: G. B. GORI, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland

- T. C. TSO, Beltsville Agricultural Research Center, Maryland: Chemistry of tobacco, and the effects of agronomy and curing.
- M. R. GUERIN, Oak Ridge National Laboratory, Tennessee: Chemistry of tobacco smoke.
- W. SELKE, Schweitzer Division, Kimberly-Clark Corporation, Lee, Massachusetts: Reconstituted tobacco sheet.
- T. EICHER and F. MÜLLER, Bayer Industries, Dormagen, West Germany: A co-tobacco material, RCN.
- C. KEITH, Celanese Fibers Company, Charlotte, North Carolina: Physical methods in modification of tobacco smoke (filters, papers and ventilation).
- W. S. CAIN, Yale University School of Medicine, New Haven, Connecticut: Sensory attributes of cigarette smoking.
- E. J. LAVOIE, Naylor Dana Institute, American Health Foundation, Valhalla, New York: The less harmful cigarette and tobacco smoke flavors.
- G. B. GORI, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland: Overview of changes in the cigarette product.

Session 4: Behavioral and Economic Issues

Chairperson: J. H. JAFFE, New York State Psychiatric Institute, New York

- S. M. SHIFFMAN, University of California, School of Medicine, Los Angeles: Reduced smoking, withdrawal symptoms, and cessation—A cautionary note.
- M. A. H. RUSSELL, Addiction Research Unit, Institute of Psychiatry, London, England: Dependency on nicotine—Relation of nicotine yield and blood nicotine levels.
- J. H. JAFFE, New York State Psychiatric Institute, New York: Preliminary observations of switchers—Some physiological and biological findings.
- J. C. MAXWELL, JR. Lehman Brothers Kuhn Loeb, Inc., New York, New York: Trends in cigarette consumption.
- J. E. HARRIS, Massachusetts Institute of Technology, Cambridge: Taxation of cigarettes according to tar and nicotine contents.
- B. RICHTER, Enviro Control, Inc., Rockville, Maryland: Macro-economics of the prevention of tobacco-related diseases.

Conference Summary: G. B. GORI, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland



Proceedings to be published in June 1980 as Banbury Report 4, *Cancer Incidence in Defined Populations*

Combined genetic and epidemiological investigations of defined groups, a number of them with cancer rates markedly different from that of the general population (according to presentations at the conference) form an important tool for quantifying the impact on cancer rates of such factors as variations in food in the diet, content of water or other drinks, or cigarettes. The conference also heard much evidence that new tools, such as computerized construction of pedigrees of such groups as the Mormons, molecular biological techniques of constructing libraries of stretches of human genetic material, and operation of national death registries, show great promise for deepening knowledge of well-defined populations in search of preventive measures against cancer. Leading in the organization of the conference were John Cairns of the Imperial Cancer Research Fund, Mill Hill Laboratories; J. L. Lyon of the Utah Cancer Registry; and Mark Skolnick of the University of Utah Medical Center.

Session 1: Cancer in Utah, Mortality Among Seventh Day Adventists

Chairperson: G. D. COPLEY, National Cancer Institute, Bethesda, Maryland

J. L. LYON, University of Utah Medical Center, Salt Lake City: Overview of studies of cancer in Utah populations.

D. WEST, Utah Cancer Registry, Salt Lake City: An assessment of cancer risk factors in Mormons and non-Mormons in Utah, with particular reference to cervix and colon cancer.

A. SORENSON, University of Utah Medical Center, Salt Lake City: Methods and strategies in nutritional epidemiology studies, using a colon cancer study as a model.

T. LOTZ, Loma Linda University, California: Death ascertainment among a defined population of California Seventh Day Adventists (SDAs) by computer-assisted matching to the California State Mortality File; and the problem of selection for comparison of mortality experience of SDAs to that of the general population.

R. L. PHILLIPS, Loma Linda University, California: Mortality from cancer of the large bowel, breast, and stomach among SDAs with differing dietary habits.

Session 2: Cancer Among Mormons and Other Defined Populations

Chairperson: C. DAVERN, University of Utah, Salt Lake City

J. E. ENSTROM, University of California, School of Public Health, Los Angeles: Health and dietary practices and cancer mortality among active California Mormons.

M.-C. KING, University of California, Berkeley: Genetic epidemiology of breast cancer in Mormon kindreds.

E. J. GARDNER, Utah State University College of Science, Logan: Prevention and cure for hereditary cancers.

A. O. MARTIN, Prentice Women's Hospital and Maternity Center, Northwestern University Medical School, Chicago, Illinois: Use of a genealogically linked data base in the analysis of cancer in a human isolate.

H. SIGVALDASON, Icelandic Cancer Registry, Reykjavik: Human health data in Iceland.

Session 3: Epidemiology and Cancer Prevention

Chairperson: J. CAIRNS, Imperial Cancer Research Fund, London, England

R. W. RAWSON, University of Utah Research Institute, Salt Lake City: The total environment in the epidemiology of neoplastic disease—The obvious "ain't necessarily so."

Discussion led by J. CAIRNS.

Session 4: Studies in Several Defined Populations, and an Examination of Associations between Radiation and Leukemia in Utah

Chairperson: R. W. RAWSON, University of Utah Research Institute, Salt Lake City

L. J. KINLEN, Cancer Research Campaign, Radcliffe Infirmary, Oxford, England: Abstinence from meat in relation to mortality among sisters in contemplative orders in the United Kingdom.

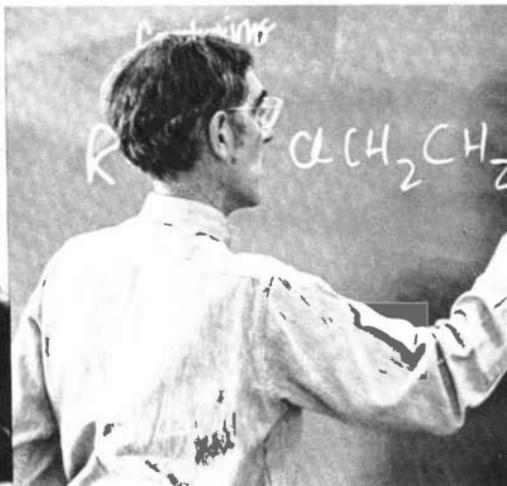
- W. NANCE, Medical College of Virginia, Richmond: The value of population-based twin registries for genetic and epidemiologic research.
- R. WARD, University of Washington, Seattle: Pedigrees and blood pressure—Genetic epidemiology in a migrant isolate, Tokelau.
- K. M. WEISS, Center for Demographic and Population Genetics, University of Texas, Houston: Studies in Laredo, Texas.
- J. L. LYON, University of Utah Medical Center, Salt Lake City: Childhood leukemias associated with fallout from nuclear testing.
- J. E. ENSTROM, University of California, School of Public Health, Los Angeles: The nonassociation of fallout radiation with childhood leukemia in Utah.

Session 5: Genealogical Studies

- Chairperson:* A. O. MARTIN, Prentice Women's Hospital and Maternity Center, Northwestern University Medical Center, Chicago, Illinois
- J. PETO, Imperial Cancer Research Fund, Oxford, England: The epidemiological study of hereditary cancer risks.
- J.-M. LALOUEL, University of Hawaii at Manoa, Honolulu: Relative merits and pitfalls of some strategies in genetic epidemiology.
- M. SKOLNICK, LDS Hospital, Salt Lake City, Utah: Genetic studies of Utah genealogy.
- J. R. HILL, LDS Hospital, Salt Lake City, Utah: Studies of coefficients of kinship for cancer in Utah Mormon genealogy.
- T. BISHOP, LDS Hospital, Salt Lake City, Utah: Analysis of the genetic predisposition to cancer in individual pedigrees.
- R. WILLIAMS, University of Utah Medical Center, Salt Lake City: Analysis of Mormon genealogical data for factors relating to heart disease.
- R. M. FINEMAN, University of Utah Medical Center, Salt Lake City: Utah registry for birth defects and genetic diseases.

Session 6: Problems of Genetic Epidemiology

- Chairperson:* E. JORDAN, National Institute of General Medical Sciences, Bethesda, Maryland
- R. L. WHITE, University of Massachusetts Medical School, Worcester: In search of DNA polymorphism in humans.
- M. SKOLNICK, LDS Hospital, Salt Lake City, Utah: Number of families needed to establish tight linkages for polymorphisms.
- T. BISHOP, LDS Hospital, Salt Lake City, Utah: Mathematical aspects of locating genes on chromosomes.
- R. S. SPARKES, University of California, School of Medicine, Los Angeles: Gene-mapping with retinoblastoma.



Chairperson: J. PETO, Imperial Cancer Research Fund, Oxford, England

L. J. KINLEN, Cancer Research Campaign, Radcliffe Infirmary, Oxford, England: The National Health Service and death registration in the United Kingdom.

J. PATTERSON, National Center for Health Statistics, Hyattsville, Maryland: Establishment of a National Death Registry in the United States.

Conference Summary: C. DAVERN, University of Utah, Salt Lake City

ETHYLENE DICHLORIDE: ECONOMIC IMPORTANCE AND POTENTIAL HEALTH RISKS, November 14 – November 17

Proceedings to be published in July 1980 as Banbury Report 5, *Ethylene Dichloride: Economic Importance and Potential Health Risks*.

Examination at the conference of the manufacture and uses of this major chemical industry intermediate, of which some 10 billion pounds is manufactured annually in the United States and most of which is converted to vinyl chloride monomer, showed that although there is a high volume of potential exposures to ethylene dichloride, largely within the chemical industry, the level of exposure is low from the industry point of view, whereas it is worrisome to researchers studying the mutagenicity of the compound (who hold that the ambient air levels likely to be inhaled by workers match up with levels sufficient to induce cancer in laboratory animals). The conference explored variations in the results of carcinogenicity tests on ethylene dichloride and other chlorinated hydrocarbons, particularly between tests by tube-feeding and by inhalation of ethylene dichloride. It also considered new information on the effects of metabolism and binding of chlorinated hydrocarbons in cells and animals, and also of age, on the outcome of short-term tests of the mutagenicity of such compounds. Leading in the organization of the conference were Bruce Ames and Kim Hooper of the University of California at Berkeley, Peter Infante of the Occupational Safety and Health Administration, and Richard Reitz of the Dow Chemical Company.

Session 1: Mutagenicity and Carcinogenicity of Ethylene Dichloride

Chairperson: R. REITZ, Dow Chemical Company, Midland, Michigan

C. MALTONI, Istituto di Oncologia e Centro Tumori, Bologna, Italy: Long-term carcinogenicity bioassays on ethylene dichloride, administered by inhalation to rats and mice.

J. M. WARD, National Toxicology Program, National Cancer Institute, Bethesda, Maryland: The National Cancer Institute bioassay of ethylene dichloride.

B. N. AMES, University of California, Berkeley: Carcinogenic potency.

K. HOOPER, University of California, Berkeley: Ethylene dichloride as a mutagen.

U. RANNUG, Wallenberg Laboratoriet, Stockholms Universitet, Sweden: The use of different metabolising systems in the elucidation of the mutagenic effects of 1,2 dichloroethane in *Salmonella*.

V. F. SIMMON, Genex Corporation, Rockville, Maryland: Review of nonbacterial tests of the mutagenicity of ethylene dichloride.

Session 2: Toxicology and Other Topics

Chairperson: P. INFANTE, Office of Carcinogen Identification and Classification, Occupational Safety and Health Administration, Washington, DC

F. SPREAFICO, Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy: Clinical chemistry of animals inhaling ethylene dichloride.

R. REITZ, Dow Chemical Company, Midland, Michigan: Pharmacokinetics and macromolecular interactions of ethylene dichloride; comparison of oral and inhalation exposures.

K. S. RAO, Dow Chemical Company, Midland, Michigan: Teratogenic and reproductive effects of ethylene dichloride in rats.

G. TER HAAR, Ethyl Corporation, Baton Rouge, Louisiana: An investigation of possible sterility and health effects of ethylene dibromide.

Session 3: Uses of Ethylene Dichloride; Worker Exposure

Chairperson: R. K. HINDERER, B. F. Goodrich Company, Cleveland, Ohio

L. GOLD, University of California, Berkeley: Uses of ethylene dichloride.

L. FISHBEIN, National Center for Toxicological Research, Jefferson, Arkansas: Uses and environmental fate of ethylene dichloride.

E. S. JACOBS, E. I. du Pont de Nemours & Company, Wilmington, Delaware: Scavengers for use in leaded gasoline.

M. N. JOHNSON, B. F. Goodrich Company, Akron, Ohio: Medical aspects of ethylene dichloride in the workplace.

R. KELLAM, Environmental Protection Agency, Research Triangle Park, North Carolina: Human exposure to ethylene dichloride—Potential for regulation as an airborne carcinogen.

B. L. VAN DUUREN, Institute of Environmental Medicine, New York University Medical Center, New York: Carcinogenicity and metabolism of halogenated olefinic and aliphatic hydrocarbons.

Session 4: Related Chemicals

Chairperson: K. HOOPER, University of California, Berkeley

W. M. BUSEY, Experimental Pathology Laboratories, Inc., Herndon, Virginia: The inhalation carcinogenesis of vinyl bromide.

H. B. PLOTNICK, Center for Disease Control, National Institute for Occupational Safety and Health, Cincinnati, Ohio: The effect of dietary disulfuram upon the toxicity of 1,2 dibromoethane.

P. INFANTE, Office of Carcinogen Identification and Classification, Occupational Safety and Health Administration, Washington, DC: Evidence for the carcinogenicity of some structural analogs of ethylene dichloride.

J. D. FABRICANT, University of Texas Medical Branch, Galveston: Evidence of the mutagenicity of 1,2 dichloroethane and related structural analogs.

P. MARLOW, Office of Carcinogen Identification and Classification, Occupational Safety and Health Administration, Washington, DC: Assessment of animal studies of ethylene dichloride and related compounds.

M. W. ANDERS, University of Minnesota Twin Cities Medical School, Minneapolis: Metabolism of dihalides to ethylene.

Conference Summary: R. K. HINDERER, B. F. Goodrich Company, Cleveland, Ohio



LABORATORY STAFF

December 1979

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GENETICS RESEARCH UNIT CARNEGIE INSTITUTION OF WASHINGTON

Alfred Hershey
Barbara McClintock



FINANCIAL STATEMENT

BALANCE SHEET

year ended December 31, 1979

with comparative figures for year ended December 31, 1978

	ASSETS		LIABILITIES AND FUND BALANCES	
	<u>1979</u>	<u>1978</u>	<u>1979</u>	<u>1978</u>
CURRENT FUNDS			CURRENT FUNDS	
<i>Unrestricted</i>			<i>Unrestricted</i>	
Cash	\$ 1,041,245	\$ 312,756	Accounts payable	\$ 94,971
Accounts Receivable	260,806	174,199	Mortgage Payable	133,200
Prepaid expenses	16,339	23,742	Due to plant funds	1,001,089
Inventory of books	123,202	135,838	Fund balance	<u>480,756</u>
Due from restricted fund	180,485	101,653		
Due from Banbury Center	87,939	14,984		
Total unrestricted	<u>1,710,016</u>	<u>763,172</u>	Total unrestricted	<u>1,710,016</u>
<i>Restricted</i>			<i>Restricted</i>	
Grants and contracts receivable	<u>2,265,333</u>	<u>2,063,598</u>	Due to unrestricted funds	180,485
			Fund balance	<u>2,084,848</u>
Total restricted	<u>2,265,333</u>	<u>2,063,598</u>	Total restricted	<u>2,265,333</u>
Total current funds	<u>\$ 3,975,349</u>	<u>\$ 2,826,770</u>	Total current funds	<u>\$ 3,975,349</u>
ENDOWMENT FUNDS			ENDOWMENT FUNDS	
<i>Robertson Research Fund</i>				
Cash	(233,575)	25,073		
Marketable securities				
(quoted market 1979—\$9,630,113;				
1978—\$8,427,386)	<u>8,418,623</u>	<u>7,978,726</u>		
Total Robertson Research Fund	<u>8,185,048</u>	<u>8,003,799</u>		

<i>Olney Memorial Fund</i>					
Cash	828	412			
Marketable Securities (quoted market 1979—\$20,332; 1978—\$22,496)	<u>26,582</u>	<u>24,123</u>			
Total Olney Memorial Fund	<u>27,410</u>	<u>24,535</u>			
Total endowment funds	<u>\$ 8,212,458</u>	<u>\$ 8,028,334</u>	Fund balance	<u>\$ 8,212,458</u>	<u>\$ 8,028,334</u>
PLANT FUNDS			PLANT FUNDS		
Investments	230,725	219,358			
Due from unrestricted fund	1,001,089	377,531			
Land and improvements	960,530	935,530			
Buildings	5,735,358	5,493,511			
Furniture, fixtures and equipment	1,103,409	857,891			
Books and periodicals	365,630	365,630			
Construction in progress	<u>88,630</u>	<u>65,012</u>			
	9,485,371	8,314,463			
Less allowance for depreciation and amortization	<u>1,951,625</u>	<u>1,545,396</u>			
Total plant funds	<u>\$ 7,533,746</u>	<u>\$ 6,769,067</u>	Fund balance	<u>\$ 7,533,746</u>	<u>\$ 6,769,067</u>
BANBURY CENTER			BANBURY CENTER		
Current funds			Current funds		
Cash	\$ 600	\$ 300	Due to CSHL unrestricted fund	\$ 87,939	\$ 14,984
Prepaid expenses	<u>95</u>	<u>95</u>	Fund balance	<u>(87,244)</u>	<u>(14,589)</u>
Total current funds	<u>695</u>	<u>395</u>	Total current funds	<u>695</u>	<u>395</u>
Endowment Funds			Endowment funds		
Robertson Maintenance Fund					
Cash	(62,591)	8,909			
Marketable securities (quoted market 1979— \$1,726,973; 1978—\$1,539,539)	<u>1,586,667</u>	<u>1,493,754</u>			
Total endowment funds	<u>1,524,076</u>	<u>1,502,663</u>	Endowment funds balance	<u>1,524,076</u>	<u>1,502,663</u>

Plant funds

Land	772,500	772,500
Buildings	412,672	412,672
Furniture, fixtures and equipment	164,416	160,700
Construction in progress	49,510	8,856
	<u>1,399,098</u>	<u>1,354,728</u>
Less allowance for depreciation	129,340	86,886
Total plant funds	<u>1,269,758</u>	<u>1,267,842</u>
Total Banbury Center	<u>\$ 2,794,529</u>	<u>\$ 2,770,900</u>
Total—All funds	<u>\$22,516,082</u>	<u>\$20,395,071</u>

Plant funds

Plant funds balance	<u>1,269,758</u>	<u>1,267,842</u>
Total Banbury Center	<u>\$ 2,794,529</u>	<u>\$ 2,770,900</u>
Total—All funds	<u>\$22,516,082</u>	<u>\$20,395,071</u>

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1979
with comparative figures for year ended December 31, 1978

COLD SPRING HARBOR LABORATORY

	<u>1979</u>	<u>1978</u>
REVENUES		
Grants and contracts	\$ 3,269,582	\$ 2,826,545
Indirect cost allowances on grants and contracts	1,499,040	1,299,409
Contributions		
Unrestricted	81,624	100,660
Restricted and capital	774,264	10,000
Long Island Biological Association	6,753	31,000
Robertson Research Fund Distribution	350,000	379,167
Summer programs	288,380	207,838
Laboratory rental	20,732	21,796
Marina rental	39,900	39,900
Investment income	102,999	45,424
Publications sales	837,763	649,041
Dining hall	319,628	272,845
Rooms and apartments	207,061	183,575
Other sources	20,019	59,175
Total revenues	<u>7,817,745</u>	<u>6,126,375</u>
EXPENSES		
Research*	3,002,771	2,583,374
Summer programs*	633,270	518,570
Library	129,752	111,479
Operation and maintenance of plant	1,011,822	796,998
General and administrative	716,487	598,880
Depreciation	405,180	366,630
Publications sales*	510,805	530,834
Dining hall*	324,093	228,767
Total expenses	<u>\$ 6,734,180</u>	<u>\$ 5,735,532</u>

BANBURY CENTER

	<u>1979</u>	<u>1978</u>
REVENUES		
Endowment income	\$ 82,604	\$ 83,424
Grants & contributions	123,000	100,000
Conference fees	—	970
Rooms and apartments	16,890	16,700
Publications	40,768	—
Transfer from Cold Spring Harbor Laboratory	40,655	12,900
Total revenues	<u>303,917</u>	<u>213,994</u>
EXPENSES		
Conferences	48,678	10,179
Publications	76,208	—
Operation and maintenance of plant	89,777	81,929
Program administration	132,522	86,949
Depreciation	40,656	38,853
Capital plant	44,371	49,826
Total expenditures	<u>432,212</u>	<u>267,736</u>
Excess (deficit) of revenues over expenses	<u>\$ (128,295)</u>	<u>\$ (53,742)</u>

TRANSFERS

Capital building projects	1,036,867	759,855
Banbury Center	40,655	12,900
Total transfers	<u>1,077,522</u>	<u>772,755</u>
Total expenses and transfers	<u>7,811,702</u>	<u>6,508,287</u>
Excess (deficit) of revenues over expenses and transfers	<u>\$ 6,043</u>	<u>\$ (381,912)**</u>

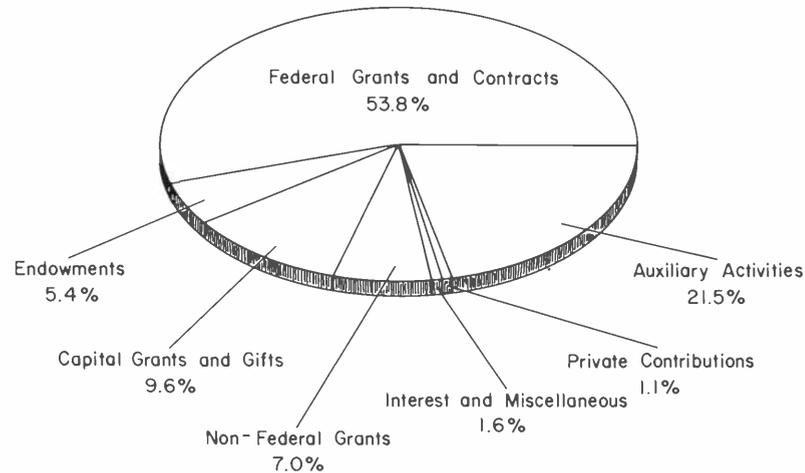
**Reported exclusive of an allocation for operation and maintenance of plant, general and administrative, library, and depreciation expenses.*

***1978 deficit primarily caused by conversion of current funds 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 ended December 31, 1979



FINANCIAL SUPPORT OF THE LABORATORY

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

The Laboratory depends upon the generous contributions of its sponsors, participating institutions, and friends for central institutional needs and capital improvements. In addition, the development of any new programs, such as year-round research in neurobiology and the marine sciences, can 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

- (1) Your broker or bank may sell the securities and remit the proceeds directly to the Laboratory.
- (2) If you wish to send stock directly to the Laboratory either (a) endorse the certificate(s) by signing your name on the back, leave the space for the transferee's name blank, have your signature guaranteed on the certificate(s) by your bank or broker, and send the certificate(s) by *registered mail* to the Laboratory, or (b) send unsigned certificate(s) with a covering letter and send under separate cover a stock power executed in blank, with signature guarantee, for each certificate, and also a copy of the covering letter (use first-class mail). Depreciated securities should be sold to establish a tax loss, then the contribution to the Laboratory should be made by check.

Bequests

Probably most wills need to be updated. Designating 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, P.O. Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8300.

GRANTS

January 1, 1979–December 31, 1979

NEW GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal Investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
National Institutes of Health	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—membrane biogenesis meeting	15,000	4/6/79– 3/31/80
	Dr. Watson— <i>C. elegans</i> meeting	13,290	1/1/79–12/31/79
	Dr. Watson—herpes virus meeting	21,250	8/1/79– 7/31/80
	Dr. Watson—advanced bacterial genetics course	25,000	7/2/79– 9/15/79
	Dr. Watson—viruses in naturally occurring cancers meeting	30,000	8/14/79– 1/15/80
	Dr. Chaconas—fellowship	13,800	8/1/79– 7/31/81
	Dr. Garrels—research	323,073	4/1/79– 3/31/82
	Dr. Sciaky—fellowship	13,800	3/1/79– 2/28/80
	Dr. Stringer—fellowship	28,000	2/1/79– 1/31/82
	Dr. Topp—research	275,806	1/1/79–12/31/81
	National Science Foundation	Dr. Watson—herpes virus meeting	5,000
Dr. Watson— <i>C. elegans</i> meeting		3,000	1/15/79–12/31/79
Dr. Watson—membrane biogenesis meeting		5,000	4/15/79– 3/31/80
W. Udry—Symposium support		5,000	5/15/79– 4/30/80
Dr. Bukhari—research		330,000	6/15/79–11/31/82
Dr. Roberts—research		210,000	12/15/79–11/30/82
	Dr. B. Zipser—neurobiology course support	60,000	7/1/79–12/31/82
Rita Allen Foundation	Dr. Watson—1979 Pain Workshop	24,500	
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—research	8,570	7/1/79– 6/30/80
	Dr. Garrels—research	8,570	7/1/79– 6/30/80
Department of Energy	W. Udry—Symposium support	8,000	6/1/79–
The Camille and Henry Dreyfus Foundation	Dr. Watson—undergraduate research participation	26,000	12/18/79– 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
Muscular Dystrophy Association	Dr. Burrige—research	25,142	7/1/79– 6/30/80
	Dr. Feramisco—fellowship	15,500	1/1/79–12/31/79
	Dr. Gordon—fellowship	2,583	1/1/79– 2/28/79

NEW GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal Investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
	Dr. E. Lane—fellowship	16,500	1/1/79—12/31/79
	Dr. B. Zipser—neurobiology course support	22,000	6/1/79— 9/30/79
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. Lin—fellowship	28,000	7/1/79— 6/30/81
	Dr. Stillman—fellowship	28,000	4/1/79— 3/31/81

CONTINUING GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
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— 3/31/80
	Dr. Watson—Symposium support	96,000	4/1/74— 3/31/79
	Dr. Watson—summer workshops	470,340	4/1/77— 3/31/82
	Dr. Watson—advanced bacterial genetics course	40,000	6/30/78— 4/30/79
	W. Udry—cancer research facility	1,411,000	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. Broach—research	144,320	8/1/77— 7/31/79
	Dr. Bukhari—career development	148,961	5/1/75— 4/30/80
	Dr. Bukhari—research	121,351	9/9/77— 8/31/80
	Dr. Chow—research	238,186	4/1/77— 3/31/80
	Dr. Gingeras—fellowship	26,400	1/1/78—12/31/79
	Dr. Hicks—research	384,703	7/1/78— 6/30/81
	Dr. Klar—research	253,000	7/1/78— 6/30/81
	Dr. Zain—research	34,572	9/30/77— 7/31/79
	Dr. D. Zipser—fellowship training	754,000	7/1/78— 6/30/81
	Dr. D. Zipser—research	355,657	5/1/74— 4/30/79
	National Science Foundation	Dr. Burrige—research and equipment	182,494
Dr. Bukhari—research		130,000	8/1/76— 7/31/79
Dr. Hicks—equipment		24,000	8/15/78— 1/31/80

CONTINUING GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
	Dr. Roberts—research	130,000	4/1/77—10/31/79
	Dr. Zain—research	65,000	8/15/77— 1/31/80
	Dr. B. Zipser—research	120,000	11/1/78— 4/30/82
	Dr. D. Zipser—research	136,000	2/15/78— 7/31/81
Rita Allen Foundation	Dr. Lewis—research	150,000	8/1/76— 7/31/81
American Cancer Society	Dr. Garrels—fellowship	10,575	9/1/78— 8/31/79
	Dr. Lewis—research	53,250	7/1/78— 6/30/79
	Dr. Zain—research	48,528	1/1/78—11/30/79
Canadian Medical Research Council	Dr. Chanonas—fellowship	24,000	8/1/78— 7/31/80
Jane Coffin Childs	Dr. Gluzman—fellowship	24,333	9/16/77— 9/15/79
The Camille and Henry Dreyfus Foundation	Dr. Watson—undergraduate research participation	20,000	12/1/78— 9/30/79
Leukemia Society of America	Dr. Engler—fellowship	25,000	11/1/78—10/31/80
Muscular Dystrophy Association	Dr. Burrige—research	25,142	7/1/78— 6/30/79
Alfred P. Sloan Foundation	Dr. D. Zipser—Biological Information Processing Workshop	17,800	5/1/78— 4/30/79
Damon Runyon-Walter Winchell Cancer Fund	Dr. Brooks—fellowship	28,560	1/1/78—12/31/79
	Dr. Hu—fellowship	26,070	7/1/78— 6/30/80
	Dr. Kilpatrick—fellowship	30,000	1/1/78—12/31/79
	Dr. Sciaky—fellowship	28,980	3/1/77— 2/28/79
	Dr. Stow—fellowship	30,000	10/1/78— 9/30/80
	Dr. Strathern—fellowship	14,990	4/1/78— 3/31/79

BANBURY CENTER

Environmental Protection Agency—Conference on Chemical Mutagens		29,000	5/11/78— 5/1/79
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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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 ship-building, 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; in 1976 the re-building of Williams House. Currently (in 1979-80) funds are being raised for the Lab's purchase from the Carnegie Institution (at a cost of \$200,000) of some 20 acres of land essential for the preservation of the Laboratory's property which the Institution no longer wishes to use.

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.

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REPORT OF THE CHAIRMAN FOR 1979

LIBA's most important activity in 1979 was that of launching a campaign to raise \$200,000 from its members over a two-year period to purchase 20 acres of land essential for the preservation of the Lab's property. At the end of the year contributions and pledges totaled more than half the amount needed to reach our goal.

The custom of giving dinner parties for the visiting scientists at the Lab's summer Symposium was successfully continued and greatly appreciated. On June 3rd, eighteen of our directors and members gave parties for over a hundred guests. This year's hosts and hostesses were:

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There were two LIBA meetings open to the whole membership. At the first on May 13th, Dr. René Dubos of Rockefeller University, the famous biologist and conservationist, gave the annual lecture in memory of our former director Mrs. Dorcas Cummings. At the second (our annual) meeting on January 27th, 1980 we were addressed by Dr. Philip Sharp (Massachusetts Institute of Technology), who told us about a wonderful new discovery (in which he has played a prominent role) that seems to have great potential for curing a number of diseases caused by viruses. The title of his lecture was *Genetically Engineering Bacteria to Produce the Human Interferon*. This meeting, which was scheduled for December 9th, had been postponed because Dr. Lewis Thomas, who was to have addressed us then, was incapacitated by an accident. He hoped to come for our deferred meeting but complications resulting from his accident put him back in the hospital. We were greatly indebted to Dr. Walter Gilbert of Harvard University who, at the urgent request of his friend Dr. Watson, agreed upon less than a week's notice to come to speak to us. We were even more indebted to his associate, Dr. Sharp, who came on 24 hours' notice in Dr. Gilbert's place when the latter had succumbed to flu! Summaries of both lectures are printed below.

On November 11th a special meeting was held for directors and their guests. The evening began with a lecture by Dr. Guenter Albrecht-Buehler describing his work on cell motility. He took his audience down to the microscopic world of the cell, describing the



Dr. Phillip Sharp and Mr. Edward Pulling at LIBA's Annual Meeting.



Williams House, rebuilt with LIBA funds

strange forces encountered there and how the cell responds intelligently to its environment. After the talk, tours were conducted through the new laboratory and computer facility set up by Dr. James Garrels for analysis of the proteins in the cell by two-dimensional gel electrophoresis. Dr. Garrels gave a demonstration in the Lab while Carter Burwell showed how the computer can analyze the data and display the results on the color TV screen. The tour, which also led the visitors through the newly reconstructed Hershey building, was followed by dinner in Blackford Hall, which was attended also by members of the Lab's scientific staff.

At the annual meeting of members and the subsequent meeting of directors, all previous directors and officers were re-elected, with the exception of Mrs.

Edward M. Shepard (the former Mrs. Joan Flanigan). Her recent marriage and new residence in Woodstock, Vermont, made her unavailable for re-election. A resolution of regret and of appreciation of her valuable services to LIBA for several years was adopted. Then Mrs. Miner D. Crary was elected to fill Mrs. Shepard's place on the Board.

I am pleased to report that the LIBA membership continues to expand and that the interest and support of the community in the Lab—our basic objective—seems also to be increasing.

January 28, 1980

*Edward Pulling, Chairman
Long Island Biological Association*



Dr. James Garrels giving lab demonstration to LIBA directors and guests



DORCAS CUMMINGS MEMORIAL LECTURE

At the May 1979 Dorcas Cummings Memorial Lecture, members of the Long Island Biological Association were privileged to hear a talk by Dr. René Dubos, who achieved worldwide fame 30 years ago for discovering the first commercially produced antibiotic. Dr. Dubos, who won a Pulitzer Prize in 1969 for his book, *So Human an Animal*, is a renowned environmentalist as well as microbiologist.

Dr. Dubos spoke of the need for mankind to establish a symbiotic relationship with the earth in order to survive. Departing from the orthodox ecologists' position that "Nature knows best," Dr. Dubos pointed out that if man interacts, rather than interferes, with Nature, both will benefit. Nature's solutions to ecological problems are sometimes clumsy, shortsighted, and inadequate. Man's reason and imagination are needed to facilitate the processes of recycling and to establish an equilibrium between population size and local resources.

"The earth is to be seen neither as a static system to be preserved unchanged, nor as a quarry to be exploited for purely economic reasons, but rather as a garden to be cultivated." This feat man has accomplished through the recycling of deposits of peat, coal, oil, and shale; by employing drainage and irrigation techniques; by terracing and developing new land from the seas; through the genetic modification of flora and the cultivation of crops from one continent to another.

Emphasizing his optimistic view of man's relation to nature, Dr. Dubos told the audience:

"We can manipulate the raw stuff of nature to shape it into environments which are ecologically sound, economically profitable, esthetically rewarding, and favorable to human health. We can insert our dreams into ecological determinism and thus enrich the earth and diversify its manifestations by bringing out its hidden potentialities.

We can be ecologically sound while humanizing the earth."



Dr. René Dubos at Dorcas Cummings Memorial Lecture

ANNUAL MEETING LECTURE SUMMARY

*Genetically Engineering Bacteria
to Produce the Human Interferon*

Dr. Phillip A. Sharp
Center for Cancer Research
Massachusetts Institute of Technology

With the development of recombinant-DNA technology in the 1970s, mankind obtained the capacity to design, isolate, and construct genes. This ability, coupled with the well-developed genetics and molecular biology of bacteria, has made it possible to use these simple organisms to produce large amounts of complicated proteins. *Biogen*, a company whose goal is to utilize this rapidly developing technology in order to produce useful products, several years ago began supporting Professor Charles Weissmann's work on the cloning and synthesis in bacteria of human leukocyte interferon.

Human leukocyte interferon was discovered in 1957 by Isaacs and Lindenman by its ability to render human cells resistant to a broad spectrum of different types of viruses. For example, interferon has been shown to block the growth of viruses such as hepatitis, influenza, poxvirus, and poliovirus. There is also hope that interferon will be of clinical use as an antitumor agent. At the moment, most of the available human leukocyte interferon is produced from white blood cells separated from blood donations in Finland by Dr. Kari Cantell's group. Even though this is the most efficient means of obtaining interferon, the process is very expensive and the source so limited that pure interferon is not available for clinical studies.

To isolate the gene for interferon, Professor Weissmann's group took messenger RNA from human leukocytes and constructed a library of 20,000 separate bacteria cultures. Each member of this library contained one type of gene from the human cell, and somewhere in this library there should have been a bacteria culture containing the interferon gene. Identifying a bacteria culture containing the interferon gene involved testing pools of members of this library. By first detecting the interferon gene in a pool of 512, then a subset of 64, then a subset of 8, and finally as a gene in a single bacteria culture, Professor Weissmann's group was able to isolate the gene specifying human leukocyte interferon. His laboratory further found that bacteria containing a complete copy of this gene synthesized a protein with interferon activity at a level of 1 to 2 molecules per cell. Even at this minuscule level, production of interferon activity in bacteria is much less expensive than from human sources. Hopefully, in the near future, there will be sufficient pure interferon produced by bacterial fermentation to bring this promising pharmaceutical compound to the stage of being useful in the treatment of man's diseases.

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