ANNUAL REPORT 1977

COLD SPRING HARBOR LABORATORY

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COLD SPRING HARBOR, NEW YORK

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DIRECTOR'S REPORT

When I was a boy in Chicago, the scientist was a poorly paid, absent-minded dreamer, very bright, if not a genius, and culturally destined never to lead the masses, much less have anything to say to any public official more important than a high school principal. Then came Oppenheimer and the atomic bomb, and physicists became men of affairs whom we and the Generals could not live without. Biologists were another matter. No one thought they posed a threat to anyone; they would grow better hybrid corn or even someday cure cancer, if they stayed by themselves and did not take on roles where they would only get in someone else's way.

I became one of them, and for twenty years it seemed I had entered the best of all possible worlds. Lots of money was dug up by Mary Lasker and her crowd, with scarcely any accountability save for the quality of our science. Even better, no one got mad at us when we didn't soon hit lots of practical medical jackpots. And so it went until Vietnam. Then came the teach-ins at which our students were told that the White House collectively had the brains of a goose, a belief soon reinforced by Lyndon Johnson when he went out to NIH to give the word that we troublemakers had better start being useful or the gravy train would end.

Being a brand-new Salk, however, is not that easy. It is not that we do not want to be do-gooders, but if it was a cinch to come up with a new vitamin that would make everyone live to 100 years of age without Social Security it would have happened long ago. Invariably, great challenges mean we don't know what to do next. Consequently, most of our research efforts are holding operations which, given time, hopefully will generate unexpected observations. With further luck we may gain a fresh outlook on some pressing human problem such as too many mosquitos or god-awful arthritis. So while generally we don't go to work thinking about what we can do next for our fellowman, we never lost any sleep worrying about whether we were in cahoots with the idle rich who preach the virtues of hard work to others and then live off more pollution. Far better to be thought unworldly and of conscience clean.

Now, however, we are in a strange mess. The word is about that we are closet big businessmen who need to be kept in check by massive regulations, if not the threat of jail. And all because the word has been spread that DNA, the stuff that makes up our genes, might do us all in. I find this total nonsense and cannot think of any potential environmental pollutant that worries me less. Everyone I know who now works with DNA feels the same, and mere mention of "NIH Guidelines" or "Memorandums of Understanding" makes our mouths froth. Under ordinary circumstances our response would be obvious—head down to Washington and tell Congress that these scaremongers are an odd coalition of environmentalists wanting to block anything new and middle-class leftists who view the new genetics as a plausible tool for further "enslaving the masses."

The heart of our trouble, however, does not lie with the far-out doomsday scenarios these pathetic souls peddle. It was we presumably responsible molecular biologists who first gave the DNA scare its legitimacy. To be sure we never said we were deeply worried, for none of us were. I don't remember experiencing even five seconds of mild anxiety prior to signing the call for a moratorium. This document, a semiofficial response to fears of genetic engineering at the service of CIA types, urged restraints on the making of certain recombinant genomes until we convened a gathering of international bigwigs at Asilomar on the Monterey Peninsula. There we were to discuss whether the 1973 breakthrough that gave us recombinant DNA was likely to generate dangerous new bugs. I didn't know how we could solve this dilemma but saw no harm in calling for a little scientific restraint in the then post-Watergate mood of self-confession. Most of us were liberal-left types from birth, and our fellow McGovernites would now see that we were not always out for quick fame and could place the general good ahead of our immediate scientific goals.

Here we were terribly naïve—the real world never stops anything it wants unless it has already smelled smoke. Though everyone at Asilomar kept saying "potential risk," experienced newspaper addicts thought differently. No one would invent phrases like P3 or EK2

unless they could measure the monster's size. The very act of setting up committees to pinpoint the "most risky" experiments could only magnify the public fear that we biologists now had our own diabolical form of the bomb.

By this stage I knew we were in way over our heads and felt that this senseless hysteria should somehow be nipped in the bud. So I argued, to the surprise of most at Asilomar, against the forming of any official guidelines that spelled out how we should work with recombinant DNA. I thought any regulations by necessity had to be capricious, and in the absence of even the slightest reason for being afraid, we were very silly to be making all this fuss. Only Joshua Lederberg and Stanley Cohen were publicly on my side, and those who privately agreed with me thought I was risking my hide by appearing so indifferent to the general good. Almost everyone reasoned that the public would not dump the matter and so went along with Sidney Brenner's eloquent plea that we must make a show of self-restraint or the lawyers and ethicists would take over and give us stupid laws that could really shut us down.

Unfortunately, the high hopes of many that Asilomar might defuse the public concern were soon dashed. Within weeks Congress got into the act, and the guys who gave us the guidelines soon found themselves in front of Senator Kennedy. Given the importance ascribed to the topic, he saw no reason to accept the argument that a matter which might affect the life or death of our society should be left in the scientists' hands. Just the opposite—it seemed the perfect occasion for the public to be asked whether they were to be the next guinea pigs for our experiments. Here the molecular biologists quickly found they had little room to maneuver. Since they were not that afraid, they could only say that there was no reason to be so concerned, and certainly no reason for specific laws to control us. On the other hand, they were tied to the Asilomar message—we need guidelines. So they were left to argue the point of how safe would it be if everyone followed the guidelines and did the "potentially most risky" experiments using specially disabled bacteria and elaborate containment facilities designed to prevent the escape of organisms bearing recombinant DNA. Not surprisingly, this message failed to reassure Kennedy, who could not believe that the Asilomar leaders would propose diverting significant amounts of scarce research funds for protective measures or risk creating a brand new bureaucracy unless they were much more scared than they publicly let out.

This and every subsequent defense of the guidelines led automatically to how we would deal with sloppy lab practices, if not downright cheating. No one would be so foolish as to suggest that mistakes will never occur or that molecular biologists are so unworldly that they cannot be dishonest. This is a point about which Congress needs no education, and once the issue was raised on Capitol Hill it became very likely that some form of punitive legislation would be enacted to ensure strict compliance with the guidelines.

If, instead, the recombinant DNA community had been divided as to whether we needed any guidelines, the final outcome would have been less clear and might have been decided by whether the Senate and the House had the gumption for still another regulatory commission. But with virtually every first-class molecular geneticist then arguing that we needed protection from ourselves, Congress would have been accused of shirking its duty if it did not seize the initiative. It then became a question of how to get a law where we, not the professional demonstrators, would control the action.

Up through today the final outcome is still unclear, and that three years have passed since Asilomar without legislation reflects more the inherent chaos of Congress than the skill with which we lobby. Though there have been moments of panic, the situation is now less out of control. The most chilling early proposal from Senator Kennedy's bailiwick was that final power should reside in a committee so constituted by law that scientists who know what DNA is must be in the minority. The vision of a "Friend of the Earth" bearing a large wig righteously telling us what not to do could be a scene from MASH. And Representative Rogers' Health Subcommittee dallied briefly with the thought that the way to keep us clean was the taint of a felony and $50,000-a-day fines, penalties which most certainly would make docile puppets even of our nation's bankers, much less our scientists.

Through these ups and downs we occasionally have had the hope that recombinant DNA would finally so bore Congress that no one would do the work necessary to see a bill through to enactment. I don't think, however, that we shall ever win this way. As long as most responsible scientists are thought to favor perpetuation of the guidelines, every incident of suspected cheating will rekindle some new hysterical outburst. Only if the scientific community en masse asserts strongly that the guidelines can go, do we have a fighting chance to weather such incidents without the possible creation of a truly imbecilic law.

I fear, however, that we academics do not have the skilled politician's knack of suddenly reversing his course without the slightest suggestion that he is soft in the brain. So the prevailing approach today is to urge the enactment of a much watered-down bill that will both keep all control over recombinant DNA research within NIH and preempt the power of local bodies to make their own decisions as to which experiments should be done. There are dreams that we might even get a sunset clause that would limit the legislation to a several-year period, after which everyone could bury the

guidelines without acute embarrassment. If all this were to come about, we could breathe easier and stop despairing that we may have closed the door in this country to many of the better experiments that we can do on the origins of cancer. For the moment, however, we must remain on guard. The final form of any legislation is often imprinted at the last moment in conference, and I see no reason for expecting that Senator Kennedy will give way on preemption. So we had best face up to the prospect that local governments will be able to justify their own bans by citing Federal law as proof that recombinant DNA poses a realistic threat.

We can only hope that those scientists whose first foray into the public arena gave us Asilomar will ask themselves whether, if Asilomar had never occurred, they would now see any compelling public health reason to propose formal restrictions on recombinant DNA research. If their answer is no, they should ask Congress for another chance to be heard. They should not delude themselves that Congress's main advice has come from the likes of George Wald. Their role has been much more of noise than of substance. At present, any legislation that we may finally get will reflect Congress's belief that they have sensed the mood of our most down-to-earth experts, and that the guidelines are what they want. I do not believe this, but I effectively speak only for myself. Now is the time for my fellow DNA workers to come forward and speak their minds.

Highlights of the Year

A remarkable research result

Nine years ago when we commenced work on the DNA tumor viruses we had two main objectives. Obviously, one was to learn how these viruses transform normal cells into their cancerous equivalents. The other was to probe more deeply the molecular genetics of eukaryotic cells. Recombinant DNA was then hardly a gleam in anyone's eye, and direct assaults on the immense genomes of higher eukaryotes were limited necessarily to highly redundant sequences such as those coding for the ribosomal RNAs. The only totally clean eukaryotic DNA available came from viruses, and we chose to work on two of the most simple examples, the very small monkey virus, SV40, and the moderately sized human adenoviruses.

At first our work went slowly compared to that of our colleagues who remained focused upon bacteria. Growing up sizable amounts of any animal virus is seldom a routine, cheap matter, and the methodologies for working with very small amounts of viral DNA were not in hand. Fortunately, the situation improved quickly. First came C_0t analysis and the restriction enzymes, then gel electrophoresis, the Southern blot, even better EM methods, and finally the incredibly fast DNA sequencing tricks. As a result, work on the molecular biology of tumor viruses now proceeds as fast as comparable work on bacterial and phage DNA.

Thus, early in 1977 the stage was set for the carrying out of experiments (independently here and at MIT) that were to revolutionize our ideas of genes and the proteins for which they code. The details of our experiments are described in the Year-round Research Section, and here I want only to relate our total amazement when we realized that the adenoviral primary transcripts were modified by a splicing process that removes one to several internal sequences. Although we had expected processing, we thought that it would occur by simple removal of sequences at the 5′ and/or 3′ ends. The counter proposal that processing occurs by removal of multiple internal sections would have been thought irresponsible speculation had not our experiments been so foolproof. Soon hints of a similar situation in SV40 emerged from NIH, and at the RNA Tumor Virus Meeting the possibility was raised that splicing generates the several classes of Rous sarcoma virus messengers. Now there is a growing belief that splicing may occur in all eukaryotes, and we expect that a multiplicity of proteins will be generated from many "single genes."

Splicing is likely to be the most important observation in molecular genetics since the 1960 discovery of messenger RNA. Immediately it has led to an explosion of experiments aimed at understanding its enzymological basis. And for many genes that previously we instinctively thought would code for a single protein, we now look to see if there are multiple products. Already it is clear that the early SV40 "A gene" codes for both "big T" and "small T," and we suspect that only through splicing will we be able to analyze correctly the transforming region at the left-hand end of the adenoviral genome.

That we have played a major role in such a fundamental discovery owes much to our Center Grant from the National Cancer Institute. It let us have an unusually large number of staff members all focusing on the molecular biology of tumor viruses. Their collaborative efforts let us quickly marshall all the key facts.

An historic Symposium on Chromatin

In June our 42nd annual Symposium had chromatin for its topic. It attracted a record three-hundred and seventy-five scientists, the large audience properly reflecting the intense efforts that now exist in so many leading laboratories to correlate eukaryotic chromosome structure and function. Happily we persuaded Francis Crick to give the opening talk, in which he cautioned all to stick to facts and avoid speculation. Most everyone followed his advice, and when the time came for Pierre Chambon to summarize, his task to range from nucleosomes through splicing was indeed formidable. He did a magnificent job and the meeting ended in a mood of exhausted euphoria.

Francis Crick at 1977 Symposium

Because our lecture hall seats only some two-hundred and thirty, we used closed-circuit TV to bring the proceedings to several other rooms. And we put up a large tent behind Blackford so that we could run two separate food lines even if it were to rain. Despite the necessary hecticness, no one complained and almost everyone stayed with the proceedings all throughout the very long morning and evening sessions.

A very pleasant break for the speakers were the dinner parties given by many of our neighbors. This tradition goes back almost two decades, and this year we wish to thank in particular Mr. and Mrs. T.S. Browne, Mr. and Mrs. Roderick Cushman, Mr. and Mrs. Norris Darrell, Jr., Mrs. William Flanigan, Mr. and Mrs. Charles Gay, Mr. and Mrs. George Hossfeld, Jr., Mr. and Mrs. Gordon Lamb, Mr. and Mrs. Samuel Parkinson, Mr. and Mrs. James Pirtle, Jr., Mr. and Mrs. Edward Everett Post, Mr. and Mrs. Edward Pulling, Mr. and Mrs. Franz Schneider, Mr. and Mrs. Richard J. Weghorn, and Mrs. Alex White.

A very full program of summer meetings and courses

Our summer session lasted almost four months, commencing in early May with a small meeting on "Parvoviruses," the tiny, single-stranded, linear DNA viruses whose replication cycle should provide key insights into how DNA duplicates. The next meeting, on "The Transformed Cell," was necessarily more heterogeneous because we wanted an overview of recent work on the phenotypes of the cancer cell. The "RNA Tumor Viruses" gathering was again mammoth, but two poster sessions kept it in bounds, and the final result was generally thought first-rate.

As soon as the Symposium ended, our courses began. Most had been given before, but two had changes of emphasis. Instead of a course covering all of Animal Cell Culture, we settled on "The Transformed Cell" to give prominence to newer techniques that look at the biochemistry of the cancer cell. Likewise, the Animal Virus offering became more restricted, with the entire emphasis on "Oncogenic Viruses." Our Neurobiology offering included one brand new course, "The Synapse." It was essentially an intense lecture-discussion course and was followed by a month-long opportunity for almost half the students to do experiments in a "Synapse Workshop" held during July in McClintock laboratory.

Meetings took over again in mid-August with a successfully large gathering on the "Molecular Biology of Yeast." The annual "Phage Meeting" was in two parts; the first, which was devoted specifically to single-stranded DNA phages, will yield in 1978 a volume in our Monograph series. Next came our "SV40, Polyoma, and Adenoviruses" meeting, again very large but well worth the intensity. Concluding the summer was our fifth Conference on Cell Proliferation, this year devoted to the "Differentiation of Normal and Neoplastic Hematopoietic Cells."

Dedication of the Banbury Conference Center

The formal opening of the Banbury Conference Center was marked by an Open House held in the late afternoon of Sunday, June 14, for members of the Long Island Biological Association. Francis Crick was the inaugural speaker and gave a short, elegant address on "How Scientists Work." He talked about the need for scientists to meet frequently with their peers who have the same objectives. Going to each others' labs is generally much too time-consuming and expensive, and the best way to sort out the meanings of new ideas and experiments is to come together at small conferences like those which we plan for the Banbury grounds. Our main guest of honor was Mr. Charles S. Robertson, whose most generous gift of the Banbury main house, outbuildings, and grounds allowed the creation of the Center. Many of his family were also able to join us, and the occasion was a most happy one. Everyone appeared to like, if not be thrilled by, the most imaginative renovation scheme which has provided what we believe to be the most striking conference center now available to biologists.

Later in the summer the lecture components of our courses on the Synapse and on Tumor Immunology were held in the Conference Center, with the students living in Robertson House. Subsequently, we had numerous discussions as to what should be the first emphasis for the Banbury Conferences, which will be held during the spring and fall. The general consensus was that we should focus on Biological Risk Assessments, particularly of agents thought to act at the genetic

Banbury Conference Center

level. Toward this end a program was developed for possible consideration by foundation and government funding sources. Hopefully, this effort will be in full operation starting in late September of 1978.

Proposed expansion of our Neurobiology teaching

We now have in McClintock and Jones laboratories first-class facilities for teaching and research in neurobiology. Thanks to much assistance from the Sloan Foundation, the Robertson Research Fund, and the Marie H. Robertson Memorial Neurobiology Fund, they are splendidly equipped and in the summer let us offer the world's most advanced program of neurobiology teaching. These facilities, however, remain essentially vacant during the remainder of the year, and the question has been raised whether we could also use them for programs of year-round research.

To discuss this problem, we assembled late in August an Ad Hoc Neurobiology Advisory Committee. It was chaired by Charles Stevens of Yale and had as its other members David Hubel of Harvard Medical School, Eric Kandell of the College of Physicians and Surgeons of Columbia University, JacSue Kehoe and Phillipe Ascher of the École Normale Supérieure, Paris, and Rami Rahamimoff of Hebrew University Medical School, Jerusalem. They pointed out that the appointment of neurobiologists to our staff would automatically preempt space that now is used for teaching. If we were to have, say, three to four full-time neurobiologists, we would seriously have to restrict teaching in either Jones or McClintock lab, and this would lead to a cut back in the number of our courses. The courses we would drop, however, could not be picked up easily by another institution and would most likely vanish. So instead of trying to put together a permanent neurobiology staff, our advisors urged that uncommitted funds for neurobiology should go toward the initiation of additional courses.

We have taken their advice ana arranged two new courses for this coming summer. One will be a lecture course on Developmental Neurobiology, to be given on the Banbury grounds. The other will be a laboratory course in Jones on the newer neuroanatomical methods being developed for use in probing the organization of the central nervous system. The starting of these new courses will be made possible by the Marie H. Robertson Memorial Fund for Neurobiology.

As for potential winter use, our committee urged that we try to attract groups of neurobiologists for joint sabbatical activities. The eventual success of that plan is likely to depend on the availability of more governmental support for our teaching efforts, thereby freeing up our own funds to support sabbatical activities. Because of the payback and other restrictions in the current legislation affecting training grants, federal monies to support our neurobiology teaching have proved most

difficult to arrange. Now our federal contract covers only one-third of the cost of these programs.

A monumental publication on the Origins of Human Cancer

Just before the New Year our 1900-page, three-book set "The Origins of Human Cancer" was published. The bringing out of these proceedings of our fourth Conference on Cell Proliferation required a prodigious effort to ensure that they would successfully record the high quality of our September 1977 meeting. These volumes represent the most complete overview yet attempted on the origins of cancer and should have a major intellectual impact on the cancer research of the next decade. Toward that end we have kept the price of the books much below commercial practices and hope that their distribution will widen correspondingly. We wish to thank both the Rita Allen Foundation and the Charles E. Merrill Trust for grants that covered part of the necessarily large production costs.

Our newest Symposium volume was also a major accomplishment. Two books were required to record "The Origins of Lymphocyte Diversity." As our Symposia grow more and more crowded with speakers, the resulting proceedings must grow accordingly. Seeing that they appear well in advance of the following year's meeting is not a routine matter, and the help of many neighbors is required to minimize the time required for proofreading.

A much bigger task than anyone predicted was the publication of the proceedings of our May 1976 "DNA Insertions" meeting. This was to be a small book, priced cheaply, and quickly in print. In the end it became a horrendously complex 800-page book, filled with masses of tables and maps, and written by authors who made major changes in proof. When it emerged late in November, we could not quite believe it had made it. The final ending, however, is a happy one. All the delays have helped create a most important book which may turn out to be a minor best seller.

We thus remain most fortunate that we have a publications group that responds cheerfully to almost any setback and which never settles for less than first-class products. Great credit must go to Ms. Nancy Ford, who this year has been most ably assisted by Annette Zaninovic, Roberta Salant, Paul Bethge, Virginia Cleary, and Thelma Sambrook.

Occupancy of the new Williams House

By frantic efforts all through the spring, the new Williams House became available late in June for use by summer course instructors and participants in our Neurobiology Workshops. The five highly imaginative apartments that it provides should radically change life along Bungtown Road and may bring back hints of the

Williams House

Edward Pulling at LIBA meeting

small village atmosphere that existed during the 1840-1860 whaling days.

The availability of the Williams apartments has let us move on to the almost complete rebuilding of Cole Cottage. Though superficially one of our older dwellings, this cottage first materialized in the early 1930s as a small, uninsulated building suitable for only summer occupancy. Subsequently, it was enlarged, given a bulky hot-air heater, and later became known for its exorbitant heating costs. Renovation efforts will be complete by early April, and already we can see that Jack Richards and his staff will pull off another project they can be proud of.

Another year of strong support from LIBA

This past year the Long Island Biological Association (LIBA) came very close to completing its fund drive to cover construction and furnishing of the new Williams House. Only $15,000 remains to be raised out of the total goal of $225,000. The raising of a sum this large, entirely from neighbors, is a most remarkable achievement, and we shall long remain indebted for this support which has been most effectively marshalled by LIBA Chairman Mr. Edward Pulling. Here I should note that Ed has now passed his 10th year as Chairman. It is impossible that anyone could have handled LIBA's affairs more effectively than he, and we are indeed fortunate to have such a remarkable friend to help direct our future.

The Annual Meeting of LIBA occurred on Sunday, December 11, the speaker being Dr. George D. Ruggieri, Director of the New York Aquarium and Osborn Laboratories of Marine Sciences. His talk on "Drugs from the Sea" gave us a most lively look at marine animals and the remarkable way they may be used for man's benefit. Departing from the LIBA Board at the conclusion of the maximum term were Mr. Duncan Cox, Mr. Arthur M. Crocker, Mr. Nevil Ford, and Mr. Angus P. McIntyre. All have been active, long-time supporters of the Lab, and we trust we can still count on their active interest in our future projects. Newly elected to the LIBA Board were Mr. S.R. Calloway, Mr. Richard Olney III, Mr. Stanley Trotman, and Mrs. Alex White.

LIBA has for over fifty years been a major factor in our past successes, and as the Laboratory Director I cannot stress too strongly how wonderful it is to know that they are on our side.

Changes in our scientific staff

In the fall we keenly felt the departure of two invaluable members of our staff. George Fey returned to Lausanne to a research position at the Swiss Institute for Experimental Cancer Research. George's presence gave us the talents of a first-class protein chemist, and we sorely miss his good-natured excellence. And Lan Bo Chen moved to the Sidney Farber Cancer Center in Boston. Lan Bo's energy is almost unlimited, and while with us he carried out many important experiments on the LETS protein and how its absence from cells is correlated with the creation of cancer.

We have lost two talented postdocs, John Hassell, who now has a position at the University of Sherbrooke, and Elizabeth Ljungquist, who joined the staff at the Karolinska Institute in Stockholm. We also miss Jim Manley, who goes to postdoc at MIT after doing most of his Stony Brook Ph.D. thesis in Ray Gesteland's lab, and Norman Maitland, who finished his University of Burmingham Ph.D. thesis here with Jim McDougall and now is in Ken Jones's lab in Edinburgh.

Promoted from Staff Investigator to Senior Staff Investigator was Louise Chow. Keith Burridge, Dietmar Kamp, and Regina Kahmann have moved up from the postdoc ranks to Staff Investigator positions.

The National Cancer Institute award for construction of a new cancer research facility

In late August the National Cancer Institute formally awarded us a construction grant of $1.5 million to help erect a new building for our cancer research efforts. The major research focus of this new facility will be the mouse. The building will provide us with extensive quarters for small laboratory animals as well as a new complex of laboratories where we shall use molecular biology to study the early developmental stages of the mouse and how failures in differentiation may lead to

the generation of cancer cells. Architectural planning is being done by the noted firm of Moore, Grover and Harper of Essex, Connecticut, and we hope to initiate construction early in 1979. Toward this end we are actively pursuing the necessary matching funds to cover the now anticipated final cost of 3.2 million dollars. Already we have received a most welcome grant of $150,000 from the Kresge Foundation.

We become more complex to administrate

Although I often fantasize that with each successive year the Lab will be easier to run, the opposite is happening. We cannot have more courses and meetings, a sparkling new conference center, a real endowment, and steadily more powerful research programs without paying the price of more administrative complexity. That we remain on top owes much to our Administrative Director, William Udry. Bill has that indispensable knack of remaining intelligently optimistic no matter how great the upheaval and never responds to complexities with money instead of sense. He has been greatly aided by our invariably helpful Comptroller, William Keen, and by the good-natured astuteness of John Maroney our Purchasing Agent. An unambiguous tribute to their operation came with our recent HEW audit. We were not seriously faulted on our grant management, and unlike many leading universities not asked to return improperly spent monies.

An indispensable Board of Trustees

The matters that we bring each year to our Board of Trustees are frequently not simple, and we continue to depend much upon their seasoned responses to a necessarily heterogeneous mix of trivia and the important. Most fortunately we have in Harry Eagle a Chairman who is near perfect in knowing the tenor of our various funding sources and so advising us when it would pay to think big. We moreover can only marvel at the speed with which Harry runs through tricky agendas. Unfortunately, this year we lost the services of Drs. Sherman Beychok, Robert Webster, Lewis Thomas, Messrs. Angus P. McIntyre, Charles S. Robertson, and Colton Wagner. All have served us well, and we shall much miss their counsel. In particular, I wish to point out the extraordinary help given by Angus McIntyre. As our Treasurer for the past four years, Angus was a bastion of sensible advice and performed many, many invaluable functions. Fortunately, we have persuaded Clarence Galston to succeed Angus as Treasurer, and we shall continue to have experienced integrity at our side.

At the conclusion of our Annual Meeting on October 12, 1977, a special dinner was held in Robertson House to honor our retiring Trustees and express our firm hope that we shall still be able to count on their able counsel. Newly elected to the Board this year as Institutional Trustees were Dr. Charles R. Cantor to represent Columbia University and Dr. Walter Guild to represent Duke University. Our new individual Trustees are Mr. Robert L. Cummings of Laurel Hollow, Mr. Walter Frank, Jr. of Locust Valley, Mrs. Robert H.P. Olney of Lloyd Harbor, and Mr. William S. Robertson of Lloyd Harbor.

The transfer of Wawepex Society assets to the Lab

Science first came to Cold Spring Harbor under the patronage of Mr. John D. Jones, a member of the illustrious Long Island Jones family who gave the name to the great beach on the south shore and which dominated commercial life at Cold Spring Harbor during the 19th century. Mr. Jones, as President of the Atlantic Mutual Insurance Company, presided over the largest marine insurers in the United States and as such was one of New York City's leading citizens. He was a man of much strength, and in early life came out for the rights of slaves, an opinion that angered certain neighbors and may have led to the burning of his house on the west shore of Cold Spring Harbor. Later he rebuilt on that site the grand Victorian house that we now call the Carnegie Dorm.

In the early 1880s he and his friend, Eugene Blackford, a leading Fulton Street fish merchant, started a fish hatchery on his land next to St. John's Church. Several years later in 1890 he gave the funds and land for the starting up of the Biological Laboratories adjacent to the hatchery. When its first permanent building, now known as Jones lab, was completed early in 1892, Mr. Jones set up the Wawepex Society to ensure long-term financial support for his fledgling scientific community. For over eighty years the Wawepex Society has flourished as an independent body which long has been one of our steadiest benefactors. The recent passage of the Foundation Reform Act, however, made unnecessarily complicated the long-term existence of such a family foundation. So over the past several years the Wawepex Society has transferred to us its assets, including the land near 25A which we had leased from them since our creation. The Wawepex Society will continue to exist, and one of its members, by bylaw a Jones family descendant, will always continue to be a member of our Board of Trustees. Since I have been the Director, Mr. Dudley Stoddard, Mr. Townsend Knight, and Mr. Bache Bleeker have been our Wawepex Trustees and all have been among our most devoted champions.

Our long period of emergency is over

For the past fifteen years we have had to work very hard—first to survive when the Carnegie Institute of

Washington began the close down of its Department of Genetics and then to grow again into a serious body for the advancement of fundamental biology. In the beginning the outcome was in doubt, and only through the efforts of many loyal friends did the Participating Institutions come into existence and join forces with our friends in this community to see that we kept going as an independent body. The first years after the reorganization were agonizing, but under John Cairns's intelligent direction the worst was passed, and we were on an upward course when I took over as Director. Initially, I focused upon the restoration of a vigorous scientific program, believing that if we could get rolling, funds would somehow materialize for our badly worn out physical plant. Naturally, we were so absorbed in catching up that our outlook was inward, with little time to think about the problems of others.

Now we are completely different. The science we do, the demanding excellence of our courses and meetings, and the high quality of our publishing program convey to the world outside the aura of a quality post-graduate university. We worry not about becoming good, but instead on how to ensure that we continue to carry out science at the highest possible level. This is a very new problem for us, and we are still somewhat uncertain how we should proceed.

To start with, I suspect that we shall have to provide more long-term stability for our staff than seemed ideal when we were just getting going. Our future is too important to rest on only a few heads, and the expectation of spending a major fraction of one's research life here is probably a necessary ingredient in developing the necessary loyalties. I suspect that tenure in the conventional academic sense is not the answer because there is no place here for anyone to go asleep intellectually on full pay. Instead, I believe we should move toward positions where our more senior staff have contracts that always have at least five more years to run. This security should allow our scientists to undertake projects which may require many exploratory years before results of deep significance materialize.

It is important here to realize that our own resources can never guarantee the funds that our scientists will need for their research. For the main part these must come from federal sources, and the long-term ability of our senior staff to continue high-powered science will be largely a function of their past track records. This situation, of course, is not unique to us and holds for virtually every important research institution in this country. We thus can hope that means will be found to reverse the current discouraging trend for most funding sources to award monies for shorter and shorter intervals. The insecurity this creates cannot be overemphasized and increasingly leads to overattention to problems whose answers we already partially know.

We must also accept the fact that we cannot go on expecting our friends to always front for our causes. We are again strong enough to help fight the battles in Washington over how the federal monies for science should be distributed. Only by joining in can we be sure that our positions are heard, and, conceivably, help lead to wiser decisions on Capitol Hill and within NSF and NIH. The time so spent will divert us from our experiments, but we should not have strong opinions unless we are willing to fight for them.

We should also be increasingly concerned with the future of Long Island. Our Cold Spring Harbor community is still one of the more attractive sites where science is now practiced in the United States. This may no longer be the case if virtually all our green fields become lost first to suburban sprawl and then to suburban blight. Unlike individual families, we do not have the choice of moving, and we would be most short-sighted if we did not fight to keep Long Island livable. We have many friends who feel as strongly, and we must work hard and effectively together.

I conclude by noting that this is my tenth report as the Director. That I still look forward so much to what we can do in the future owes immeasurably to my wife Liz. We married just as I took over, and her steadfast desire never to do a job half-well has been a major factor in keeping our sights so high.

March 31, 1978 — *J.D. Watson*

YEAR-ROUND RESEARCH

MOLECULAR BIOLOGY OF TUMOR VIRUS

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Our major areas of interest are (1) how the various products of the early region of the SV40 genome cause cells to enter and settle in the transformed state; (2) adeno-SV40 hybrid viruses; (3) SV40 as a vector for the insertion of genes into mammalian cells; and (4) the structure and synthesis of specific RNAs coded by adenoviruses.

Integration and expression of SV40 products in relation to transformation

There is a wealth of evidence indicating that two conditions must be fulfilled for stable transformation to occur. First, the cells must acquire the sequences of the early region of the SV40 genome. Usually these viral DNA sequences recombine with those of the host and are then passed on to the cell's descendants like any other part of the cellular genome. In the past, part of the work of this section has been to decipher the structure and arrangement of the integrated viral genes in sets of isogenic transformed cell lines. Comparative analyses at the molecular level have shown that integration of viral DNA does not occur at a special location in either the host or viral genome (Botchan et al., *Cell 9:*269 [1976]). Thus, there was no reason to expect that SV40 should be integrated exclusively into a specific chromosome. Nevertheless, C. Croce and coworkers, on the basis of segregation studies with somatic cell hybrids, have claimed that human chromosome 7 carries a site that is preferentially used for integration of SV40 DNA. In the past year, in collaboration with R. Kucherlapati, and J. K. McDougall (of the Mammalian Cell Genetics Section), we have analyzed the arrangement of SV40 DNA sequences in a series of somatic cell hybrids formed between SV40-transformed human cells (GM637) and mouse line B82. We find that, as in other human cells transformed by SV40, a fraction of the cells in this population are spontaneously induced and are replicating free viral DNA. By a two-cycle purification procedure involving a sucrose gradient fractionation and an agarose gel electrophoresis separation, the replicating free viral DNA can be removed from the chromosomal DNA. Our hybridization analysis shows that there is one stable insertion of SV40 DNA in cell line GM637. The same SV40 viral insertion was detected in somatic cell hybrids derived by cell fusion between GM637 and mouse line B82. Furthermore, in ten hybrid clones examined, free SV40 DNA could not be observed. This result is not surprising as the hybrid clones had lost many human chromosomes, and therefore the genes needed for viral replication had probably been lost. By coordinating a careful karyotic analysis of these hybrid clones with an SV40 T-antigen analysis, we concluded that the single insertion of viral DNA resides in chromosome 8 in the parent line GM637. Although additional independent transformed cell lines will have to be examined, it now seems quite clear that SV40 insertion into human DNA is not chromosome-specific, as sites have been mapped to chromosomes 7, 8, and 17.

The second prerequisite for transformation is that the integrated set of viral early genes must be expressed. So far, two polypeptides have been identified as products of the SV40 early region — the well-known T antigen (m.w. 94,000) and a recently discovered 17,000-dalton moiety (small t). It is securely established, by several lines of evidence, that transformation of nonpermissive cells by SV40 requires the presence of functional T antigen. First, data obtained both here and in A. van der Eb's laboratory have shown that transformation can be achieved by infecting cells with subgenomic fragments

of viral DNA (obtained by the use of restriction endonucleases) which span the entire *A* gene; no fragment of DNA that contains only a segment of the gene carries transforming activity (Graham et al., *Cold Spring Harbor Symp. Quant. Biol. 39:*637 [1975]; W. Topp, unpubl.). Second, cells infected at the nonpermissive temperature with viruses carrying temperature-sensitive mutations in the *A* gene (tsA mutants) do not become transformed; furthermore, temperature-shift experiments indicate that the *A*-gene product begins to be required soon after infection. Third, cells transformed at the permissive temperature by tsA mutants of SV40 exhibit a partially temperature-sensitive phenotype (Tegtmeyer, *J. Virol. 15:*613 [1975]; Brugge and Butel, *J. Virol. 15:*619 [1975]; Martin and Chou, *J. Virol. 15:*599 [1975]; Osborn and Weber, *J. Virol. 15:*636 [1975]; Kimura and Hagaki, *Proc. Natl. Acad. Sci. 72:*673 [1975]). Fourth, the product of the *A* gene (T antigen) is always present in transformed nonpermissive cells (Risser and Pollack, *Virology 59:*477 [1974]). Fifth, revertants of cells transformed by SV40 have either lost the SV40 DNA or contain a nonfunctional viral set (Steinberg et al., *Cell 13:*19 [1978].

During the past year we have shown that small t also plays a role in transformation. A series of SV40 mutants was isolated which had suffered deletions of varying lengths between map positions 0.54 and 0.59. Mutants with deletions mapping within this region showed little or no change in their ability to grow in a range of permissive and semipermissive cells. Thus, it is clear that the deleted sequences are not necessary for viral replication; neither do they encode structural information for T antigen — even the largest deletion caused no detectable change in the size of T antigen measured by SDS-acrylamide electrophoresis. Similar results have been reported for the mutant dl884 by Shenk et al. (*J. Virol. 18:*664 [1976]) and Tegtmeyer (cited in Cole et al., *J. Virol. 24:*277 [1977]). In addition, we found no difference in the T-antigen levels measured by complement fixation in CV1 cells 48 hours after infection with mutant or wild-type virus.

Although T antigen appeared to be unaffected by deletions in the 0.54 – 0.59 region, the small t protein, first described by Prives et al. (*Proc. Natl. Acad. Sci. 74:*457 [1977]), was either reduced in size in extracts from cells infected with the mutants or absent entirely. This constitutes strong evidence that part of the coding sequence for small t protein lies between positions 0.54 and 0.59 on the genome. More importantly, the deletion mutants are defective in their capacity to transform certain lines of cultured cells. Thus, when compared by the same assay, rabbit fibroblasts and the revertants of rat-1 SV40 transformants were more readily transformed by the deletion mutants than the original rat-1 line or NIH Swiss 3T3 cells. In addition, a striking effect was found when the ability of the mutants to transform was measured by different assays. Thus, the efficiency of transformation of rat embryo fibroblasts by the deletion mutants was indistinguishable from that by wild-type virus when the assay was carried out at low cell density, i.e., by plating cells out at low dilution after infection and scoring for the formation of densely staining colonies. However, when the cells were not subcultured after infection, so that transformation required formation of a dense focus of cells in a monolayer, transformation by the deletion mutants was not above background levels.

A possible explanation for this assay-dependence became clear during a closer examination of the properties of the rat embryo fibroblast lines transformed by mutant and wild-type virus. All lines displayed a transformed morphology, and there was little difference in the abilities of wild-type and mutant transformants to grow in low and high serum. However, all 13 lines of mutant transformants reached a saturation density of $2-3 \times 10^5$ cells/cm^2, only about twice that of the nontransformed parent; whereas in similar experiments cells transformed by wild-type virus reached significantly higher saturation densities ($4-6 \times 10^5$ cells/cm^2; Risser and Pollack, *Virology 59:*477 [1974]). In our experiments all but one of the wild-type transformants sloughed off the plate before a saturation density was reached. Moreover, only one out of thirteen mutant transformants grew in semisolid medium, whereas five out of seven wild-type transformants gave measurable colony formation. It is not surprising, therefore, that the mutants appear not to transform when assayed by dense-focus formation on a monolayer of nontransformed cells or by growth on semisolid medium. From these results it seems that transformants derived from the deletion mutants are generally less transformed than those derived from wild-type virus and generally resemble in their properties the minimal transformants described by Risser and Pollack (*Virology 59:*477 [1974]). Studies are now underway to determine whether cells transformed by the mutants are tumorigenic.

The major problem that we now face is to define precisely how small t and T antigen interact with the cellular machinery to produce the transformed phenotype. No data are yet available for small t, although it is interesting to speculate that the protein may be a protein kinase or a protease. In the past twelve months, however, we have gained significant insight into the nature of the interaction between T antigen and specific sequences of nucleic acid. Progress actually began with the discovery that cells infected with the Ad2-SV40 hybrid Ad2$^+$D2 produce large quantities of a 107,000-dalton phosphorylated protein which is specifically immunoprecipitated by anti-T sera and shares extensive peptide homology with authentic SV40 T antigen (J. Hassell et al., *J. Mol. Biol.* [in press]; R. Tjian,

unpubl.). This hybrid virus greatly facilitated the difficult task of purifying large quantities of biologically active T antigen because large quantities of HeLa cells infected with Ad2⁺D2 could be grown in suspension cultures. In addition, late after infection host protein synthesis is inhibited by Ad2, but the infected cells continue to produce and accumulate protein related to SV40 antigen protein. Thus, 1 mg of highly purified 107,000-dalton protein can be isolated from 16 liters of infected HeLa cells in 3 days by a simple procedure involving nuclear elution followed by three standard chromatographic columns. Because the 107,000-dalton polypeptide encoded by Ad2⁺D2 is a fusion protein containing some adenovirus-coded amino acids at its amino terminus and most of the SV40 *A*-gene sequences at its carboxy terminus, it is called the D2 hybrid protein.

To check that the purified protein retained at least some of the biological activity generally attributed to authentic SV40 T antigen, microinjection experiments were carried out. After injection into individual cells in culture by means of a glass capillary, the D2 hybrid protein was shown to induce the synthesis of cellular DNA and to provide helper function for the growth of Ad2 in monkey cells (R. Tjian et al., *Proc. Natl. Acad. Sci.* [in press]). With the reassurance that the purified protein was biologically active, we then asked whether it could bind to specific regions of the SV40 genome.

Purified protein was allowed to bind radio-labeled SV40 DNA, and the DNA-protein complex was then digested with an excess of pancreatic DNase I to remove all DNA sequences not shielded by the protein. The 107,000-dalton protein was found to bind to SV40 DNA and to protect three fragments from nuclease digestion. These protected fragments consist of double-stranded DNA approximately 30, 75, and 120 nucleotides in length. Hybridization, endonuclease cleavage, and pyrimidine tract analysis of these DNA fragments indicate that all three protected sequences arise from a region near the origin of SV40 DNA replication and consist of tandem binding sites extending approximately 60 nucleotides on either side of position 67. At low concentrations of D2 hybrid protein, the preferred binding site is a stretch of 30 nucleotides located at about position 66; at high concentrations of protein, binding occurs at adjacent sites extending the protected sequence toward position 68 (Fig. 1). Thus, the D2 hybrid protein binds in a sequential manner to tandem recognition sites located within a sequence of 120 nucleotides which is likely to encompass the origin of DNA replication and may contain the start of early and late transcription.

More recently, specific bases (A and G), which lie within the protected sequences and are in close contact with the protein, have been identified by using dimethyl sulfate in a modified DNA sequencing reaction as described by W. Gilbert and A. Mirzabekov (pers. comm.). These studies should yield a better picture of the actual binding and recognition sequences within the protected fragments. Finally, experiments have been initiated to determine the functional role of the multiple binding sites. These experiments involve detailed binding and sequencing studies of DNA replication mutants constructed from viable viruses containing two origins of replication (T. Shenk, *Cell* [in press]). We intend to extend this approach to catalog, identify, and clone the *cellular* DNA sequences with which T antigen acts. With some luck, such studies may lead to a clearer understanding of the role of T antigen in transformation.

We have also begun to examine the role of T antigen in transformation of permissive cells. By analogy with results obtained by others with nonpermissive cells (cited above) one might have predicted that simian cells infected at the nonpermissive temperature with tsA mutants of SV40 would be neither killed nor transformed. Surprisingly, when an early SV40 mutant, tsA 209, was used to infect CV1 cells (a permissive established simian line) at 40.5°C, no viral DNA synthesis was detectable; after several weeks, however, transformation was observed, as assayed by formation of dense foci on cell monolayers. The efficiency of transformation was about 0.1%.

The properties of one established line isolated from a dense focus are:

1. All cells are T-antigen-positive.
2. The T antigen has an electrophoretic mobility similar to that of T antigen extracted from lytically infected cells.
3. Cells are permissive to superinfection with either SV40 virions or DNA.
4. The cells do not contain free SV40 DNA after incubation at the permissive temperature (33.5°C).

More lines have been isolated and their properties are under investigation. We hope that these cell lines may be useful in investigating, at the molecular level, an interesting biological phenomenon — the rescue of SV40 from transformed cells. Although a single active copy of the viral early region is sufficient to sustain transformation, cells usually contain additional sequences of viral DNA. In many cases the complete viral genome is present: when lines of cells transformed by SV40 and free of infectious virus are fused with simian cells, infectious SV40 becomes detectable a day or so later (Watkins and Dulbecco, *Proc. Natl. Acad. Sci. 58:*1396 [1968]; Koprowski et al., *Proc. Natl. Acad. Sci. 58:*127 [1968]). Our current model for the mechanism by which rescue of viral DNA from the integrated state might occur is shown in Figure 2.

Our recent data show unequivocally that the genetic source for this rescued virus is the integrated copy. On

Hind III, Hae III

A A G C T T T T T G C A A A A G C C T A G G C C T C C A A A A A A G C C T C C T C A C T A C T T C

1, 10, 20, 30, 40

T T C G A A A A A C G T T T T C G G A T C C G G A G G T T T T T T C G G A G G A G T G A T C A A G

Alu I, Hae III, Hae III, Bgl I, Hae III

T G G A A T A G C T C A G A G G C C G A C G C G G C C T C G G C C T C T G C A T A A A T A A A A A

50, 60, 70, 80, 90

A C C T T A T C G A G T C T C C G G C T C C G C C G G A G C C G G A G A C G T A T T T A T T T T T

Bgl I

A A A T T A G T C A G C C A T G G G G C G G A G A A T G G G C G G A A C T G G G C G G A G T T A G

100, 110, 120, 130, 140

T T T A A T C A G T C G G T A C C C C G C C T C T T A C C C G C C T T G A C C C G C C T C A A T C

Late Transcription

Bgl I

1 — 20 — 40 — 60 — 80 — 100 — 120

.66 .67 .68

Early Transcription

Figure 1
Sequence of nucleotides at the binding site of D2 hybrid protein. The sequence of 140 nucleotides in the region of the SV40 origin of DNA replication was reproduced from the published data of Dhar et al. (Proc. Natl. Acad. Sci. 74:*827 [1977]). Endonuclease cleavage sites of the indicated restriction enzymes are represented by arrows (↑↓). The 30-, 75-, and 120-nucleotide protected fragments are represented by (••••••), (------), and (———), respectively. The boxed areas in the lower diagram represent a possible arrangement of binding sites deduced from the pattern of protected fragments. The large numbers on the bottom represent coordinates on the conventional SV40 DNA map, and the leftward and rightward arrows represent the directions of early and late transcription, respectively. (Reprinted, with permission, from Tjian,* Cell 13:*165 [1978].)*

the one hand, by using sensitive blotting hybridization techniques, we can show that no detectable free viral DNA is carried in the transformed cell lines (less than one copy in 1 out of 50 cells); on the other hand, after cell fusion, between 30% and 60% of the heterokaryons form infectious centers. This quantitation effectively rules out the suggestion that rescue of virus reflects a low-level, persistent infection (or plasmid state) of SV40 in a small proportion of these transformed rodent cells.

A curious problem is raised by this phenomenon in light of the finding that the attachment of SV40 to the host chromosome is not site-specific. How then is the viral DNA specifically recognized for excision if there is apparently nothing particular about its attachment sequences? How does the viral DNA recyclize to form infectious SV40 DNA? Some recent observations have given some insight into this problem. When tsA-transformed rat cells are fused with simian cells, no free viral DNA is detected in the Hirt supernatants when the heterokaryons are incubated at 41°C. However, after shift to 32°C, free viral DNA is found to be present. Moreover, there is a five- to tenfold increase in viral DNA still associated with chromosomal DNA which precedes the detection of free viral DNA. We believe, therefore, that the first steps of viral excision involve a T-antigen-dependent activation of the viral DNA replication in situ (steps a–e in Fig. 2). The simian cells would then provide replicative functions not present in nonpermissive rat cells. This polytenized structure would be unstable, especially if replication elongation were not a continuous process. Eventually, a newly initiated replication fork would meet an old one, and a free linear molecular containing SV40 DNA would be generated (step f). Sterically free to recombine with itself, the SV40 DNA may then re-form a circle. In this regard we have found that cell lines that contain tandem

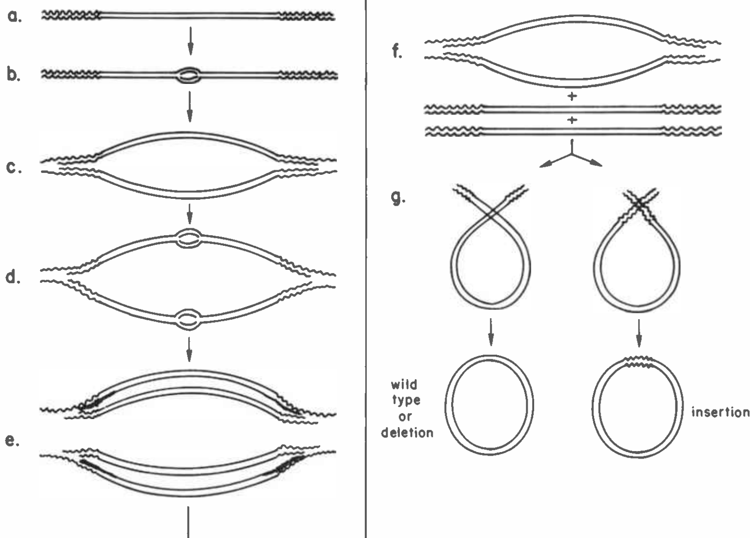

Figure 2
A model for SV40 viral excision from chromosomal DNA.

duplications of integrated SV40 DNA yield infectious virus with an efficiency 100 to 1000 times greater than lines that show no detectable duplications. In one cell line (14B) where the integrated viral DNA has been carefully analyzed and shows no evidence of terminal duplication, no infectious virus could be rescued. However, free, noninfectious viral DNA, heterogeneous in size, could be detected in the Hirt supernatants of heterokaryons made between 14B and CV1 cells. This heterogeneous viral DNA has been used in a DNA infection as a helper for tsA DNA replication in monkey cells. Plaques were picked and it was found that the virus stocks retained insertions of rat DNA. In view of these findings we can then speculate (step G) that, in the absence of tandem duplication, SV40 DNA recyclizes by illegitimate recombination to form deletion or insertion variants. An exciting extension of this reasoning, which may be very useful for experiments involving SV40 as a cloning vehicle, is that the SV40 origin sequences excise with nonviral sequences of DNA attached to them. In support of this notion we have observed excision and replication of fragments of SV40 DNA in heterokaryons formed between CV1 cells and rat cell lines transformed with the *Bam-Hpa*II fragment of SV40 DNA. In short, then, all of our circumstantial evidence supports the ideas suggested by our replication-excision model. We intend to obtain more direct data on the mechanisms of excision by studying SV40 viral DNA excision from adeno-SV40 hybrid viruses and by a biochemical investigation of the factors present in CV1 cells that allow for this replication.

Adeno-SV40 hybrid viruses

We reported last year that Ad2$^+$ND1 host-range mutants that have lost the ability to grow on monkey cells are chain-terminator mutants. The mutants *140*, *162*, and *71* fail to synthesize the 30K proteins specified in part by the SV40 sequences they contain and instead produce fragments of 19K *(140, 162)* and 10K *(71)*. In

collaboration with Ray Gesteland and Jim Lewis of the Protein Synthesis Section, we found that the synthesis of the polypeptide fragments directed by mRNA isolated from mutant-infected cells and translated in mammalian cell-free systems was altered when the in vitro system was supplemented with yeast suppressor tRNA. The mRNA of mutant *71* is translated in the presence of yeast ochre tRNA into wild-type-size 30K protein as well as the 10K fragment. mRNA from mutants *162* and *140* yield large amounts of 30K protein in the presence of amber tRNAs. We are now trying to assay in vivo the activity of suppressor tRNAs by microinjecting them into mutant-infected monkey cells and determining whether the yield of virus is thereby increased.

We also reported last year that revertants of the chain-terminator mutants could be isolated which had regained the ability to grow efficiently on monkey cells. Whereas revertants of *162* produced 30K protein and had genomes identical to Ad2⁺ND1, some revertants of ochre mutant *71* had suffered a deletion (revertant Δ7a) or contained a partial duplication (revertants dp2a and dp1a) of the region of the SV40 insertion. It was suggested that the deletion of Δ7a (which eliminates the SV40 sequences that map between positions 0.28 and 0.21) removes the ochre triplet, leaving only the C'-terminal end of the 30K protein which is required for helper function (see Fig. 3). This deletion now turns out to be only one of a family of deletion revertants that have been isolated from mutant *71*. So far, five viable and one defective mutant *71* deletion revertants have been obtained, as well as one viable deletion revertant from amber mutant *140* (see Fig. 4). The genomes of the revertants have been analyzed by analysis with restriction enzymes and heteroduplex mapping and the proteins they produce have been studied in vivo and in vitro. The deletions carried by the viable mutants range in size from 50 to 300–400 bp. None of these viruses synthesize either the 30K protein or the mutant fragment. Rather, they all synthesize new proteins, all of which are smaller than 30K. The larger the deletion, the smaller the protein that it produces. The defective deletion revertant produces a new protein of 112–115K, presumably because the C-terminal end of the 30K protein has become fused to a large adenoviral protein such as hexon or 100K.

We have also isolated an additional mutant *71* revertant, dp3a, which, like dp1a and dp2a, carries a partial duplication consisting of a fusion between the beginning of the adenoviral fiber gene and the distal part of the SV40 insertion. All of the revertants with rearranged genomes were isolated by growing the mutants at moderately high multiplicities (10–50 pfu/cell) in monkey cells, and the structures of their genomes suggest that they have arisen as a result of unequal crossing-over (see Fig. 3). This further suggests that the SV40 sequences in Ad2⁺ND1 can recombine at low frequency with any region of the Ad2 genome. The recombinant may be recovered provided that the new virus has a large enough genome to be packaged and that the distal part of the SV40 sequences is expressed. We have examined this possibility further by isolating from another Ad2-SV40 hybrid virus, Ad2⁺ND5, revertants that grow on monkey cells. Although Ad2⁺ND5 contains SV40 sequences that extend from map position 0.39 to 0.11 and produces a 42K protein (Kelly et al., *J. Virol. 12:*643 [1973]; Mann et al., *J. Virol. 24:*151 [1977]), it fails to grow on monkey cells. By nitrous acid mutagenesis and by passage at 10–50 pfu/cell in monkey cells, we have obtained variants of ND5 that grow on monkey cells. The viruses grown at moderately high multiplicities are all defective and have suffered deletions. This suggests that the region in Ad2⁺ND5 needed for helper function

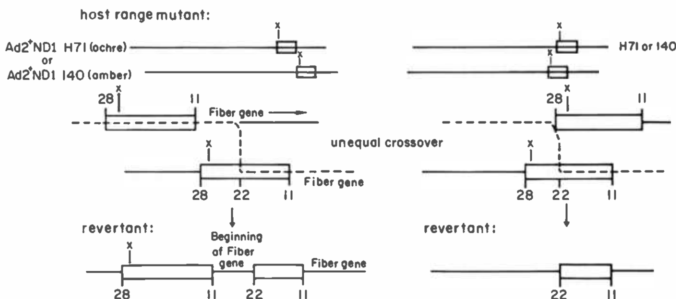

Figure 3
Model for the generation of revertants that carry deletions and duplications.

	Revertant specific proteins		DNA
	in vivo	SV40 selected mRNA translated in vitro	
Ad2⁺ND1	30K	30K	
71	(10K)	10K	
140	19K	19K	
1. deletions: 71ʳΔ			
Δ 3b	29K	29K	Δ vv small (~50 bp)
Δ 4a	28K	28K	Δ < 1-150 bp
Δ 5a	–	not done	Δ 1-150 bp
Δ 6a	23K	23K	Δ > 1-150 bp
Δ 7a	–	18K	Δ 3-400 bp
Δ 8a	115K	–	Δ large: defective virus
2. duplications: 71ʳdp			
dp 1a	58K	58K + 10K	Bam ~5% Bam; SV40 SV40
dp 2a	23K	23K + 10K	~2.5%; SV40 SV40
dp 3a	21K	not done	~2.2%; SV40 SV40
3. deletions: 140ʳΔ			
Δ 50a	25K	25K	Δ < 1-150 bp
4. other: 140ʳ			
1a-1	29K	not done	no change
3a-1	29K	not done	no change

Figure 4
Revertants of Ad2⁺ND1 host-range mutants 71 and 140.

is intact. The nitrous acid-induced revertants have genomes identical to Ad2⁺ND5, as determined by analysis with restriction enzymes, and produce the 42K protein when SV40-specific revertant mRNA is translated in vitro.

We are presently engaged in studies to determine the factors responsible for the growth (or lack of it) of various mutants and revertants in monkey cells. We are concentrating our efforts on analyzing the synthesis of a unique class of viral mRNAs whose 5′ ends consist of adenoviral sequences and whose 3′ ends consist of SV40 sequences (hybrid mRNA). This approach has been made possible by the development of a technique (sandwich hybridization) in which RNA is hybridized to defined fragments of viral DNA bound to nitrocellulose filters in such a way that the 3′ end of the RNA protrudes as a single-stranded tail. The DNA sequences complementary to the tail sequences can then be detected by a second round of hybridization using ^{32}P-labeled SV40 DNA. The high sensitivity of the technique has led to the observation that the single hybrid mRNA synthesized at late times following infection of monkey cells with Ad2⁺ND1 hybridizes not only to sequences adjacent to the SV40 insertion in Ad2⁺ND1, but also to other discrete regions of the Ad2 genome. This observation, along with similar discoveries in other laboratories, has led to the conclusion that RNA sequences (leaders) derived from multiple, noncontiguous areas of the viral genome become linked during or after transcription. Similar studies carried out with a hybrid mRNA synthesized during the course of lytic infection with Ad2⁺ND1 dp2 have revealed the presence of four discrete leader sequences associated with sequences at the 5′ end of the mRNA. We have been able to demonstrate that, in the cytoplasm of infected cells, two classes of hybrid mRNA exist which differ from one another in the presence or absence of the leader sequence immediately adjacent to the coding sequences of the mRNA. We have isolated both forms of the hybrid mRNA and have shown that each is able to function in an in vitro protein synthesis system.

Our current hypothesis is that the leader sequences are rather stable processing intermediates which lie at the end of the chain of events that occurs when precursors are fashioned into mRNAs. We are currently engaged in experiments to test this idea.

SV40 as a vector for the insertion of genes into mammalian cells

A eukaryotic vector system is being developed for the study of gene expression. Our aim is to link eukaryotic cloned genes to defective SV40 and to propagate the recombinants in the presence of a complementing temperature-sensitive SV40 helper virus, with the following question to be answered: Can the genes introduced into the defective virus be expressed during the lytic infection cycle, or in cells stably transformed with such a recombinant? For a start, we have chosen to link histone genes of the sea urchin *Psammechinus miliaris* to SV40 because these genes are very well characterized. They were first isolated by density gradient centrifugation (Birnstiel et al., *Proc. Natl. Acad. Sci. 71:* 2900 [1974]) and then by cloning (Clarkson et al., *Nucleic Acids Res. 3:*2617 [1976]). The DNA sequence of a large, continuous segment of the H2B histone gene has been published (Birnstiel et al., *Nature 266:* 603 [1977]). More than 3300 nucleotides of the 6000-bp repeat are known to date, including the whole coding sequence, or large sections of it, for all five histone genes, as well as prelude and trailing sequences and segments of the AT-rich spacer DNA (W. Schaffner et al., *Cell* [submitted]).

For the construction of an appropriate SV40 vector (Goff and Berg, *Cell 9:* 695 [1976]), a DNA fragment has been purified consisting of the origin of replication and the whole early region, but lacking the late functions. This fragment was elongated by terminal transferase with about 100 dAMP residues at each end (Roychoudhury et al., *Nucleic Acids Res. 3:*863 [1976]). Cloned 6000-bp histone DNA was fragmented to an average size of 2000 bp and was elongated by the same enzyme with about 100 dTMP residues. Poly(A)-elongated SV40 "early" fragment was annealed to poly (T)-elongated histone DNA.

The resulting SV40-histone DNA recombinants were then used in the presence of DEAE-dextran to infect CV1 monkey cells which had been infected previously with a high multiplicity of SV40 temperature-sensitive in the early function (tsA 209). The cells were overlayed with agar medium and kept at high temperature. Cells that had received both a tsA and A^+-histone DNA recombinant could support viral growth, resulting in the formation of a plaque. To date, 54 plaques which showed hybridization to ^{32}P-labeled histone DNA have been identified by the blotting technique of Villareal and Berg (*Science 196:*183 [1977]). The virus of the corresponding agar overlay has been recovered. Eight such recombinant clones have been purified by replaquing. Experiments are in progress to find out whether sea urchin histone genes are expressed during the lytic cycle in monkey cells.

Another approach is the study of histone gene expression in (nonpermissive) rodent cells transformed by recombinant SV40. Starting from two recombinant virus clones, nine transformed cell lines have been obtained and subcloned. All of them are positive in an immunoassay for T antigen. They are currently being tested for the presence of sea urchin-specific DNA and for expression of the histone genes.

Structure and synthesis of specific RNAs coded by adenoviruses

Work has continued on both the virus-associated (VA) RNAs and the 9S messenger RNA which codes for polypeptide IX of adenoviral particles. Despite the heterogeneity of VA RNAs isolated from adenovirus-infected cell cytoplasm, there are just two genes in the viral DNA which give rise to those small, abundant transcripts. The genes are close neighbors, separated by only 80 bp and—unique in the viral genome—are transcribed by the host cells' RNA polymerase III. Studies of the RNAs themselves and of the corresponding region of the viral DNA in this laboratory and in that of S. Weissman have elucidated most of the sequence of each of these two genes. As anticipated from earlier hybridization and fingerprint data, the sequences of VA RNA_I and VA RNA_{II} show little homology. By contrast, the regions preceding the 5′ terminus of the RNAs contain a number of similar clusters of nucleotides, although the sequences as a whole differ greatly. The similarities presumably reflect the function of these regions, which both groups thought to be involved in the initiation of transcription. Other genes, from sources as disparate as yeast and *Xenopus,* which are also recognized by polymerase III share the same clusters of nucleotides, an observation that strengthens the idea that these sequences may play a general role in the DNA:protein recognition process.

Unlike the genes for other structural constituents of the adenoviral particle, the gene for polypeptide IX is located in a portion of the genome that is transcribed into RNA in the early phase of the infectious cycle, before the beginning of viral DNA synthesis. The gene also shares with one other virion component, polypeptide IVa_2, the distinction of lying outside the region encoding the long nuclear transcript that gives rise to most of the "late" mRNA species. As a further corollary, the 9S RNA product of this gene lacks the tripartite leader sequence common to the other "late" messengers. These properties suggest that the synthesis of polypeptide IX might be controlled by a mechanism distinct from that which operates over the other "late" mRNAs. Experiments carried out with H. Persson and U. Pettersson uphold this hypothesis. Polypeptide IX is first detectable in adenovirally infected cells before the other "late" proteins, but after the appearance of au-

thentic "early" proteins. Furthermore, unlike the other "late" proteins, its production is not prevented by interference with viral DNA replication. On the basis of these findings, another class of adenoviral products has to be added to the well-established "early" and "late" categories. By analogy with phage T4, we propose to include polypeptide IX in a class of "quasi-late" proteins, which are defined as products whose syntheses do not require viral DNA replication absolutely but accelerate markedly in the postreplicative phase of the lytic cycle.

The revelation of unexpectedly complex patterns of overlapping and partially overlapping viral transcripts, the result of work from several laboratories employing varied techniques, has underlined the need for a more detailed analysis of translationally active messenger RNAs. Using cell-free translation systems to identify their coding ability, we have begun to characterize mRNAs with respect to size and sequence content.

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PROTEIN SYNTHESIS

R.F. GESTELAND, J. Atkins, R. Bigelis, J. Broach, M. Fey, M. Harter, B. Hoffman, D. Klessig, J. Lewis, J. Manley, C. McGill, P. Reichel, R. Silver, J. Strathern, N. Wills, C. Woldron

Suppressors

We have further characterized yeast suppressor tRNAs using the cell-free synthesis of bacteriophage Qβ synthetase as an assay system. The serine-inserting suppressor tRNAs from the allelic ochre and amber resessive lethal suppressor mutants have been purified to 50–90% homogeneity. The combination of BD cellulose chromatography and Sepharose 4B chromatography results in material that has two components, as seen on acrylamide gel electrophoresis, one of which is the serine-inserting suppressing species. The identity of the second component is not yet known and has so far proven difficult to purify from the suppressor species.

We have also been investigating a yeast UGA suppressor (from Gerry Fink of Cornell University) and have been using the readthrough of the normal UGA terminator at the end of the beta chain of rabbit globin as an assay. This UGA suppressor tRNA is strongly retarded on BD cellulose. A second assay—production of the Qβ coat protein readthrough—does not give a positive response to this tRNA. We are currently looking at the protein chemistry of the products from the globin readthrough to identify which tRNA is involved.

Cloning

Using serine tRNA as a probe, we have identified three clones among the recombinant DNA collection produced by Peter Wensink of Brandeis University and David Botstein of the Massachusetts Institute of Technology that have serine tRNA sequences. In addition, using the Southern procedure, the serine tRNA probe reveals 12–14 restriction fragments from DNA of the suppressor-carrying cells, and we are cloning these fragments in *E. coli*. The known sequences for the serine tRNAs make strong predictions about the presence of sites for various restriction enzymes in the DNA of these clones and should provide valuable information for identification of the various species. Once these suppressor-containing clones are in hand, they will be tested for activity by reintroduction into yeast cells and will be used to try to construct vectors that may express these genes in mammalian cells with the hope of producing suppressor cell lines.

Nonsense mutants of Ad2-SV40

We have identified two amber and one ochre mutant among the host-range mutants of Ad2-SV40 hybrid virus isolated by Terri Grodzicker of the Tumor Virus Section. These mutants were selected to be defective in the SV40-encoded function that permits the hybrids to grow in monkey cells. Thus, mRNA from the infected cells purified by hybridization to SV40 DNA was translated in a cell-free system to which various suppressing tRNAs could be added. The SV40-specific message from the wild-type infected cells encodes a 30,000 m.w. protein. Two of the mutants produce a 19,000 m.w. protein, which when translated in the presence of amber suppressor tRNA produces some of the wild-type 30K protein. Another mutant makes a 10,000 m.w. fragment, which when suppressed with ochre tRNA gives wild-type product. Hence, we are convinced that ochre and amber nonsense mutations of adeno-SV40 hybrid virus are in hand and will provide a biological assay for potential suppressor cell lines. We are now trying to microinject suppressing tRNAs into mutant-infected monkey cells to see whether these tRNAs can function in vivo and whether serine inserted at these positions can give a product with biological activity.

Prokaryotic translation

With the arrival of John Atkins as a visitor in the laboratory, we have reinvestigated the old problem of translation in vitro of RNA phage RNAs that results in the synthesis of some small fraction of protein longer than any of the known genes. In particular, the synthetase of either R17 or MS2, which has a molecular weight of 63,000, is translated in vitro in predominantly this form but also gives rise to a 5% component that has a molecular weight of 66,000. Examination of this reveals that additional, longer components are present, and by translation under various conditions with various tRNAs, we have tentatively concluded that the only viable explanation for the existence of these longer polypeptides is that at some low frequency ribosomes can frame-shift, thus avoiding the normal termination site and giving rise to proteins longer than normal. It is necessary to invoke both +1 and −1 frame-shifting to explain the results. The level of frame-shifting is greatly enhanced by addition of purified wild-type threonine tRNA (obtained from Brian Reed, University of California at Riverhead) to the cell-free system, and this enhancement is diminished by inclusion of increasing amounts of a specific proline tRNA. Hence, the tentative model is that threonine tRNA is causing frame-shifting of both the + and − types of proline condons, and that if sufficient proline tRNA is present to compete, the frame-shifting is not observed. Experiments done to date are all circumstantial with respect to supporting these ideas, and we are currently setting out to do appropriate protein chemistry experiments to test the frame-shift model. In addition, we are setting up a genetic system that we hope will be able to answer whether such phenomena occur in vivo and are utilized.

Host-range mutant 1 Ad2 that multiplies on monkey cells

A variant (hr400) of Ad2 that overcomes the block to multiplication of the wild-type Ad2 on monkey cells has been selected, after nitrous acid mutagenesis, by sequential passage on monkey cells. Its growth properties are similar on human and monkey cells and comparable to those found for wild-type Ad2 on human cells (Klessig, *J. Virol. 21:*1243 [1977]. An extensive comparison of the genome of hr400 with that of its parent by restriction endonucleases, electron microscopy, and hybridization analysis failed to detect any differences, which suggests that a point mutation may be responsible for its increased host range. SV40 sequences, which in certain Ad2-SV40 hybrid virus (e.g., Ad2^{+}ND1) allow adenovirus to multiply efficiently in monkey cells, were not detected in hr400. Whereas the synthesis of many of the late Ad2 proteins is markedly inhibited in monkey cells, hr400 can fully express all of its late genes in both monkey and human cells. Thus, the mutation affects directly and/or indirectly the expression of most or all of the late viral genes. The mutation has been mapped by a modified marker-rescue technique to within 16% of the viral genome (coordinates 59−75). Its position suggests that it may alter a protein (100K) whose function may be involved in the metabolism of the late viral mRNAs, since the 100K protein has been reported to bend to ribosomes (Russell and Blair, *J. Gen. Virol. 34:*19 [1977]) and/or associate with RNA in the form of RNP particles (Lindberg and Sundquist, *J. Mol. Biol. 86:*451 [1974]).

Common leader on late Ad2 mRNAs—RNA splicing

The analysis of the 5′ termini of two late Ad2 mRNAs which encode the fiber and 100K proteins has been continued. After obtaining highly purified mRNA by hybridization to restriction endonuclease fragments of Ad2 DNA followed by electrophoresis on polyacrylamide gels containing 98% formamide, the 5′ terminal oligonucleotides generated by RNase T_1 digestion of the messengers were selected by dihydroxyboryl-cellulose chromatography. Both mRNAs gave an identical 5′ undecanucleotide with the general structure $7mG^{5'}ppp^{5'}A^{m}C^{(m)}U(C_4U_3)G$. This undecanucleotide could be removed from the mRNA by mild RNase treatment after hybridization to DNA fragments containing the main coding sequence of the messenger. In contrast, a small region defined by *Bal*I E (14.7−21) protected this undecanucleotide from RNase. A second region contained within both *Hin*dIII B (17−31.5) and *Hpa*I F (25.5−27.9), although unable to protect the undecanucleotide, hybridized to both fiber and 100K mRNAs and protected a similar sequence of 100 to 150 nucleotides. These observations indicate that both mRNAs contain a long common sequence complementary to at least two different sites on the Ad2 genome remote from the start of these two genes. This suggests that these mRNAs are chimeric molecules, parts of which are encoded at, at least, three noncontiguous sites on the Ad2 genome. Electron microscopic analyses by Chow et al. (*Cell 12:*1 [1977]) indicate that the common leader sequence is found on eight different late mRNAs, including fiber and 100K, and that parts of it are encoded at three noncontiguous segments on the viral genome (at coordinates 17, 20, and 27). A general mechanism for the biosynthesis of such chimeric mRNAs from larger precursor molecules is shown in Figure 1. Its basic feature is that two or more noncontiguous segments of RNA within the large precursor molecule, which will become adjacent, are brought together by looping out the intervening sequences and then covalently joined via an intramolecular ligation reaction (splicing).

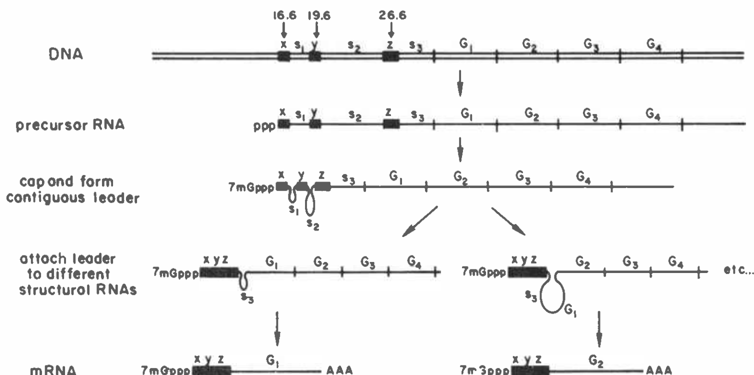

Figure 1
The model diagrammed above presents a mechanism by which different segments of an RNA molecule encoded at widely spaced sites on the DNA are brought together to form a contiguous leader sequence, which is then attached to various structural RNAs encoded downstream. The numerical coordinates indicate the region on the Ad2 genome encoding the three segments (XYZ) of the leader. These three segments are brought into close proximity by looping-out of the intervening sequences (S_1 and S_2) and are then covalently joined by intramolecular ligation. This leader is coupled to various structural RNAs (G_1-G_4) encoded downstream by another looping-out of any intervening sequences (S_3, S_3+G_1, or $S_3+G_1+G_2$, etc.) followed by intramolecular ligation and removal of distal messenger sequences. sequences.

Ribosome-binding sites on Ad2 messenger RNA

Jim Manley has investigated whether the leader sequences found on late Ad2 messenger RNAs are involved in ribosome binding. ^{32}P-labeled mRNA was bound to ribosomes in a reticulocyte lysate in the presence of diphtheria toxin and sparsomycin to inhibit ribosome movement. After loading with RNase, the protected fragments were sized, fingerprinted, and hybridized to restriction fragments of Ad2 DNA. Sequences corresponding to the 5′ ends of genes could be identified, but neither the capped oligonucleotide nor the leader sequences that map from position 17 through 27 were found. Hence, these structures that are found on the 5′ end of messenger sequences are not an intimate part of the initiation site.

Purification and cell-free translation of Ad2 mRNA

Our efforts have continued toward producing a more complete map of the Ad2 genome by using hybridization to a specific fragment of Ad2 DNA to purify one or a few mRNAs, which are then identified according to the protein produced upon cell-free translation of the mRNA. A few changes in our experimental procedures, such as using higher multiplicities of infection when preparing early Ad2 mRNA and substituting KOAc for KCl in cell-free translation, resulted in the recognition of the additional early proteins 13K, 14K, 17K, and 21K. Increasingly these experiments have benefited through collaboration with others here at Cold Spring Harbor. For example, Ad2 mRNA that had been characterized by cell-free translation, and in some cases greatly enriched by hybridization purification, has been, in collaboration with Louise Chow and Tom Broker of the Electron Microscopy Section, mapped precisely by R-loop analysis in the electron microscope. A summary of our current knowledge of mRNA map positions and of the proteins encoded by each mRNA is given in Table 1.

Our previous experiments mapping late Ad2 mRNA by hybridization to specific DNA fragments were partially hampered by the fact that many late mRNAs seemed to hybridize to two sections of the genome. The majority of the mRNA for a given protein was selected by hybridization to a DNA fragment specific for that protein. However, smaller amounts of many mRNAs were also selected by fragments from near the left end of Ad2 DNA. In particular, each of the late Ad2 mRNAs encoded to the right of 29 map units exhibited some hybridization to fragments within the internal 17–19

Table 1
Coordinates of AD2 mRNAs

Protein	Map units of Ad2 DNA: segment of DNA used to select mRNA	Map units of Ad2 DNA: mRNA map coordinates by R loop	Strand orientation
Early 40–50K	0–4.4	1.3–4.0	R
Early 15K	4.4–17.0	(5.0–6.4	R
		5.0–11.1)	R
Late IX	7.5–17.0	9.8–11.1	R
Late IVa_2	7.5–17.0	11.2–14.9(?)	L
Late IIIa	29.1–40.9	—	
[Late III	29.1–59.0	—	
Late V]	29.1–59.0	—	
Late P-VII	40.9–59.0	—	
Late P-VI	40.9–70.7	49.5–51.9(?)	R
Late II	40.9–70.7	51.9–62.2	R
Early 72K	58.5–70.7	62.4–67.9	L
[Late 100K			
Late 38K	58.5–83.4	(68.0–78.6	R
Late P-VIII]		68.0–82.8)	R
[Early 15.5K			
Early 14K	75.9–83.4	(78.6–82.8	R
Early 13K]		78.6–86.2)	R
Late IV	83.4–98.5	86.2–91.5	R
[Early 21K			
Early 19K	89.7–98.6	91.5–96.8	L
Early 17K			
Early 11K]			

Brackets indicate proteins whose mRNAs have not been ordered with respect to each other, or which may be translated from the same mRNA.

Parentheses indicate mRNAs that hybridize to overlapping regions so that one or both may be the mRNA for the protein(s) whose gene(s) map to that region.

map units. This complementarity was not shown by early RNAs or by those late mRNAs mapping to the left of 17–19 map units. Ashley Dunn (Mammalian Cell Genetics Section) and John Hassell (Tumor Virus Section) have used a different technique to show that several mRNAs of Ad2-SV40 hybrid viruses are each homologous to two or more widely separated regions of the genome. These observations appear much more sensible in light of the demonstration by Dan Klessig, Louise Chow, and Tom Broker (Electron Microscopy Section) and Richard Gelinas and Rich Roberts (Nucleic Acid Chemistry Section) that several, if not all, Ad2 late mRNAs mapping to the right of 29 contain at their 5′ ends a sequence of 100 to 200 nucleotides coded for by three different regions of the genome, mapping at 16.6, 19.6, and 26.6 map units. The implications of these findings are not yet clear, but the organization and expression of Ad2 genetic information are apparently considerably more involved than previously thought.

Characterization of revertants of Ad2⁺ND1 host-range mutants

Amber and ochre translation-termination mutants of Ad2⁺ND1 have been isolated by Terri Grodzicker. These mutants synthesize, respectively, only 19K and 10K fragments of the Ad2⁺ND1-specific 30K protein and grow only on human (not monkey) cells. In collaboration with Eugene Lukanidin, George Fey, Denise Galloway, Terri Grodzicker, and Joe Sambrook (Tumor Virus Section) and Sayeeda Zain (Nucleic Acid Chemistry Section) we have been characterizing revertants of these mutants. Cell-free translation of purified "30K" mRNA from cells infected with revertants determined for each revertant whether the revertant protein was the Ad2⁺ND1 30K, the mutant 19K or 10K, or something different from either. This information was correlated with studies of the revertant DNA, and in some cases the peptide composition of the revertant

protein was studied. In brief, these experiments show that only a small part of the C-terminal region of 30K, which is missing in the amber and ochre mutants, is necessary for growth on monkey cells. This result arises from comparison of the many ways in which the ability to grow on monkey cells is regained in different revertants. In some cases no gross changes in DNA structure are evident, and the revertant synthesizes the wild-type 30K. In other cases a region of the DNA, presumably including the mutation, is deleted so that a protein smaller than 30K is synthesized by the revertant. The revertant protein (18K–29K in various revertants) presumably contains both the N- and the C-terminal regions of the wild-type 30K but lacks a region in the middle. In still other cases the region of the DNA coding for the C-terminal of 30K is duplicated and inserted into another region in the genome. The resulting protein has the C-terminal of 30K but an entirely different N-terminal sequence. In this model genetic system the virus clearly utilizes a variety of different strategies to regain expression of the portion of the 30K gene required for growth on monkey cells.

Studies of Ad2 early proteins

Many Ad2 early proteins can be identified in extracts of cells that have been treated with cycloheximide to enhance synthesis of early Ad2 mRNA and then labeled with [^{35}S]methionine. Using SDS-polyacrylamide gel electrophoresis to analyze cell extracts, proteins of 11K, 14K, 15K, 19K, 21K, and 72K were found in extracts of infected, but not mock-infected, cells, and these correspond in electrophoretic mobility to proteins synthesized in vitro using purified early Ad2 mRNA. Two-dimensional gel electrophoresis reveals, in addition, six polypeptides of 38–50K and acidic (pH 5.9–6.0) isoelectric point not present in mock-infected cells and similar in apparent molecular weight and isoelectric point to the 40–50K synthesized in vitro. Furthermore, a comparison of peptides produced by partial enzymatic proteolysis shows that the six 38–50K polypeptides synthesized in infected cells are much related in peptide composition to each other and to the 40–50K synthesized in vitro using mRNA complementary to the leftmost Ad2 early gene, which is associated with the transforming ability of Ad2. The chemical basis for the electrophoretic heterogeneity of this protein is unknown (why are there six polypeptides of 38–50K for the product of one gene with a maximum coding capacity of 36K?). However, the identification of 40–50K in infected cells enables us to use two-dimensional gel analysis as an assay to guide the purification of this protein. These experiments are in progress.

Publications

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MAMMALIAN CELL GENETICS

J. McDOUGALL, C. Casserly, C. Copple, A. Fabrizi, K. Jones, S. Levings, N. Maitland, C. Mutt, E. Paul, M. Shevchuk, C. Stewart, M. Szadkowski, R. Tepper, D. Whitfield

During this year we have concentrated on emphasizing that part of our program which deals with mechanisms of tumor induction and the investigation of more sensitive methods for detecting viral nucleic acid sequences in human tumors. With regard to the in vivo experimental aspects of tumor induction, we continued to be limited by the lack of suitable animal facilities at Cold Spring Harbor but were lucky to have the collaboration of Phil Gallimore at Birmingham and Raju Kucherlapati at Princeton, in whose laboratories studies could be carried out in the athymic nude mouse.

In attempts to develop the in situ hybridization technique further, the contribution of Ken Jones from Edinburgh was of great value and it was a pleasure to have him in this laboratory for part of the year. The cooperation of Dr. Cecilia Fenoglio at the College of Physicians and Surgeons of Columbia University was invaluable.

Transformation by adenoviruses

We have continued the studies on the tumorigenicity of adenovirus-2 (Ad2)-transformed cell lines, in collaboration with Phil Gallimore and Lan Bo Chen (Harvard).

A number of in vitro characteristics were examined in an attempt to define one or more correlating with the ability to produce tumors in adult athymic nude mice. The results indicate that high saturation density, increased protease activity, decreased doubling time, and anchorage-independent growth are frequently features of Ad2-transformed cell lines but are not essential for a cell line to be tumorigenic. Three of the seven adenovirus-transformed lines failed to uphold the hypothesis that anchorage-independent growth correlates with tumor formation in adult athymic nude mice. Ad2/F17 (efficiency of plating [EOP] 5.25%) and Ad5/HEK FG (EOP 30.3%) both show anchorage-independent growth in methyl-cellulose but were nontumorigenic in the adult nude mouse, and Ad2/F19 (EOP 0.02%) shows anchorage dependency in this system but produced two slow-growing tumors which, although benign in nature, did not regress, being excised when 2.5 cm in diameter.

If anchorage-independent growth reflects a potential for growth in vivo, then there should be a correlation between both plating efficiency and/or colony size in methyl-cellulose and the latent period and/or tumor incidence in the nude mouse. This was not observed in our study even for Ad2 lines that show anchorage-independent growth.

From their study of SV40-transformed rat embryo cell lines, Pollack et al. (*Proc. Natl. Acad. Sci. 71*:4792 [1974]) provided evidence that elevated levels of plasminogen activator accompanied loss of anchorage dependency, and they suggested that this proteolytic activity may have a significant role in tumor invasion and metastatic spread. The significance of fibrinolytic activity for malignancy has recently been questioned by the observation of Pearlstein et al. (*Cancer Res. 36*:1475 [1976]) that some normal cells—e.g., cells from calf bladder—produce high levels of protease as measured by fibrinolysis. In the same report Pearlstein et al. observed that loss of LETS protein from transformed cells was not due to their increased synthesis of protease. We have confirmed this finding in that the nontumorigenic line Ad2/F17 has high fibrinolytic activity and yet produces a massive three-dimensional network of LETS protein. It is possible that proteolytic enzymes different from plasminogen activator may be associated with a cell line's malignant behavior, and, with this in mind, we have recently investigated Ad2/50A further. This cell line is the most malignant and the highest producer of fibrinolytic activity of the Ad2-transformed rat lines;

however, exponentially growing cultures of Ad2/50A do not hydrolyze overlays of purified rat tail collagen even after prolonged incubation (10 days at 37°C). This preliminary finding implies that collagenase activity is not associated with this cell line's aggressive infiltration of normal tissues.

Ad2-transformed cells expressing normal levels of LETS protein either grow poorly in vivo or respond to the control processes that inhibit the growth of normal cells in vivo. This could explain a previous observation that tumorigenicity in syngenic rats did not always appear to be a reflection of the antigenic status of a cell line. Cell line Ad2/F4 is heterogeneous for the expression of LETS protein, and it can be postulated that the requirement for additional anti-thymocyte serum-induced immunosuppression before this line produces tumors in syngenic rats is a reflection of the time it takes for LETS-protein-negative cells to establish themselves in vivo, the latent period of this line in nude mice being 19 days. The finding that all three Ad2/F4 nude mouse tumor lines show a dramatic reduction in LETS-protein-positive cells and are highly tumorigenic when inoculated into newborn syngenic rats lends some support to this theory.

LETS protein therefore appears to have a major role in determining the in vivo behavior of Ad2-transformed cell lines. We are continuing to study the role of LETS protein in other transformation systems and in human tumor sections.

Adenovirus-12 replication

A series of hybrid cell lines derived from fusions of mouse and human cells has been established in culture in order to examine the factor(s) that permit replication of this serotype in human cells, but presumably are absent or repressed in the cells of other mammals in which this virus cannot replicate but can transform. The hybrid cell lines carry subsets of the human genome in various combinations. Initial results suggest that the block to virus replication in mouse cells is dominant in the hybrids examined so far.

Transformation of human cells by SV40

In collaboration with Raju Kucherlapati and Mike Botchan (Tumor Virus Section), we are analyzing a number of human cell lines transformed by SV40 to determine the integration sites in the human genome. In one such cell line we have shown that the presence of integrated SV40 DNA and the expression of virus-coded T antigen are associated with the retention of human chromosome 8 in mouse/human hybrids. This increases the number of human chromosomes (from different SV40-transformed cell lines) with integration sites for SV40 DNA to four (nos. 2, 7, 8, and 17). This finding is consistent with the concept of random integration sites for viral DNA.

Transformation by herpesvirus type 2

We have previously reported transformation of TK^- cells to a TK^+ phenotype by the transfection of specific restriction endonuclease-derived HSV2 DNA fragments. We have continued to study these biochemically transformed cells to obtain information about the control of viral gene expression in such cells. The initial experiments indicated that growth of the transformed cells on medium where TK gene expression was not required for cell survival did not affect gene expression, but more careful study revealed that a small fraction of the transformed cells had ceased to produce viral TK under these nonselective conditions. Revertant TK^- cells were subsequently produced by growth in back-selective medium containing 5-bromodeoxyuridine and were investigated for reversibility of the loss of TK expression. The results raise the possibility that viral TK expression is under some form of cellular control in these cells.

Detection of virus mRNA in tumor sections

The application of molecular hybridization techniques to the detection of viral DNA integrated into host-cell genomes has provided identification of specific transforming regions of the chromosomes of smaller DNA viruses. Such methods are based upon hybridization in solution or hybridization in situ to DNA transferred from gels to nitrocellulose. Although it is true that these approaches have advanced our knowledge of experimental systems and provide a sound biochemical basis for developing an understanding of the mechanisms of transformation, there have been no unequivocal studies implicating these smaller DNA viruses (e.g., SV40, adenovirus) in the etiology of human tumors, and, indeed, the results of some recent studies argue that the evidence is strongly against such an involvement.

In the case of the larger DNA viruses, e.g., herpesviruses (particularly Epstein-Barr virus and herpes hominis type 2), the epidemiologic and serologic data present stronger arguments for an etiologic association with human tumors. However, the fact that the size and complexity of herpesvirus genomes are of a higher order than those of SV40 and adenovirus genomes does raise greater problems in terms of detection of putative integrated viral sequences. If the minimum quantity of DNA required for transformation is of a size similar to that established for the smaller viruses, then the target for hybridization represents only 1–2% of the herpesvirus genome. Probes presently in use do not provide an

efficiency of detection at this level and are therefore of limited value, even when used to examine a cell population *wholly* composed of cells retaining, say, 1–2% of the virus DNA molecule. Furthermore, there is as yet no herpesvirus fragment identified as containing "transforming genes," a prerequisite for obtaining increased hybridization efficiencies in searching for integrated viral DNA.

Examination of tumor tissue, as opposed to transformed cell lines, is complicated by a number of factors not encountered in experimental tissue-culture systems. Tumor, or putative tumor, samples may be small in mass, especially in the case of biopsies, and will certainly consist of both neoplastic and normal tissues, with the normal frequently present as the dominant type. Extraction of DNA or RNA from such samples will certainly result in a dilution of the nucleic acid sequences from any particular cell type and could provide false negatives. Outgrowth of cells in culture may also be inefficient in terms of a preferential selection of cells able to grow in vitro; the resulting cell line may not be representative of the original tumor cells.

To overcome these factors. we propose to approach the problem of detecting viral sequences by further developing the technique of in situ cytological hybridization of viral DNA to virus-specified messenger RNA. The primary advantages of the method are that (1) direct examination of individual cells in smears or sections can be achieved, and (2) the target sequences should be amplified as mRNA, provided that any integrated viral DNA is transcribed. Experience with experimental transformations using DNA viruses argues strongly that, if viral sequences are present, then there is transcription of at least part of the DNA. Thus, a transformed cell line of Ad2 (rat) origin containing 14% of the Ad2 genome in six copies (each amounting to 1.6×10^6 daltons [5.3 kb]) of transcribed DNA has been used to provide a model system of viral insertion sequence detection. Viral mRNA was detected after a 4–6 week exposure using an in-vivo-synthesized DNA probe (^{3}H-labeled whole virus genomal DNA). The level of autoradiographic signal was at saturation at the time of examination, indicating a very much shorter practical time for detection. Similar cells recently have been shown by direct analysis to have a number average of about 300– 400 mRNA molecules of viral origin per cell (Flint and Sharp, *J. Mol. Biol. 106:*749 [1976]). Assuming a total transcript, this is about 1.8×10^4 kb mRNA (or approximately 0.5 times as much as in the case of globin mRNA in the fully expressing cell). Experiments (C. Godard and K.W. Jones, ms. in prep.) using AKR-MuLV cDNA to detect 70S RNA tumor virus genomes in leukemia cells have confirmed the value of the technique in this area as well and have led to a number of technical improvements to the method.

Herpesvirus-transformed cells have been examined in our laboratory using the in situ cytological hybridization method to detect virus mRNA, and we are continuing these studies in experimental HSV2-induced tumors.

With the foregoing as a basis, we have commenced a program in which we intend to examine biopsies from cases of cervical carcinoma and premalignant cases of dysplasia and carcinoma in situ. Our preliminary results from experiments in which an HSV2 [^{3}H]DNA probe has been hybridized to frozen sections and impression smears indicate specific hybridization to only some areas of the sections. Pathology results on serial sections from the positive biopsies show that HSV2 hybridization is found only to cells in the neoplastic tissue or in macrophages and lymphoid cells associated with areas of abnormality. Results from similar attempts at hybridization to other human tissue and other neoplasms have so far been negative. We must stress that the numbers tested so far are small. We cannot at present rule out the possibility that these preliminary findings indicate replicating virus DNA in the neoplastic cells. Should this prove to be the case, it would be surprising to find that HSV2 grows preferentially in these cells when the expectation is that all cells in the biopsy would be permissive. Hybridization of other nick-translated viral DNA probes, i.e., Ad2, SV40, and bacteriophage lambda, to the same tissues gave negative results.

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MOLECULAR GENETICS

D. ZIPSER, E. Cheng, R. Kahmann, D. Kamp, D. Kwoh

Bacteria recognize a wide variety of mutant and altered proteins as abberant and degrade them. This degradation occurs in actively growing bacteria and apparently is not related to the degradation of proteins induced by starvation. Over the years, our laboratory has used nonsense mutations in the Z gene of the *lac* operon as a model system to study this degradation. The degradation of long nonsense fragments is totally dependent on an energy supplied to the cell. It is instantly blocked by the addition of cyanide or other respiratory poisons but is unaffected by protein-synthesis inhibitors such as chloramphenicol. The degradation of shorter nonsense fragments is greatly slowed by the addition of energy poisons but is not completely stopped. In the past year we have continued our study of a class of mutations that affects the degradation of proteins in *E. coli*. These mutants were originally isolated in our laboratory several years ago by Ahmad Bukhari, and in the past year their genetics and physiology have been actively studied and analyzed in cooperation with Susan Gottesman at the National Institutes of Health. We have shown unambiguously that all of the mutations isolated by us so far that affect degradation are synonymous with a class of mutations identified previously by the characteristic that they make the cells mucoid. These mucous-producing mutations are called *lon*, and so we have changed the name of all protein-degradation-inhibiting mutations to *lon*. This is a significant finding because *lon* has long been known to have an extremely polytropic phenotype affecting many features of the cell, including cell-wall properties, DNA replication, the ability to repair UV damage, and the inducibility of a variety of enzymes. The action of *lon* on β-galactosidase nonsense fragments is virtually the same as the action of energy poisons such as cyanide. These *lon* mutations block the degradation of long nonsense fragments, such as X90, completely but only slow the degradation of the shorter fragments, such as 545. This year we have been able to demonstrate that, in the *lon* background, whatever degradation of nonsense fragments occurs is completely insensitive to the addition of cyanide. Thus, we have come to the conclusion that the *lon* mutation blocks all energy-dependent degradation in *E. coli* but does not affect energy-independent degradation. We have known since the visit of Charles Miller two years ago that the *lon* mutation does not affect any of the known proteases of *E. coli*, and this statement is still true. If *lon* affects a protease, this protease is still unknown.

Clearly, a study of protein degradation requires that the reaction should be studied in cell-free conditions. We have been trying for a long time to carry this out and the studies continued this year. So far, they have been a complete failure. Since the result is negative, we do not know the cause, but we do know that it is not the result of an obvious error. For example, energy-dependent degradation does not take place in an in vitro system capable of synthesizing β-galactosidase starting with DNA. In addition, we have shown this year that energy-dependent degradation is blocked instantly by the addition of toluene to growing bacteria under conditions in which sufficient ATP and other metabolities are present in the external media to allow cells to continue DNA synthesis. These conditions of toluene plus added ATP are sufficient to allow the degradation of lambda repressor by the X protein, as demonstrated by Jeffrey Roberts at the University of Wisconsin. We feel quite confident that the X protein, or the *recA* function, is not involved in the degradation of nonsense fragments, as this degradation occurs in rec^- cells and, in addition, probably does not occur under in vitro conditions in which lambda repressor is degraded by the product of the *recA* gene. Thus, although we have failed completely in our attempts to run energy-dependent degra-

dation of nonsense fragments in vitro, our experiments this year have convinced us of the importance of continuing this effort to find out what mysterious ingredient or structure is missing from our in vitro work.

We have had considerably greater success in studying energy-independent degradation of nonsense fragments. Small nonsense fragments are degraded in the absence of energy, and this degradation continues in in vitro systems. For example, extracts of *E. coli* rapidly degrade the smaller N-terminal peptides of β-galactosidase which are detectable by complementation and are called "auto-α." These peptides are produced by autoclaving β-galactosidase and can be sensitively assayed in a complementation system. In our work this year we have shown that when β-galactosidase is autoclaved, a series of peptides ranging in size from about 3000–4000 m.w. up to about 20,000 m.w. are produced which are active in complementation. When these peptides are mixed with crude extracts of *E. coli*, the smaller size class is degraded rapidly, whereas the larger size classes are virtually untouched, the cutoff being at about 7000 m.w. We have confirmed that this is in fact due to protein degradation by partially purifying the fragments involved and following their disappearance from acrylamide gels. Using the degradation of small auto-α as an assay, we have been able to purify the enzyme that carries out the reaction and have called it "protease III." Protease III is present in actively growing *E. coli* as well as in extracts of *lon* mutations. Its purification was very difficult because it binds strongly to other proteins but only weakly to various ion-exchange columns. It required a rather elaborate purification procedure that took considerable time to work out. The other factor complicating its purification was that it is present in very low amounts in cells. While it is still too early to make an accurate estimate of its concentration, it could be as low as 10 molecules per cell. The enzyme has a large molecular weight, about 110,000, is active as a monomer, and, when purified, is quite stable under proper conditions and is very active. The purified enzyme has a size selectivity. It degrades practically no proteins larger than 7000 m.w., although there may be specific exceptions to this, and degrades many peptides smaller than 7000 m.w. Degradation appears to be to fragments too small to retain on acrylamide gels. We have not yet determined the mechanism of degradation.

During the year we have also developed a variety of strategies for isolating mutations in protease III but so far we have not obtained a mutant in this enzyme. A mutant in protease III is extremely important because, in addition to being able to remove this background from our in vitro systems to allow us to have a higher sensitivity for finding the energy-dependent system in vitro, mutants should also throw light on any relationship between the energy-dependent and energy-independent systems.

Cloning the left end of phage Mu and the repressor and operator

The genetic maps of the lytic and lysogenic forms of phage Mu are the same. The left end of the virion DNA has a short segment of random bacterial DNA attached to it. Extensive mapping of phage Mu has indicated that the repressor gene, called the "C gene," is the first identifiable Mu function on the left end. When phage Mu is cut with the restriction enzyme *Hind*III, the extreme left-end piece is about 1000 base pairs long and contains the short, random, bacterial DNA segment. During the past year we have cloned this fragment on the amplifiable plasmid pMB9 using the method of adding poly(A) and poly(T) tails with the enzyme terminal transferase. We believed these clones would be useful for two purposes: first, to try to isolate the previously undetected Mu-repressor protein, which mapping indicated probably resided in this fragment; and second, to have material for use in sequencing the ends of phage Mu. These plasmids would also serve as a source of sequence material for sequencing the left end. Clones were obtained which rendered their host immune to Mu. Examination of the biological properties of these strains indicated that many of them overproduced repressor. The level of repressor was so high that even though the cloned repressor was from a temperature-sensitive gene, high temperatures did not remove all traces of repression. This pattern is virtually identical to that found when clones that overproduce lambda temperature-sensitive repressors are studied. By transferring the plasmids carrying the cloned repressor gene to minicell-producing strains, we were able to identify on acrylamide gels a band of 25,000 m.w. which was clearly correlated with the presence of Mu immunity. Minicells are ideal for this because the only proteins produced in them are directed by the small plasmids present in the strain, as the chromosomal DNA does not enter the minicell itself. Having identified the repressor protein as a band on a gel, and being able to obtain it virtually radioactively pure by lysing minicells, we proceeded to see if it would bind specifically to Mu-operator-containing DNA. For this we used sucrose gradients to separate DNA-bound repressor from soluble labeled proteins. Each fraction of the gradient was then analyzed on an acrylamide gel; in this way we could follow the binding of repressor to DNA in a very straightforward and unambiguous fashion. These experiments showed that at low salt concentration repressor would bind to any DNA but that at appropriate higher salt concentration it bound only to DNA carrying Mu operators. For example, at the appropriate salt concentration repressor did not bind to lambda DNA or to DNA from phage D108 (which is homologous to Mu everywhere except in the immunity regions), but it did

bind to wild-type Mu DNA. We also found that Mu repressor at the appropriate salt concentration did not bind to the original plasmid pMB9, but it did bind to the plasmid which was used to synthesize it, i.e, the plasmid on which the repressor gene had been cloned. This indicates that the operator maps very close to the repressor gene, not an unexpected result in view of our knowledge from other phages.

We have also cloned segments from the right-hand end of Mu which contain the boundary between the right-hand end of Mu and the large section of bacterial DNA present at that end. These segments will enable us to sequence the Mu bacterial boundary at the right end, and, together with the previously described left-end clone, we should be able to sequence both ends of Mu. Sequencing has already begun on a left-end clone, but we do not yet have sufficient data to be able to define the unique sequences of Mu.

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INSERTION ELEMENTS AND PLASMIDS

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Transposition of DNA sequences is one of the most exciting topics in molecular genetics today. Many laboratories around the world are examining the structure and functioning of transposable elements in prokaryotic organisms. Intriguing cases of inserts in eukaryotic genes are also beginning to raise some interesting questions concerning the organization and evolution of eukaryotic genomes. It is too early to tell, however, whether these inserts are a manifestation of the complex phenomenon of transposable elements.

A fundamental property of the prokaryotic transposable elements is that they have "active" ends. Various genetic rearrangements—deletions, inversions, duplications—can be initiated at the ends. The ends can also link up with unrelated host genes to form complex transposable units called transposons. The temperate bacteriophage Mu, the focus of our work, also has "active" ends whose behavior is strikingly similar to the behavior of the ends of transposable elements. Thus, the mechanism by which Mu DNA is inserted into the host DNA is interesting not only because of the random integration of Mu, but also because it is a model system for studies on transposition of DNA sequences.

During the last two years, work in our laboratory on the structure and integration of Mu DNA has led us to propose that integration of Mu DNA follows its replication. We have continued to test the replication-integration hypothesis in detail. In addition, we have begun an analysis of the Mu proteins involved in the replication-integration cycle of Mu and are determining the nucleotide sequences at the ends of prophage Mu DNA. The sequencing studies should answer the questions of whether there is any peculiarity at the host sites at which Mu DNA is inserted and whether or not the two ends of prophage Mu contain identical sequences in inverted order, as is the case with the transposable elements.

Mu integration and replication

The problem of circles

Whether or not Mu DNA is converted to a covalently closed, circular form after infection of host cells has been a major point of discussion. Mature Mu DNA has a unique structure. It contains random host sequences at both ends; thus, a special mechanism would be required for fusion of Mu ends. Last year we reported that Mu DNA is, in fact, not converted to a circular form. To examine this question further, we infected Mu-sensitive, or Mu lysogenic cells in which Mu does not replicate, with ^{32}P-labeled Mu particles, extracted the DNA at various times after infection, and subjected the DNA samples to electrophoresis in 0.3% agarose gels. On these gels Mu DNA can be separated from host DNA. No unusual form of Mu DNA could be detected in these experiments. In parallel experiments with bacteriophage P2, whose DNA is about the same size as Mu DNA, conversion of ^{32}P-labled P2 DNA to a circular form could be demonstrated readily. These results strengthen our conclusion that initial events in the infectious cycle of Mu do not include circularization of infecting Mu DNA.

Integration of infecting Mu DNA

The experiments discussed in last year's annual report indicated that the infecting Mu DNA is not a good substrate for integration and that it is probably replicated before any integration can take place. We have tested the ability of ^{32}P-labeled Mu DNA to integrate into the host chromosome under various conditions. The main approach was to infect cells with labeled phage particles and then separate the host DNA and the phage DNA on 0.3% agarose gels. The phage band and host band were then cut out and analyzed for radioactivity in the scintillation counter. These experiments showed that integration of Mu DNA occurs slowly, reaching a maximum of 20% to 30% of the input label (subtracting the background of label associated with host DNA) about 40–50 minutes after infection. This result indicated that some integration of infecting phage DNA does take place. However, this integration is blocked when host DNA synthesis is inhibited either by pretreating cells with mitomycin C or by temperature

shift of *E. coli* mutants temperature sensitive for DNA synthesis. Furthermore, integration of infecting Mu DNA does not take place if Mu amber *A* or *B* mutants, which are blocked in DNA replication, are used for infection. These observations are consistent with our hypothesis that Mu DNA replication is necessary for its integration and imply the intriguing possibility that host DNA replication might also be involved in Mu integration.

Mutants blocked in DNA packaging

By using the technique of separation of Mu DNA from host DNA on 0.3% agarose gels, we have been able to determine which of the Mu mutants available in our collection are defective in packaging. We have found that the mutants defective in head morphogenesis fall into two groups: those that are normal for the DNA packaging reaction and those that are blocked in the appearance of mature phage DNA. The packaging-defective Mu mutants would be very useful for studying the structure of replicating Mu DNA and maturation precursors of Mu DNA.

X Mutants

We have shown that, unlike some other well-known temperate bacteriophages, prophage Mu DNA is not excised upon induction and must therefore replicate in situ (Ljungquist and Bukhari, *Proc. Natl. Acad. Sci. 74:*3143 [1977]). Paradoxically, a mechanism does exist by which the prophage DNA can be cut out of the host DNA. This excision occurs at low frequency and can be detected if the prophage carries mutations that eliminate the killing functions of Mu. We have termed the excisable defective prophages the *X* mutants. The *X* mutations have been shown to be caused by insertions near the left end of Mu DNA.

Nature of insertions

The Mu *X* mutants isolated by us originally all carried the 800-base-pair transposable element IS*1*. We have now found that the X mutations can also be caused by the 1200-base-pair transposable element IS5. The identity of IS5 has been established by restriction endonuclease cleavage analysis and DNA-DNA hybridization. IS5 insertions are located in the same region as the IS*1* insertions in the previously described *X* mutants. The properties of the Mu *X* IS5 mutants are being examined further.

Mechanism of excision

Why is the excision of Mu DNA detected in these special cases at a low frequency and not after induction of a prophage? We think excision of Mu DNA as seen with the *X* mutants is a by-product of the Mu replication-integration cycle. If the replication-integration cycle is initiated but cannot proceed normally, a prophage DNA is rendered susceptible to excision. It is still not clear whether the insertions in the *X* mutants play any role in the excision process.

Excision of Mu *cts X* mutants is blocked by a secondary Mu c^+ prophage but can occur in the presence of phage D108, a heteroimmune, Mu-like phage. This observation implies that Mu gene expression, which can be blocked by Mu repressor but not by D108 repressor, is necessary for *X* excision. This is consistent with the previous finding that gene *A* of Mu is required for the excision process to occur. In a strain diploid for Mu *cts X*/Mu *cts X* mutants, excision of one X prophage does not lead automatically to the excision of the other. This result can be interpreted to mean that excision of Mu DNA is not an efficient, instantaneous process.

Products of excision

Excision of Mu *cts X* mutants can be precise (10^{-6} to 10^{-7} per cell) or imprecise (10^{-5} to 10^{-6} per cell). When a Mu *cts X* prophage is located in the *Z* gene of the *lac* operon, Mel^+ revertants arise at a frequency 10- to 100-fold higher than the frequency of Lac^+ revertants. Reversion of cells to Lac^+ requires precise excision (restoration of activities of both the *lacZ* and *lacY* genes), whereas reversion to Mel^+ requires restoration of only *Y*-gene activity and thus indicates imprecise excision. We have confirmed by extensive deletion mapping that most Mel^+ revertants have deletions in the *Z* gene. Surprisingly, about 20% of the Mel^+ revertants were found to have retained Mu DNA at its original site in the *Z* gene. Perhaps the *Y* gene is now linked to a Mu promoter because of rearrangement of Mu sequences proximal to the *Y* gene. In some cases we have been able to show a rearrangement in prophage sequences by DNA-DNA hybridization. In other cases, where no rearrangements of prophage sequences can be seen, perhaps rearrangements involving transposable elements have occurred independently of Mu DNA and have generated a new promoter in the vicinity of the *Y* gene. These observations open up the possibility of using Mu to promote expression of the nearby genes.

Mu-induced modification of DNA

During the course of our routine cleavage of Mu DNA with restriction endonucleases, we found that Mu DNA is not cut to completion with the enzyme *BalI*. Furthermore, from the Mu *X* and Mu *X cam* mutants, *BalI* generated fragments whose presence could not be explained on the basis of known sites for *BalI* cleavage in Mu DNA. These fragments turned out to be partial fragments that were present in approximately equimolar amounts. We have concluded that, after induction, Mu DNA is modified in such a manner that a given *BalI* recognition site is affected in about 50% of the molecules. This modification requires the *mom* function of Mu, as well as the *dam* (deoxyadenosine methylase modification) function of *E. coli*, as suggested by Ariane Toussaint (University of Brussels). In the case of Mu *X cam* mutants, a *BalI* recognition site within the

gene for chloramphenicol resistance can be unmasked only if the Mu X *cam* mutants are grown in *dam*⁻ cells. Since the *dam* and *mom* functions act in concert, it must mean either that the specificity of the *dam* function is changed by mom or that *mom* requires *dam* as an activator.

The G segment of Mu DNA

Experiments by June Scott's group at Emory University and by Ariane Toussaint and her associates at the University of Brussels have shown that Mu and bacteriophage P1 can exchange their host-range properties. Since the homology between Mu and P1 is apparently restricted to the invertible segments (termed the G segment in Mu and the C segment in P1), it can be inferred that these segments encode at least some components of the host-range system. Our colleagues at Cold Spring Harbor have shown that only one G orientation, called the "flip" or plus orientation, gives rise to viable phage particles (Kamp et al., *Nature 271*:575 [1978]). If the prophage carries the "flop" or minus orientation, no plaque-forming phage particles are produced upon induction. We have extended these observations further by examining the adsorption properties of the Mu particles made by induction of a lysogen. In these lysates, only the phage particles with the "flip" orientation of G can adsorb to the cells properly. Therefore, upon infection, only Mu particles with the "flip" G orientation are obtained. The simplest explanation for this observation is that the Mu component needed for adsorption to *E. coli* cells is made only when Mu is in the "flip" orientation. This case provides an interesting example of the "flip-flop" control of gene expression. We are examining the nature of this regulation of gene expression further.

Transposable elements

Several simple transposable elements, called the IS elements, have been identified in the genome of *E. coli*, and in the F factor, and in various R plasmids. We have systematically examined the presence of some of the elements in several different bacterial species. The results so far show that IS*1* is present in multiple copies in all species belonging to the family Enterobacteriaciae. However, we have so far not detected IS*1* or IS2 in several other bacterial species.

Last year we determined that IS*1* is present in the genome of bacteriophage P1. All P1 strains apparently have one copy of IS*1* at the same site. We detected an additional copy of IS*1* in at least one strain. Analysis of the chloramphenicol resistance transposon Tn9, which contains IS*1* at each end, in a P1 *cam* strain (originally isolated by Mitsuhashi's group in Japan) has shown that Tn9 is located at exactly the same site as the *IS*1 found in P1. This structure of Tn9 in this P1 cam strain appears to be interesting (Fig 1). Tn9 is present in this strain in tandem copies, such that two copies share one IS*1* in the middle.

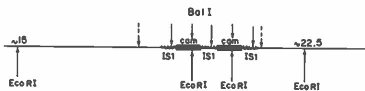

Figure 1
Proposed structure of Tn9 in P1 cam. At least two Tn9 copies in tandem are present in the P1 cam *strain in use in our laboratory. The figure shows the P1 segment (defined by the* EcoRI *cleavage sites at coordinates 15 and 22.5) containing Tn9. The arrows pointing upward indicate* EcoRI *cleavage sites; the arrows pointing downward indicate* BalI *sites. The* BalI *cuts, outside of* Tn9, *are indicated by dotted arrows. Recently, L. MacHattie (pers. comm.) has shown that many tandem copies of Tn9 can be generated in bacteriophage λ, beginning from one Tn9 copy.*

Visiting colleagues

Dr. Ariane Toussaint, from the University of Brussels, spent some time with us examining the properties of her Mu mutants that carry insertions in the β segment, near the right end of Mu DNA. These studies led to the identification of an insertion in Mu as IS2 element.

Dr. Anthony Garro, from Mt. Sinai School of Medicine, New York, initiated experiments on the excision process of ϕ105 prophage from *Bacillus subtilis* cells in order to compare the process of ϕ105 induction with that of prophage Mu induction.

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ELECTRON MICROSCOPY

T.R. BROKER, L.T. CHOW, J.A. Engler, B.A. Kilpatrick, J.M. Scott

Adenovirus-2 RNA transcripts

We have used electron microscopic RNA-loop mapping to determine the chromosome coordinates of the Ad2 RNA transcripts isolated at early and late times from the cytoplasm of productively infected human KB cells, some details of which were described in last year's report. R loops are formed by hybridizing RNA to the complementary DNA sequences in partially heat-denatured double-stranded DNA. In the presence of 70–80% formamide, the RNA:DNA hybrids are more stable than DNA:DNA duplexes. After the annealing mixture is cooled, loops of displaced DNA remain opposite DNA:RNA hybrids and, when observed by electron microscopy, constitute distinctive landmarks by which the map positions of the transcripts are determined. Many of the individual transcripts have discrete lengths and well-defined termini and have been correlated with the Ad2 proteins mapped previously by cell-free translation of enriched messages (Lewis et al. *Cell 7:* 141 [1976]; *Cell 12:*37 [1977], Harter and Lewis, *J. Virol.* [1978, in press] (Fig. 1).

Alternation of DNA template strands in different regions can be observed directly in R loops in which the 3′ ends of RNAs complementary to opposite strands converge, as seen at map positions 11.0, 62.3, and 91.5 (cf. Fig. 2d). Interestingly, these strand switches separate blocks of early and late genes, and there is no detectable nontranscribed region between the blocks at the switch points.

Families of RNAs from the four map intervals 4.9–11.0, 38.8–49.9, 49.9–62.2, and 67.9–82.8 contain common sequences. The longer transcripts are complementary to two or more adjacent genes, notably RNA with coordinates 4.9–11.0 (proteins E15K and IX), coordinates 49.9–62.2 (proteins pVI and hexon), coordinates 67.9–78.2 (proteins 100K and pVIII), and coordinates 96.8–91.5 (proteins E11K, E17K, E19K, E21K, and E24K). Shorter RNAs from these same regions generally have the same 3′ end but different 5′ ends. This heterogeneity suggests that the signals governing RNA length determination during transcription or processing are not absolute. In at least two cases the 5′ end of an mRNA appears to be located within the adjacent gene. The 5′ terminus at coordinate 74.1 for pVIII mRNA seems well within the gene for nonvirion protein 100K (67.9–76.1); despite ambiguity in the relative map order of the genes for penton (III) and minor core (V), either the predominant 5′ terminus at coordinate 38.8 or the minor species near coordinate 42 is within the penton gene. These observations imply that protein-encoding sequences might also serve as mRNA processing sites.

Ad2 leader sequences and spliced transcripts

Biochemical studies by Richard Gelinas, Dan Klessig, Sayeeda Zain, Richard Roberts, John Hassell, Ashley Dunn, and Jim Lewis at Cold Spring Harbor Laboratory have accumulated evidence that the 5′ ends of late Ad2 transcripts are not encoded next to their respective genes. These 5′ ends seem to be complementary to a remote portion of the genome, within the *Hin*dIII B restriction endonuclease fragment (coordinates 17.0–31.5). When we prepared R loops from late cytoplasmic RNA, about 200 nucleotides at the 5′ terminus of one-third of the transcripts observed were not hybridized to the DNA and formed visible branches.

In a collaborative effort with Richard Gelinas and Richard Roberts, we established that the 5′ terminal sequences of all late mRNAs were encoded at several remote sites. The approach we took was to anneal separated strands of the *Hin*dIII B restriction fragment to late polysomal mRNA loops preformed in Ad2 DNA. The rightward-transcribed DNA strand (R strand) of the *Hin*dIII B fragment paired with the 5′ branches present

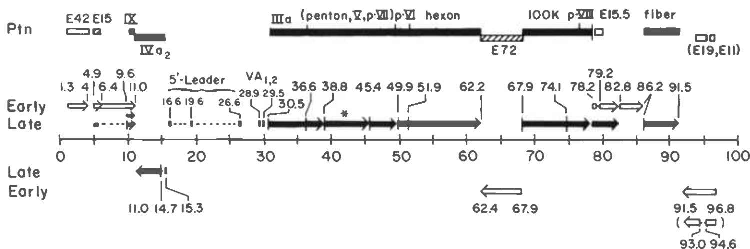

Figure 1
Transcription and translation maps of Ad2 (Broker et al. 1977; see below for additional references). Assignments are based upon EM RNA-loop mapping (Chow et al. 1977a,b) and upon cell-free translation of purified mRNAs (Lewis et al. 1975, 1976, 1977). A tripartite leader sequence derived from coordinates 16.6, 19.6, and 26.6 is coupled to the 5′ end of each species of late, polysomal R-strand transcript from the interval 29−92 at the positions indicated by vertical bars on the arrows. At least some fiber mRNA has a fourth leader component derived from coordinates 78.6−79.1. The L-strand transcript from 15.1−11.0 has a 5′ leader from coordinate 15.6. Another composite late transcript has sequences from 4.9−6.0 linked directly to those from 9.6−11.0, while a simple species of the same family consists of just those sequences from 9.6−11.0. Dashed lines designate sequences absent in the mature polysomal mRNA. Arrowheads represent 3′ termini. In the intervals 36.6−49.9, 49.9−62.2, 67.9−78.2, and 78.2−86.2, families of transcripts of different lengths share common 5′ and/or 3′ termini. The asterisk (*) designates that additional 5′ ends are seen near coordinate 42.7. Each of the two viral-associated (VA) RNAs extends rightward for 0.45 map units from the 5′ ends indicated (Söderlund et al. 1976). Spliced early RNA transcripts that map from 78.2 to 86.2 and from 96.8 to 91.5 were first reported by Kitchingman et al. (1977), as were recombinant transcripts of E72K mRNA (not shown). The lengths of the protein blocks indicate the necessary coding capacity of the DNA. Some protein-mRNA assigments are provisional, and gene products within parentheses have not been ordered unequivocally. Hatched blocks represent proteins made both early and late.

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at each of nine different R loops located in the interval 30.5−91.5. Quite unexpectedly, the restriction fragment was constrained into a looped configuration, suggesting that two short segments of the DNA located 2400 nucleotides apart were complementary to the contiguous sequences at the 5′ end of each of the mRNAs. When the R strand of the longer *Bam*HI B restriction fragment was similarly hybridized to the 5′ termini of the mRNAs in R loops, a third short DNA segment complementary to the RNA was revealed by the formation of two loops (1000 bases and 2400 bases long) in the DNA fragment (Fig. 2a). Separated strands of other restriction fragments derived from all portions of the Ad2 genome were also tested. The results were completely consistent with the conclusion that sequences on the rightward-transcribed DNA strand at coordinates 16.6, 19.6, and 26.6 encode a total of about 150−200 nucleotides found at the 5′ terminus of at least 12 late transcripts, all of which are synthesized from the R strand downstream from the leader.

Further EM experiments showed that mRNA from the distal gene in the series (for the fiber protein) had a fourth leader component. This was noted because fiber mRNA hybridized occasionally to the same DNA duplex in two distinct intervals: from coordinates 86.2 to 91.5, where the coding portion of the gene is located, and also from 78.6 to 79.1, immediately to the left of the nonessential early region of the chromosome. The RNA formed a bridge and constrained the duplex DNA between 79.1 and 86.2 into a loop (Fig. 2b). In such structures the 5′ terminal portion of the fiber mRNA was still capable of hybridizing to DNA sequences at coordinates 16.6, 19.6, and 26.6. Studies in collaboration with Ashley Dunn indicate that not all fiber mRNA has

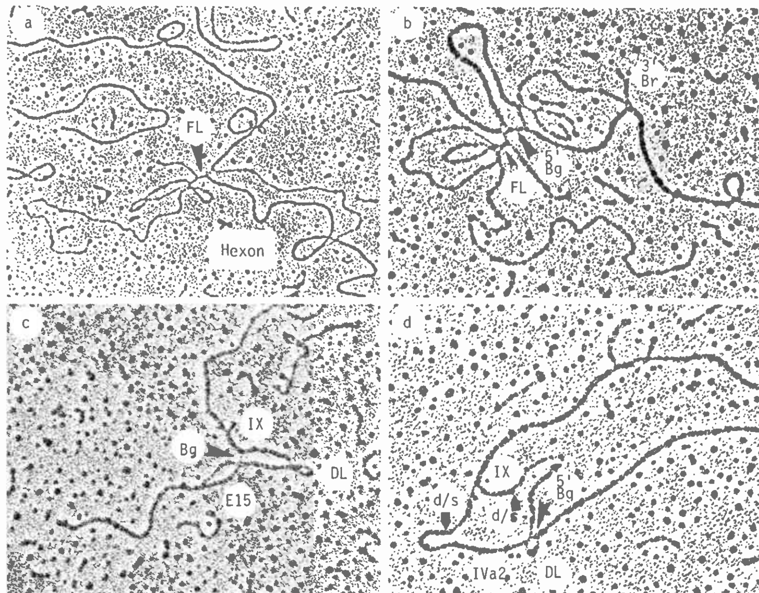


Figure 2

R loops exhibiting the four classes of recombinant Ad2 late mRNAs.

(a) The R strand of the BamHI B DNA restriction fragment annealed to the 5' end of mRNA for the hexon protein is constrained into two loops (FL) because sequences at coordinates 16.6, 19.6, and 26.6 are all complementary to the RNA leader. At least 12 other late mRNAs transcribed from the R strand form the same complex, three-component hybrid.

(b) The primary R loop corresponding to the fiber-encoding sequences extends from coordinates 86.2 to 89.9, with a characteristic branch (3' Br) of RNA displaced at the 3' end (89.9 – 91.5), presumably due to stable RNA secondary structure at 89.9. Near its 5' end, a bridge (5' Bg) of RNA formed a secondary R loop at coordinates 78.9 – 79.1, and in addition paired with the BamHI B fragment at three positions (FL), as in panel a.

(c) A mosaic late transcript consists of sequences from genome coordinates 4.9 – 6.0 (E15K gene) linked directly to RNA transcribed from 9.6 – 10.9 (peptide IX; a late hexon-associated virion component). It formed a double R loop, with the intervening DNA (6.0 – 9.6) constrained into a duplex DNA loop (DL) by an RNA bridge (Bg) as in panel b.

(d) The convergent R loop revealing the switch in transcribed strands consists of mRNA for peptide IX (9.7 – 11.0) annealed to the Ad2 R strand and the neighboring mRNA for virion component IVa2 (15.1 – 11.0) annealed to the L strand. The double-strand/single-strand (d/s) junctions are indicated. The 5' leader for IVa2 mRNA has formed a bridge (5' Bg) from coordinate 14.7 to its complement at 15.6 and has constrained the DNA into a loop (DL) similar to those illustrated in panels b and c.

the fourth leader component, but all do have the tripartite leader. The cause of this variability is not yet understood.

RNA-bridge structures were also observed elsewhere. We found a late RNA transcript complementary to a region considered previously to encode only early proteins. It consists of sequences from coordinates 4.9 to 6.0 linked directly to those from coordinates 9.6 to 11 (Fig. 2c). Another late transcript is a subset of that mosaic RNA and is complementary to a shorter segment of DNA from 9.6 to 11.0 (Fig. 2d). The short species, but not the mosaic RNA, encodes late protein IX, a virion component (Pettersson and Mathews, *Cell 12:* 741 [1977]). The relations among the variously sized early and late transcripts of interval 4.9 – 11.0 are not yet fully established.

The left late transcript complementary to the L strand has been assigned provisionally to virion protein IVa2. It also has a recombinant structure, with a short leader component from coordinates15.6–16.0 attached to the coding sequences from 15.1 to 11.0 (Fig. 2d). Interestingly, the first segment of the major R-strand leader and the late L-strand leader for IVa2 message are in close proximity in the interval 16.0–16.5, which suggests a late promoter region from which transcription diverges.

In conclusion, all late mRNAs encoded by Ad2 are recombinant with respect to the genome from which they are transcribed. This startling fact has abruptly changed our conception of what a eukaryotic gene is and how its expression is controlled. It provokes many intriguing questions about the generality of RNA splicing, the nature of the nucleotide sequences and enzymes involved, and the purposes and consequences of the processing. Is late RNA splicing catalyzed by a viral enzyme? Does it serve to differentiate late mRNAs from host and early viral mRNAs to allow selective loading onto ribosomes for translation? Does it serve to bypass protein termination codons that may be present in precursor RNA? Does RNA splicing occur uniquely and reproducibly or, for instance, in the cases of the fourth leader of fiber and the early mRNAs, are there alternative patterns that allow the eventual synthesis of families of related proteins? On the presumption that only one late message can be derived from each long nuclear precursor RNA (since the leader sequences are represented only once per transcript), what recombination mechanism assures that each of the nine late mRNAs is produced in relatively equal abundance? The simple concept of intrastrand deletion events would incorrectly predict that the joining of leader to message would be inversely proportional to the distance separating the nine possible combinations. A new type of recombination may have to be sought which is relatively insensitive to distance. What process—transcription termination, simple cleavage of precursor RNA, or splicing of polyadenylate to one of the nine messages in nuclear precursor RNA—determines the 3′ end of late mRNAs? Have leader sequences diverged among the various adenovirus serotypes, and, if so, might not this be a reason why genetic recombinants between different classes of adenoviruses cannot be obtained? Presumably, nucleotide sequences near the leaders, as well as near the 5′ end of each gene, must be matched to allow splicing. Many of these challenging problems will be addressed in the forthcoming year.

Bacteriophage Mu DNA G segment and β region: Structure and function

The DNA from temperate bacteriophage Mu is a linear duplex about 38 kilobase pairs (kb) in length. A 3-kb segment of its DNA, designated G, can be present in either orientation relative to its neighboring segments to the left (α, 30.7 kb) and to the right (β, 1.7 kb). The Mu G segment is flanked by short (less than 0.05 kb) inverted duplications at which a hypothetical Mu function *(gin)* promotes G-segment inversion by intramolecular recombination. When phage are grown by induction, the G segment can be found in either orientation (the [+] and the [−] orientations) with roughly equal frequency. In contrast, when phage are grown by lytic infection, more than 98% of the progeny have G in the (+) orientation.

We have carried out a variety of experiments on the structure, functions, and phylogenetic distributions of the invertible G segment, as detailed below.

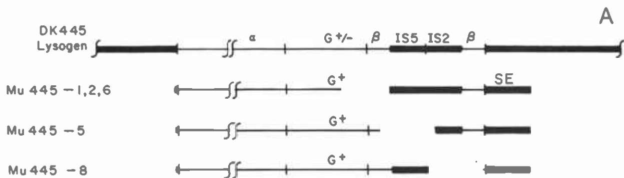
Comparison of the G inversion in phages Mu, P1, and P7

Phages P1 and P7 are related to each other but are unrelated to Mu. Nonetheless, both contain an invertible segment which is homologous to the Mu G segment. P1 has inverted duplications of 0.6 kb in length flanking the G segment. In contrast, we found that P7 has only one flanking segment of 0.6 kb, and the other segment has a small (0.3 kb) internal deletion, so it is only 0.3 kb long. The P7 G segment, like Mu-G DNA, is present in only one orientation when phage are grown by infection. In contrast, the P1 G segment is found in either orientation when phage are produced by lytic infection or by induction of lysogens. We have postulated that the long inverted duplications flanking the P1 and P7 G segments are functionally homologous to the Mu β segment and are part of the *gin* system, which promotes the G *in*version (see below).

Isolation and characterization of viable deletion/substitution Mu mutants

An *E. coli* strain DK445 lysogenic for Mu was found by electron microscopy to contain an insertion of 2.6 kb in the middle of the prophage Mu β region. Seven independent viable mutant phages have been isolated from it. Each of them has been characterized by EM heteroduplex studies and was found to have a deletion and/or substitution in the G β region (Fig. 3a). The lengths of the bacterial DNA normally linked to the β end were found to decrease or increase in response to the increase or decrease of the Mu genome caused by the deletion/substitution in these mutants. These findings strongly support the concept of a headful packaging mechanism for Mu phage.

The G segment of the parental Mu prophage in DK445 inverts normally. However, five of the mutants have their G segment locked in the (+), or the lytic,


A
DK445
Lysogen
α
G+/-
β
IS5
IS2
β
Mu 445 -1,2,6
G+
SE
Mu 445 -5
G+
Mu 445 -8
G+

B
Mu vir/Mu 445-1
/Charon 1
λ_R
IS5
IS2
Mu SE
λ_R
IS2
Mu SE
IS5
Mu vir
λ_L
Mu α

orientation because one of the inverted duplications flanking G is deleted. Since these phage are viable, we conclude that the inversion of G from the (+) to the (−) orientation is not essential for the Mu life cycle. A sixth mutant, designated 445-5, has an intact G segment but no longer inverts its G segment. This mutant has a small deletion/substitution in the β region; presumably, this deletion has damaged the *gin* function. G inversion in this mutant can be achieved in *trans* by a wild-type helper phage. Therefore, we have located at least part of the Mu G inversion *(gin)* gene in the left half of β and believe that it encodes a diffusable enzyme.

The infectivity of phage Mu depends on the orientation of the G segment

Although mature Mu 445-5 *gin*$^-$ with the G segment in the (+) orientation is viable, mature Mu 445-5 *gin*$^-$ G(−) produced by induction of the lysogen is not infectious. Using the *gin*$^-$ G(+) and *gin*$^-$ G(−) mutants as controls, the G-segment inversion of wild-type Mu was examined thoroughly. We have found that the efficiency of an induced lysogen culture to yield infectious centers is directly proportional to the percentage of phage in the lysate with the G segment in the (+) orientation. We take this to mean that the inversion of G occurs predominantly or exclusively in the prophage and does not occur to any appreciable extent after heat induction of the prophage. Thus, each cell produces either G(+) or G(−) phage, but not both. The amount of each type of phage in the lysate reflects the proportion of G(+) and G(−) prophages in the population at the moment of heat induction. We can conclude also that wild-type Mu phage with G in the (−) orientation is not infectious. This explains why the G segment can be observed in either orientation when Mu is grown by induction but only in the (+) orientation when it is grown lytically.

Although Mu G(−) phage do absorb to bacterial cells, their defect cannot be restored by providing the *gin* function from inside the cell. This indicates that the DNA of the phage does not penetrate the cell wall, a conclusion which is strengthened further by the failure of marker rescue from a G(−) phage after infection.

We have evidence that an essential gene, designated *S*, which has previously been mapped to the left of G is, in fact, at least partially located within the G segment. Inversion of G from the (+) to the (−) orientation would then split the gene and inactivate it; alternatively, inversion might separate the gene from its promoter.

S$^-$ phage particles are similar to G(−) phage particles in that they too show no killing of the host. Both G(−) phage and S$^-$ phage inhibit growth of the G(+) *gin*$^-$ phage during coinfection. Furthermore, a G(−) prophage does not seem to complement *S* when introduced into a Mu *S*$^-$ lysogen. We are presently trying to construct an *S*$^-$ *gin*$^-$ double mutant to verify this observation. We have tried unsuccessfully to restore infectivity to G(−) phage by growing them in the presence of the viable Mu 445-5G(+) phage. Therefore, the effects of G inversion are more complicated and cannot be explained solely by *S* inactivation; a *cis*-acting function might also be involved.

Mu gin

We have studied the potential of other recombination systems to substitute for Mu *gin* in catalyzing the inversion of the Mu G segment. *E. coli rec*, λ *red*, λ *int*, and ϕ80 *int* systems all fail to complement; P1, P7, and D108, a phage largely related to Mu, complement Mu *gin*$^-$ phage. We have shown that G inversion is not affected by an *E. coli himA* mutation, which blocks the integration of certain phages. We have cloned the right-end *Eco*RI B fragment of Mu wild type with a phage lambda vector. This hybrid phage expresses *gin* upon infection as well as in the lysogenic state. We have used this phage to study the kinetics of G inversion: a strain lysogenic for the defective Mu 445-5G(−) phage was superinfected with λgt-Mu *Eco*RI B and tested at various times after infection for production of viable Mu phage. The rate of G inversion was found to be 0.1% to 1% per phage generation.

A 6000-base-pair *Sal*I-*Eco*RI fragment overlapping the G segment and *gin*, derived from Mu 445-8, was cloned into pBR322. This plasmid expresses *gin* and we intend to use it to study the *gin* protein and other proteins made from this region of Mu DNA in an *E. coli* minicell system.

P1 gin

The P1 *Eco*RI A fragment has been cloned with a lambda vector. This fragment contains the P1 G segment and *gin* function. A series of deletion mutants of this hybrid phage was isolated after EDTA treatment of the phage

Figure 3

(a) A graphic representation of the Mu prophage in E. coli *strain DK445 and some of the substitution/deletion phage derived from it. The segments of Mu DNA are designated as α (30.7 kb), G (3.0 kb), β (1.7 kb), and SE (heterogeneous bacterial DNA that forms split ends in heteroduplexes). The prophage has an insertion of 2.6 kb in the middle of the β region, which consists of adjoining bacterial insertion elements IS5 and IS2. The mutant phages retain all or part of the insertion. Caps represent deletions in the mutant DNAs.*

(b) *A three-component heteroduplex demonstrating the presence of IS5 in Mu 445-1. The heteroduplex of Mu 445-1 and Mu vir DNA contains a substitution loop. The IS5 in lambda charon 1 has hybridized to the substitution loop at the IS5 resident in Mu 445-1.*

and is presently being analyzed with restriction enzymes. The goals of this work are to determine the precise location of the P1 *gin* function and to isolate P1 *gin*⁻ mutants. We hope that these experiments will provide clues as to the relatedness of G segments and *gin* functions as well as to their origins.

Studies on the Mu mom *System (modification of Mu DNA)*

We have studied the effect of G on the system of Mu *mom*, a DNA modification function. Both Mu 445-5G(+) and Mu 445-5G(−) phage are mom^-, as determined by restriction enzyme cleavage patterns of the Mu DNA. Because the original prophage in DK445 and all deletion phages derived from it are mom^-, the *mom* gene must map to the right of *gin*, or largely or entirely to the right of the insertion. The mom^- phenotype is due either to the destruction of *mom* gene by the insertion or to the block of transcription caused by the insertion in the 445 series of Mu phages.

We have also determined the specificity of *mom* by comparing restriction endonuclease digests of mom^+ and mom^- DNAs. A 5-base-pair sequence $(\frac{G}{C})A(\frac{C}{G})XY$ is recognized. The sequence is highly degenerate in positions 4 and 5. In the absence of host *E. coli dam* (methylase) function, no *mom* activity can be detected. However, the *dam* recognition sequence (GATC) does not seem to overlap those for *mom*. Therefore, *mom* does not act as positive regulatory protein to turn on the *dam* methylase.

Identification of bacterial insertion sequences IS5 and IS2 in viable Mu mutants and IS5 in a λdarg transducing phage

Evidence from restriction endonuclease analyses and EM heteroduplex mapping shows that the 2.6-kb insertion in the Mu prophage which generated our viable Mu mutants consists of bacterial insertion element IS5 linked directly to IS2. Some of the deletion/substitution mutants contain this tandem IS5 : IS2, others have either IS5 or part of IS2 (Fig. 3b).

Using EM heteroduplex methods, we have also found that an insertion in the *argB* gene of a lambda transducing phage (λ*argB*2) is IS5. Demonstration of two additional, independent examples of IS5 helps establish it as a bona fide insertion element. Our physical results, taken together with genetic information obtained by others (W. Szybalski and N. Glansdorff, pers. comm.), suggest that IS5, like IS*1*, exerts a polar effect on transcription in either orientation.

Bacteriophage lambda attachment site (att)

We have developed an in vivo assay for active attachment sites that is based on our observation that a plasmid containing a 450-base-pair long *Hin*dIII *Bam*HI fragment spanning the λ *att* site can be integrated into phage lambda DNA, packaged, and excised after infection of a recipient cell. This process has an absolute requirement for *att* on the plasmid and *int* on the superinfecting lambda phage. By in vitro manipulations, a special lambda vector has been constructed that has deleted an *Sst*I and an *Hin*dIII fragment to the left of the attachment site but is int^+ and xis^+. This vector can accommodate an insertion of 12,000 base pairs. The test has been used for two purposes: (1) the detection of plasmids carrying small attachment-site fragments and (2) the detection of attachment sites from other origins.

Using the assay described above we have identified two chimeric plasmids that are active in the attachment-site assay: one was obtained from a shotgun cloning of *Proteus vulgaris* DNA using pMB9 as a vector; the other contains the right-end *Eco*RI fragment of Mu 445-1 in pMB9.

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NUCLEIC ACID CHEMISTRY

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Adenovirus-2 mRNA

The oligonucleotide $m^7G^{5'}ppp^{5'}m^6AmCmU(C_4U_3)Gp$ appears to be the only capped T_1 oligonucleotide found at the 5′ end of late Ad2 mRNAs. When hybrids between Ad2 mRNAs and Ad2 DNA are treated with ribonuclease T_1, this capped T_1 oligonucleotide is released, which suggests that it is not complementary to sequences lying immediately adjacent to the main coding region of the mRNA. Experiments carried out in collaboration with Louise Chow and Tom Broker of the Electron Microscopy Section have provided direct evidence for this interpretation by showing that many late Ad2 mRNAs are mosaic molecules composed of sequences encoded at several positions on the Ad2 genome. When RNA displacement loops (R loops) are formed between Ad2 mRNAs and Ad2 DNA, sequences at the 5′ terminus of each mRNA are available for hybridization. If single strands of restriction endonuclease fragments of the Ad2 genome are added to these R loops, complex tertiary hybrids are formed between the R strands of fragments containing short sequences from map positions 16.6, 19.6, and 26.6 and the 5′ ends of all late mRNAs mapping to the right of map position 30. The resulting structures show that a typical mRNA contains a leader sequence whose 5′ end is encoded at map position 16.6, which is covalently joined to sequences encoded at map positions 19.7 and 26.7 and has a total length of 150 to 200 nucleotides. This leader is in turn covalently joined to a main coding segment which is transcribed from a region of the Ad2 genome previously characterized as a structural gene. These unexpected findings require that a novel mechanism must exist for the biosynthesis of these late viral mRNAs. A wealth of data from the laboratory of James Darnell at Rockefeller University suggests that the primary transcription product is a large RNA molecule which is initiated between map positions 15 and 20 (perhaps at 16.6) on the Ad2 genome and may extend as far as the right-hand terminus of the genome (map position 100). If such a transcript is the precursor of these late viral mRNAs, as seems likely, then the production of these mRNAs will require a series of cleavage and joining events to produce the observed structures. Experiments are in progress to determine the nature of the sequences present at these junctions and to isolate the enzyme(s) involved in their formation.

One particular mRNA, that encoding the major structural protein fiber, is being studied in detail. The 5′ end of the main coding region of this mRNA lies within Ad2 sequences immediately adjacent to one of the junctions between Ad2 and SV40 sequences in the nondefective hybrid virus $Ad2^+ND1$. DNA sequences from this region of the genome have been obtained previously. Experiments carried out in collaboration with Dan Klessig and Jim Manley of the Protein Synthesis Section have shown that within 200 nucleotides of this junction sequences occur which are present on fiber mRNA and which are also present in ribosome-protected fragments of fiber mRNA. Direct DNA sequence analysis of this region reveals that two of the three possible reading frames contain termination signals for protein synthesis. The third frame contains a long stretch of in-frame codons preceded by an initiation codon. Immediately following this initiation codon within the DNA sequence is a sequence which, if transcribed, would lead to the presence of a long T_1 oligonucleotide in fiber mRNA. A long oligonucleotide of the predicted composition has been found when fiber mRNA is hybridized to a restriction fragment containing this region of the genome and has also been found in the fragment of fiber mRNA protected by ribosomes. Furthermore, experiments to assess the extent of hybridization between this region of

the DNA and fiber mRNA suggest that the mRNA is complementary to sequences lying no more than 30 nucleotides to the left of this initiation codon. Presumably, this represents the point at which the leader sequence is joined to the main coding region of fiber mRNA. Experiments are in progress to define exactly the point of divergence between sequences present on the DNA and sequences present on fiber mRNA.

Terminal structure of the adenovirus-2 genome

The complete sequence of the inverted terminal repetition of Ad2 is now available. This repetition is 102 nucleotides long and contains a number of repeated sequences whose significance is unknown. It is perhaps noteworthy that the terminal 40 nucleotides are extremely AT-rich, whereas the internal section is highly GC-rich. If, as seems likely, replication begins at the very end of the molecule, the AT-rich nature of the termini would thus allow for relatively easy strand separation. Several stable secondary structures can be drawn from the primary sequence, including one that would allow a self-priming mechanism for DNA replication. Since the sequence of the inverted terminal repetition is both unique and nonpalindromic, any self-priming mechanism would cause scrambling of the sequences at the termini of the DNA. Such scrambled sequences are not present either in viral DNA or in free nuclear DNA, and thus it is unlikely that DNA replication proceeds by a self-primed mechanism.

Restriction endonucleases

The collection of restriction endonucleases continues to expand. Nearly 130 such enzymes are now known, with almost 60 different specificities. New endonucleases include enzymes from several strains of *Agrobacterium tumefaciens* (which may be of great biological interest), *Bpa*I and *Bpa*II from *Bordetella parapertussis*, *Cpe*I from *Corynebacterium petrophilum*, *Pvu*I and *Pvu*II from *Proteus vulgaris*, *Sfa*NI from *Streptococcus faecalis*, *Tgl*I from *Thermopolyspora glauca*, *Xni*I from *Xanthomonas nigromaculans*, and *Xpa*I from *Xanthomonas papavericola*. In addition, second enzymes have been found in two strains of *Moraxella nonliquefaciens* (*Mno*II and *Mnn*II) and in *Thermus aquaticus* (*Taq*II).

Recognition sequences have been deduced for a number of restriction endonucleases, including *Bcl*I (T↓GATCA) from *Bacillus caldolyticus* and its isoschizomer *Cpe*I; *Bpa*I (GT↓$^{C}_{A}$$^{T}_{G}$AC); *Blu*I from *Brevibacterium luteum*, *Xho*I from *Xanthomonas holcicola*, and *Xpa*I from *Xanthomonas parahaemolyticus* (C↓TCGAG) *Pvu*II (CAG CTG); *Sal*I from *Streptomyces albus* G (G↓TCTAC); *Sac*II from *Streptomyces achromogenes*, *Sst*II from *Streptomyces stanford*, and *Tgl*I (CCGC↓GG); and *Xba*I from *Xanthomonas badrii* (T↓CTAGA). In addition, the recognition sequence for *Mnl*I from *Moraxella nonliquefaciens* has been deduced as CCTC; however, the site of cleavage is known only to lie within 5 to 10 nucleotides 3′ of this sequence. This enzyme is of considerable interest for DNA sequence analysis as it is expected to cleave DNA at twice the frequency of enzymes such as *Hae*III which recognize a tetranucleotide palindrome. It is becoming increasingly apparent that many different kinds of recognition and cleavage processes are possible for the type-II restriction endonucleases and studies of their mechanisms of action should provide much valuable information about protein-nucleic acid interactions.

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CELL BIOLOGY

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Most types of cancer could be eliminated surgically if it were not for the metastasizing cells. Therefore, the understanding of migration of mammalian cells seems of great importance for the understanding of many types of cancer, as well as, of course, for the understanding of normal cell behavior, e.g., during embryogenesis.

Recently, we have developed a new technique which allows migrating animal cells to record their own movements on a glass substrate which is densely and evenly covered with colloidal gold particles, ranging in size between 0.2 μm and 0.4 μm. The migrating cells remove these particles from the substrate during their displacement and in this way leave a particle-free track behind. We suggested that these tracks be called "phagokinetic tracks." The convenience of studying such tracks and their large numbers have helped us to make several observations which point to the possibility that animal cells are in command of their migration —their pathways follow preset programs which can be revised however, in a predictable way upon collision with another cell.

The first, and still strongest, evidence for predetermination in cellular migration comes from the observation that the track of one daughter 3T3 cell appears predominantly as the mirror image or identical copy of the track of the other daughter cell. We described this observation in last year's annual report. This year's work concentrated on other aspects of predetermination of migration and on attempts to localize in 3T3 cells a putative "control center of movement" responsible for such programming. As will be pointed out below, we believe at present that the centrioles are part of this postulated "control center." If substantiated, this "control center" might offer a new approach to cancer cells, because one can imagine that transforming events may affect this "control center" and unleash less controlled motile activities, thus, perhaps, leading to the metastasis of such cells.

Cell-line characteristic track patterns

Different cell lines or single-cell clones of established cell lines produce characteristically different tract patterns. In this context, we use the term "cell-line characteristics" in the same sense as it is commonly used to describe culture morphologies of different cell lines. Upon changes in culture conditions, the appearance of the track patterns may change, but so do culture morphologies. We found that the track patterns of a cell line are in this sense representative. In a given cell line the morphologies of single cells can differ considerably from one another; so did track patterns of single cells.

As a preliminary observation, we should like to note that transformed 3T3 cells, even in the presence of 20% calf serum, seem to produce much shorter phagokinetic tracks than the parental cells.

Track patterns formed by cells from the second or third generation

From the finding of symmetry between the tracks of two daughter 3T3 cells, the question arose whether such predetermination can go beyond one generation.

Therefore, we searched for track patterns produced by second- or third-generation descendants of a single cell. Unfortunately, such track patterns are rarely readable because the descendant cells or other cells may cross the "ancestor" tracks too often. Screening about 20 preparations, we found several track systems that suggested that descendant cells up to the third generation can reproduce the track patterns of their ancestors.

This finding strengthens the notion that migration is predetermined and controlled by 3T3 cells.

Collision behavior of 3T3 cells

The movements during a collision between two migrating homologous cells have been known for quite some time (Abercrombie and Heaysman, *Exp. Cell Res.* 6:293 [1954]; Trinkaus et al., *Exp. Cell Res.* 64:291 [1971]; Weiss, *Int. Rev. Cytol.* 7:391 [1958]). Invariably, a cessation of ruffling at the impact area is followed by the formation of retraction fibers and the expression of a new ruffling lamellipodium somewhere else at the cell's circumference. The study of phagokinetic tracks of colliding cells seems to offer an additional aspect to this complex cellular behavior. The new lamellipodia do not seem to form just "somewhere else" on the cell circumference, but in such places and in such successions that the cell eventually moves away from the impact area as if it were reflected.

Within 100–200 μm from the collision area the angles between ingoing tracks and a central line appear equal to the angles of the outgoing tracks. Unfortunately, based on the tracks alone, one cannot be completely certain that two migrating cells actually collided at the point of closest approach of either track, although one can argue that if it were otherwise the tracks should have crossed.

To make more certain that two cells were at the point of closest approach of their respective tracks at the same time, one can use nonmigrating cells, such as BSC-1 cells, as one collision partner. We plated 3T3 cells together with BSC-1 cells on a particle-coated cover slip and screened for collision patterns 1 or 2 days later. Again we observed that the 3T3 cells formed ingoing tracks within 100–200 μm from the impact area which appeared as if reflected. The target BSC-1 cell, however, did not seem to change its position after the impact and in most cases remained centrally located inside its particle-cleared area.

In view of the many hours duration of a cell-cell collision, the above finding suggests that the colliding cells reorient their path in a predictable, and therefore nonrandom, way.

Actin patterns and phagokinetic tracks

The front and rear ends of a cell can be determined by the track direction (they are indicated as "f" and "r" in Fig. 1). According to this definition, the major actin-containing bundles stretched between the rear end of the cell and the nucleus (Fig. 1). The front end showed bundles only rarely.

We examined the actin patterns and corresponding tracks of 113 individual 3T3 cells. In 61% of the cases the symmetry axis of the main bundle stretched parallel to the current (Fig. 1a) or previous segment of the corresponding track. In 39% of the cases no relationship between bundles and track segments could be determined because the segments were either too short or irregular. Sometimes the cells had too many different bundle directions or were rounding up.

Tubulin patterns and phagokinetic tracks

Most of the cytoplasmic microtubular patterns appeared in the front half of the cells. The density of fluorescent fibers in the rear end seemed to be reduced unless diffuse fluorescence around the accumulated gold particles made it impossible to detect single fibers. Due to the complexity of the cytoplasmic microtubular patterns, a relationship between them and the track directions was not obvious.

Most helpful for the search for structures that are conspicuously oriented to track segments, however, was the fluorescent-labeled rodlike structure which was described by Osborn and Weber (*Proc. Natl. Acad. Sci.* 73:867 [1976]). In electron microscopy studies, Wheatley (*J. Anat.* 110:367 [1971], *J. Anat.* 113:83 [1972]) had shown earlier that 50—75% of 3T6 cells contained one, single, mostly intracellular cilium growing out of a centriole ("primary cilium") whose shaft was embedded in a vacuole.

Screening 101 3T3 cells on plain glass, we found a single, primary cilium in 87% of them. Invariably, it was located close to the nucleus. As far as one can tell from light microscopy, using an objective lens (N.A. 1.4) with a small depth of focus (approx. ± 0.7 μm), in 61% of the cells the cilium was straight and ran approximately parallel to the substrate. In 14% of the cells it was bent, and in 12% it was oriented vertically to the substrate.

The cilia frequency in 3T3 cells plated on gold-particle-coated glass substrates was lower than on plain glass (53%). This reduced frequency may be explained by a reduced visibility of cilia on such substrates due to internalized gold-particle clusters or a high background level of diffuse fluorescence around the nucleus. There may be other, as yet unknown, factors influencing the expression of cilia.

Comparing the orientation of the primary cilium with the direction of the phagokinetic track in 258 cells, we found that where the structure could be detected it ran approximately parallel to the substrate and the current segment in 73% of the cells (Fig. 1b). In the remaining 27% it was vertical, or bent, or pointed in a direction that did not seem related to the track direction. The cilium could be detected near the cell bottom or close to the dorsal surface (Fig. 1b). In cells that extended sideways, the primary cilium remained oriented parallel to the segment just completed.

The interpretation of the above results is complicated by the difficulty in distinguishing between cause and effect in the relationships described between the directions of the primary cilia, the main actin bundles, and the track segments. Nevertheless, we should like to submit the following possibilities.

The main actin-containing bundles stretched predominantly from the rear end of the cells to beneath the nucleus and ran parallel to the current segments of the tracks. It seems possible, therefore, that the fibers of the main bundles became organized close to the substrate in parallel lines, and that the nucleus and the bulk cytoplasm moved along them as if on "rails." Conceivably, the bundles could lengthen farther during this process. According to this model, the used parts of the rails were left behind, converged in the rear, and eventually were dissembled during the tail retraction.

This notion of cellular displacements on "rails" offers a reason why the main bundles in 3T3 cells, which are, in fact, microfilament bundles (Goldman et al., *Exp. Cell Res. 90*:333 [1975]), are located near the substrate and are often tied to the substrate by adhesion plaques (Goldman et al., *Cell Motility*, p. 217. Cold Spring Harbor Laboratory, New York [1976]). Such features are implied by the term "rail" in the first place. Movement along rails would also allow the nucleus and bulk cytoplasm to move parallel to themselves, thus possibly reducing the chance of entangling the complex cytoplasmic architecture during displacement. Furthermore, it would explain the observation that 3T3 cells, if considered random walkers, move persistently during short time intervals of 2.5 hr (Gail and Boone, *Biophvs. J. 10*:980 [1970]).

Most striking is the observed relationship between the orientation of primary cilia and current track segments. There are various possible interpretations of this relationship, ranging from the assumption that the cilium shaft is passively oriented by certain cytoplasmic forces that act during cellular displacement, to the assumption that the direction of the cilium determines the direction of a migrating 3T3 cell by an as yet unknown mechanism. One could also think of the primary cilium as a "sensor" or mechanotransducer which is aligned with the "intended" direction of movement so as to yield to, and thus detect, cytoplasmic movements deviating from this direction. There is also the possibility that the cilium itself is of little relevance for the move-

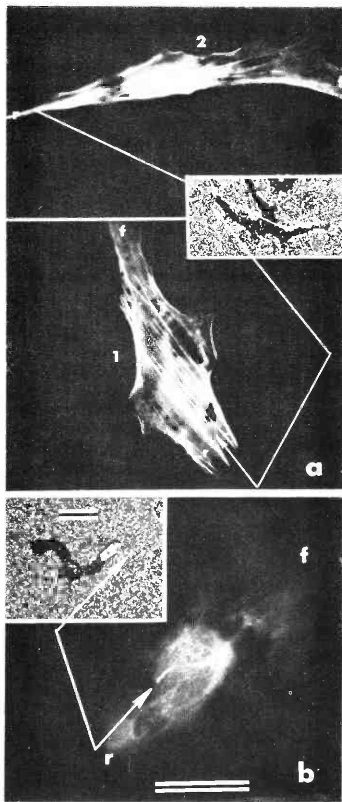

Figure 1
Parallels between the directions of the main actin-containing bundle (a) *or the primary cilium* (b) *and the current direction of displacement in 3T3 cells.*

The inserts show dark-field light micrographs of the same cells (white clusters) in their phagokinetic tracks as those seen in fluorescent light microscopy. Inserts and fluorescence micrographs are shown in identical orientations relative to the observer. Bars in b *represent 20 μm and 100 μm. The connected arrows indicate the observed parallels between the actin bundles or the primary cilium and the respective track directions. The letters "f" and "r" designate the front and rear ends of the cells as derived from the track directions. The black clusters in the fluorescence micrographs are internalized clusters of gold particles.*

(a) *Pair of daughter cells with the main bundles parallel to the current track segment. The bundles of cell 2 converge due to the tail formation.*

(b) *Primary cilium (arrow) oriented parallel to the current direction of a 3T3 cell. The focus in the fluorescence micrograph is raised to the level of the cilium, thus producing only a blurred image of the whole cell.*

ment of cells. One may consider it simply a convenient light microscopic marker for the direction of the axis of the centriole out of which it grew. In other words, the parallels between the direction of primary cilia and current track segments described here may have to be interpreted as a geometric relationship between the direction of migration and the orientation of one centriole in migrating interphase cells. On-going experiments, which will be described in next year's annual report, support the interpretation that it is not the cilium, but rather its basal centriole, that is involved in the control of directional changes in migrating 3T3 cells.

Local inhibition of centripetal particle transport where LETS protein patterns appear on 3T3 cells

The assay of phagokinetic tracks described above is based on the phenomenon that a cell encountering a particle removes it from the substrate and transports it centripetally to the perinuclear area of the cell where it is phagocytosed. Therefore, we are very interested in this general phenomenon of centripetal particle transport which has been described by Marcus (*Cold Spring Harbor Symp. Quant. Biol. 27*:351 [1963]), Abercrombie et al. (*Exp. Cell Res. 62*:389 [1970]), and Harris (*Exp. Cell Res. 73*:519 [1972]). We found that the so-called LETS protein, which recently has attracted much attention, seems to be related to the expression of centripetal particle transport along the surface of 3T3 cells.

Originally discovered as a major surface glycoprotein of molecular weight 220,000–250,000, which disappeared upon cell transformation, LETS protein on the dorsal surface of fibroblastic cells shows several intriguing aspects. The protein distribution patterns, or at least their antigenic availability, seem to originate from cell-cell contact areas. Later on, the protein can cover large cell-surface areas in the form of intertwined streaks, indicating a major nonhomogeneity in the cell-surface composition.

Assuming a constant centripetal flow, one might expect that fibrillar surface components like LETS protein would eventually have to orient themselves parallel to the radial flow lines. The complex distribution patterns of LETS protein in all cell lines investigated so far, however, seemed hard to reconcile with a radial centripetal flow. One may suspect, therefore, that the flow has ceased in areas covered with LETS protein.

Therefore, we allowed 3T3 cells to pick gold particles from the substrate and transport them centripetally. Subsequently, we fixed and stained them for LETS protein using indirect immunofluorescence. We found that cells that were able to accumulate the gold particles all around the nucleus in the form of a ring showed only a few patches of LETS protein or none at all, whereas cells with extended patterns of LETS protein still had the particles scattered randomly all over their surfaces. We also observed quite a few cells with incomplete rings of centripetally accumulated gold particles. In many such cases, the ring segment marked a cell-surface sector without LETS protein, whereas the complementary sector contained extended LETS-protein patterns.

These observations suggest that the particles cannot move radially into or inside areas where LETS protein covers the cell surface in the form of extended patterns. One would expect, therefore, that removal of LETS protein from the cell surface would increase the centripetal transport of particles.

During a mild treatment of a 3T3 cell culture with trypsin, the predominant component removed from dorsal cell surfaces is LETS protein; yet the cells show no detectable shape alteration in response to this treatment. In contrast, similarly treating the cells with thrombin does not affect the LETS protein (Teng and Chen, *Nature 259*:578 [1976]). Treating 3T3 cells in this way with trypsin or thrombin prior to exposing them to particles resulted in a considerable increase of centripetal particle transport in the trypsin-treated cells as compared with the thrombin-treated cells.

It seems, therefore, that the centripetal transport of surface-attached particles and, perhaps, the surface flow itself are locally inhibited by those surface networks that are built predominantly of LETS protein.

LETS protein and the extracellular matrix

In last year's annual report we described our studies on the large, external, transformation-sensitive (LETS) protein. This is a prominent extracellular glycoprotein associated with the surfaces of many cell types, including fibroblasts, myoblasts, and epithelial cells. Our main approach to studying this cell-surface component involved the use of specific antisera and immunofluorescent microscopy. In some transformed cells LETS protein is expressed only in regions of cell-cell contact, whereas in normal cells at confluence it is distributed over the whole cell surface and appears to be a major component of the extracellular matrix. We have extended the study of LETS protein and the extracellular matrix by means of both scanning and transmission electron microscopy. Figure 2 shows a scanning electron micrograph of a chick embryo fibroblast after 4 days in culture. The cell is surrounded and overlaid with an extensive matrix of fibers of varying dimensions. If a comparable cell is reacted first with antibody against LETS protein, next with a fluorescent antibody, and then viewed by fluorescent microscopy, an equivalent matrix extending both over and beyond the cell is found to be brightly fluorescent, indicating the presence of LETS protein. Further evidence that this extracellular matrix

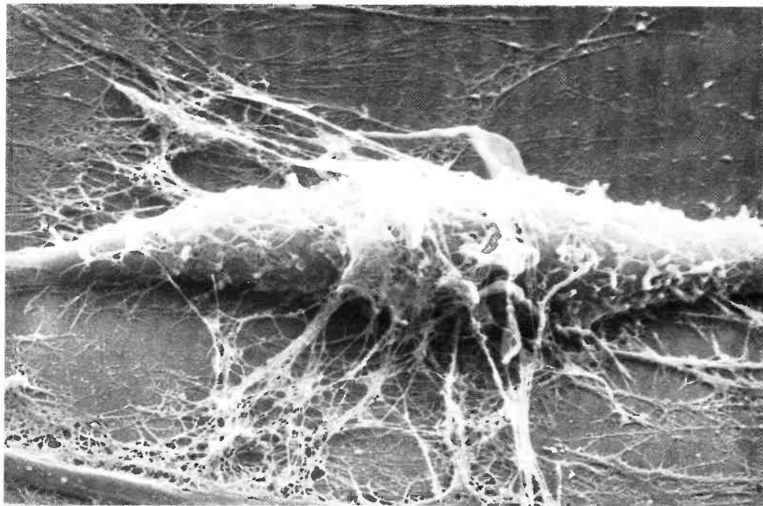

Figure 2
A scanning electron micrograph of a chick embryo fibroblast after 4 days in culture. Note the extracellular fibrillar network extending over the cell. This network is rich in LETS protein.

contains LETS protein as a major constituent has come from transmission electron microscopy. Cells were again treated with antibody against LETS protein but then with a ferritin-conjugated second antibody directed against the first. The electron-dense ferritin molecules could be located in the electron microscope and confirmed the abundance of LETS protein in the fibrils of the extracellular matrix surrounding normal cells in culture.

To study LETS protein and the extracellular matrix further it would be desirable to isolate the matrix free from the cell and cellular debris. To this end we have followed and modified procedures of cell extraction with nonionic detergents which have been found by others to leave LETS protein and much of the cytoskeleton behind while extracting most other cellular components (Hynes et al., *J. Supramol. Struct. 1:*189 [1976]; Brown et al., *J. Supramol. Struct. 5:*119 [1976]). Previously, such extractions were performed at or near neutral pH, but we have found that extraction in 1% NP-40 at pH 9.6 followed by 0.3 M KCl extracts most of the cellular components, including microfilaments, intermediate filaments, and the nucleus, leaving behind what appears to be only the extracellular matrix. Analysis of this matrix has confirmed that the major component is LETS protein. Some actin and myosin were also detected in the gels, but immunofluorescent microscopy showed that these were not part of the matrix but were present as a random, speckled pattern in between the fibers of the matrix.

Experiments have been performed to examine the response of cells to the extracellular matrix prepared as described above. It was found to have a marked influence on the morphology of certain cells when these were plated on it. A line of Chinese hamster ovary cells, for example, that normally possess a rounded-up morphology, when plated on these matrix preparations spread and became spindle-shaped. Currently, we are uncertain as to the role of LETS protein in effecting this morphological transition, but various other substrates, such as collagen, fibrinogen, and polylysine, did not induce it.

Hormones and LETS protein

In most experiments where the expression of LETS protein has been studied, serum is routinely present in the culture medium at concentrations between 5% and

10%. While studying the effect of thrombin on the LETS protein of mammalian cells we noticed that serum-free control cultures frequently lost the fibrillar LETS network as compared with cultures in 10% serum. This prompted us to investigate whether hormones normally present in serum might affect the expression of LETS protein. Many hormones and growth factors have been tested, and one, epidermal growth factor (EGF), has been found to have a marked effect on the expression of LETS protein. Confluent cultures of 3T3 cells in medium supplemented with 10% calf serum have massive fibrillar networks of LETS protein, as detected by immunofluorescence. If the medium of such cells is replaced by medium containing 0.7% serum, after 2 days most of the LETS network disappears or deteriorates significantly. When such cultures are supplemented with EGF at 1 ng/ml, the extensive network of LETS protein is restored within 30 hours. This effect has been found with many cell types, including secondary fibroblasts from mouse, rat, and hamster, and with the established cell lines NIL-8 and BHK. An exception, however, was found in the case of chick embryo fibroblasts, where the fibrillar LETS network remains unaffected even when the cells are cultured in serum-free medium for 2 days.

EGF can stimulate the growth of many fibroblasts, such as 3T3 cells. We have investigated whether this stimulation of growth can be dissociated from the stimulation of LETS-protein production. At high doses of EGF, significant growth stimulation does not occur; however, LETS-protein production is enhanced considerably. Evidence such as this, which indicates different dose-response ranges, suggests that the two effects can be dissociated. Little is known about the way hormones like EGF function, and we hope that this identification of a biochemical response to EGF will open up new directions for research into how such hormones affect their cellular targets.

The cytoskeleton

Our work on the distribution and behavior of contractile and cytoskeletal proteins in nonmuscle cells has continued. Recently, we have been concentrating on a line of gerbil fibroma cells that have a very flat morphology on glass and that reveal arrays of comparatively narrow stress fibers. These properties make them ideal for immunofluorescent microscopy. In previous studies the distributions of myosin and α-actinin along the stress fibers have been shown to be periodic. We have extended this observation and have shown that the distributions of these two proteins alternate and are complementary. This is reminiscent of the distributions of these proteins in skeletal muscle myofibrils and supports the theory that the arrangement of actomyosin in these nonmuscle stress fibers is sarcomeric. A most intriguing observation with these cells has been that we have also seen, by immunofluorescence, an intermittent distribution of actin in some fibers. This may suggest that even the actin is distributed periodically in nonmuscle "sarcomeres." This actin periodicity, however, has been observed only with a low frequency, but this may be because in most stress fibers there is an overlap of the actin filaments obscuring the periodicity. Such an overlap might depend on the state of contraction of the stress fiber during fixation, the average state being a contracted one. Alternatively, this occasional periodic distribution of actin may indicate that only some fibers have a sarcomeric arrangement of actin and that this occurs as a result of a particular physiological state in localized regions of certain cells.

The ultrastructure of these gerbil fibroma cells has been studied by electron microscopy. Such studies have shown that these stress fibers, like those in many other fibroblasts, are bundles of tightly packed microfilaments with an intermittent distribution of electron-dense structures (dense bodies). A comparison between the electron micrographs and the immunofluorescent images reveals that the fluorescent units of the α-actinin pattern have the same size, shape, and spacing along the stress fibers as the dense bodies, whereas the fluorescent units of the myosin pattern have the same dimensions as the spaces between the dense bodies. From this we have concluded that the dense bodies are the sites of α-actinin localization and that the spaces between them are the sites of myosin localization. No indication of a periodic actin distribution has as yet been found at the EM level, but here we are limited by the small number of cells that can be examined by this technique.

Intermediate filaments

During our work with antisera against various structural proteins we have routinely screened the sera of rabbits before immunization to test for any preexisting immunological activity. In one nonimmunized rabbit an antiserum was found that reacted strongly with a system of filaments in various cell types, such as fibroblasts, epithelial cells, macrophages, and neuroblastoma. A gerbil fibroma cell reacted with this antiserum is illustrated in Figure 3. These filaments are distinct from the actin microfilament bundles made visible by an antibody against actin and they are not affected by brief treatment with cytochalasin B. Although resembling microtubules somewhat, these filaments can be distinguished both by a comparison of their respective locations during cell division and by their responses to the drugs colchicine and vinblastine. During cell division the mitotic spindles bind antibodies against microtubules, whereas the system of filaments reactive with this unknown antiserum is found generally dispersed

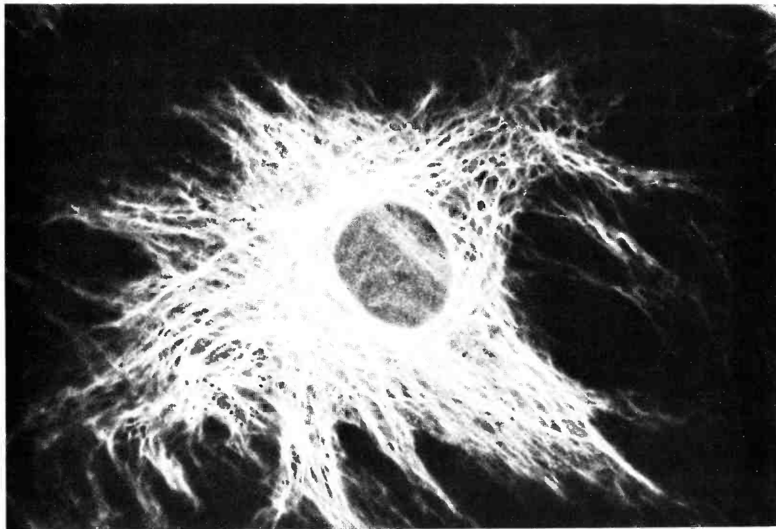

Figure 3
Indirect immunofluorescent micrograph of a gerbil fibroma cell stained with an autoimmune rabbit antiserum. The fibers reacting with this antiserum are the intermediate (10 nm) filaments.

through the cytoplasm everywhere except at the spindles. In response to treatment of the cells with colchicine or vinblastine, the filaments reacting with this antiserum condense to form a compact perinuclear coil of fibers, a distribution and behavior in agreement with that described previously for intermediate (10-nm) filaments studied by electron microscopy. A comparison of electron micrographs and immunofluorescent patterns from parallel cell cultures has provided further evidence that this antiserum reacts with intermediate filaments.

To identify the antigens reacting with this antiserum we have used the technique of immunoautoradiography on SDS gels, the development of which was described last year. Two reactive polypeptides have been identified in both 3T3 cells and the gerbil fibroma cells, having apparent molecular weights of about 56,000 and 30,000 daltons, respectively. As far as we know, this is the first time an autoimmune antiserum has been analyzed in this way.

Antigen and glycoprotein detection in SDS gels

Last year we described the methods we had developed for analyzing both antigens and glycoproteins directly in SDS gels by their binding of labeled antibodies or lectins, respectively. We have continued to improve the techniques and they have been put to a variety of applications. As described above, the technique of detecting antigens in SDS gels has been used to analyze an autoimmune serum reactive with the intermediate filaments of nonmuscle cells. A different application has been found in collaboration with R. Bigelis (Protein Synthesis Section) who has been working on the yeast *his4* gene complex, which codes for a multifunctional protein that catalyzes three steps in the pathway of histidine biosynthesis. An antibody raised by R. Bigelis against this protein will detect the 95,000-dalton polypeptide in SDS gels. We have varied the antibody technique by replacing the second antibody with radioiodinated staphylococcal protein A. This protein A has the property of binding to the Fc portion of the immunoglobulins of many species. In the experiments with the antibody against the *his4* protein we could detect in gels not only the 95,000-dalton protein, but also the fragment polypeptides from those mutant strains bearing nonsense, frameshift, and deletion mutations in this region. Many of these polypeptide frag-

ments are unstable, and because they are degraded rapidly they have not been detected by conventional immunoprecipitation techniques. However, with immunoautoradiograpy on SDS gels the immunological reaction occurs after the proteins are resolved in the gel rather than before (as in conventional immunoprecipitation), and since proteolytic degradation can be kept to a minimum, this has allowed us to identify mutant proteins that were previously undetectable.

Cell-surface glycoproteins and development

The cell surface must play a critical role in development, and yet surprisingly little is known about most of the proteins and glycoproteins that are present on cell surfaces. The general ignorance concerning cell-surface molecules results largely from inadequate analytical techniques. One technique that may contribute information in this area is the identification of specific glycoproteins in SDS gels by their direct binding of labeled lectins, which has been mentioned above and which has been developed independently in several laboratories. During the last year we have applied this technique to the analysis of glycoproteins in two differentiating systems in which cell-surface events must take place. Glycoproteins have been analyzed from the cellular slime mold *Dictyostelium discoideum*. This interesting creature spends most of its life cycle as a free-living amoeba feeding on bacteria. When the food supply is exhausted, however, the amoebae respond by aggregating and forming a fruiting body, with sporulation being the end result. During aggregation the cells become more adhesive and bind selectively to each other. Clearly, changes at the cell surface must occur. After aggregation, the mass of cells will differentiate, giving rise to spore cells that are raised up above the substrate on a pedestal of stalk cells. When the glycoproteins from successive stages in the developmental cycle are analyzed, some pronounced changes are revealed with some lectins. With Concanavalin A many reactive glycoproteins are found over the complete molecular weight range. During development some of these glycoproteins remain constant, whereas others appear or are lost at particular stages in the sequence. The free-living amoebae have very few glycoproteins that react with the wheat germ agglutinin, but during their differentiation several prominent reactive glycoprotein gel bands appear. Similarly, lectins with a specificity for fucose do not reveal any reactive glycoproteins either in the free-living amoebae or during the early stages of development. Late in development, however, and possibly corresponding to the stage of spore-cell differentiation, several reactive bands appear. Little, if any reaction was found with lectins specific for terminal galactose residues or terminal *N*-acetyl-galactosamine. This was somewhat surprising as a lectin isolated from *Dictyostelium* by several laboratories has a specificity for galactose. The expression of this lectin is developmentally regulated and it appears as the amoebae become more adhesive. It has therefore been implicated in the aggregation process. Since it would be of considerable interest to identify the receptor for this protein, we have purified and radiolabeled it and have then applied it back to fixed SDS gels. To our surprise we have not yet detected any reactive material in these gels. We suspect that the receptor for this *Dictyostelium* lectin may be a glycolipid. This might escape detection even if it migrated in the gel, as it would very likely be lost during the fixation procedure. More work is needed in this area.

Another system we have been exploring is muscle development. This is of interest because much is known about the biochemistry of the internal muscle structural proteins and it is a system that can be studied conveniently in tissue culture. Single-celled myoblasts mature in culture, aligning with fellow myoblasts and fusing to form multinucleate myotubes. Clearly, the cell surface must be important both during myoblast recognition and in the subsequent fusion process. After fusion, the myotubes acquire new surface properties, such as the appearance of acetylcholine receptors. We have been analyzing this sequence of myogenic development using a rat myoblast cell line and have been analyzing the glycoproteins of these cells before and after fusion. Some interesting changes have been revealed. In an attempt to determine the functions of some of these alterations we are currently trying to raise antibodies against those components that change with development. Specific antibodies will permit localization studies and will also enable us to probe the possible functions of some of these components. It would be interesting, for example, to raise an antibody that would block fusion or that would disrupt other events in this sequence of myogenic development.

Publications

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Albrecht-Buehler, G. and L. B. Chen. 1977. Local inhibition of centripetal transport where LETS protein patterns appear on 3T3 cells. *Nature* **266:**454.

Bigelis, R. and K. Burridge. 1978. The immunological detection of yeast nonsense termination fragments on sodium dodecyl sulfate-polyacrylamide gels. *Biochem. Biophys. Res. Commun.* (in press).

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Chen, L.B. 1977. Alteration in cell surface LETS protein during myogenesis. *Cell* **10**:393.

Chen, L.B., N. Maitland, P.H. Gallimore, and J.K. McDougall. 1977. Detection of the large, external transformation-sensitive protein on some epithelial cells. *Exp. Cell Res.* **106**:39.

Chen, L.B., R.C. Gudor, T.-T. Sun, A.B. Chen, and M.W. Mosesson. 1977. Control of a cell surface major glycoprotein by epidermal growth factor. *Science* **197**:776.

Chen, L.B., A.Murray, R.A. Segal, A. Bushnell, and M.L. Walsh. 1978. Studies on intercellular LETS glycoprotein matrices. *Cell* (in press).

Gordon, W.E., III, A. Bushnell, and K. Burridge. 1978. Characterization of the intermediate (10 nm) filaments of cultured cells using an autoimmune rabbit antiserum. *Cell* **13**:249.

———. 1978. The cytoplasmic matrix of cultured nonmuscle cells. I. Immunofluorescent and ultrastructural studies of actin, myosin, and α-actinin in stress fibers. (Submitted)

POSTGRADUATE TRAINING PROGRAMS

SUMMER 1977

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.

THE TRANSFORMED CELL, June 12–July 1

INSTRUCTORS

Pollack, Robert, Ph.D., State University of New York, Stony Brook
Hynes, Richard, Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANTS

Feldman, Jonathan, B.A., State University of New York, Stony Brook
Lanza, Robert, B.A., University of Pennsylvania, Philadelphia

In vitro transformation of mammalian cells by oncogenic viruses or by environmental agents has served in the last decade as a central model for the earliest steps in the process of oncogenesis. This new course presented an integrated series of laboratory exercises, informal discussions, and guest lectures designed to provide each student with a chance to carry out and to review critically the many different assays that detect differences between normal and transformed cells. Wherever possible, these assays were also made to serve as starting points for examinations of the molecular events underlying them and of their relationship to in vivo processes such as initiation, promotion, angiogenesis, invasion, and metastasis. Exercises included SV40 virion and DNA transformation of murine precrisis fibroblasts and cell lines, selection of phenotypic revertants, karyotype analysis, immunofluorescent localization of viral antigens, preparation of chick embryo myoblasts and fibroblasts, transformation by ts and wt RSV, fibrinolysis, HAT selection, gel electrophoresis and localization of LETS by antibody- and lectin-staining on gels, immunofluorescent localization of cytoskeletal proteins and of LETS, sugar transport, and explantation of tumors from the nude mouse.

Special thanks for their contributions of time and material to these exercises go to Bill Topp, Keith Burridge, Bettie Steinberg, Kate Nyman, Andrew Lo, Seung-Il Shin, Dan Rifkin, Bill Brinkley, Bob Goldman, Guenter Albrecht-Buehler, and Peter Morrell.

PARTICIPANTS

Alexander, Stephen, Ph.D., Sidney Farber Cancer Institute, Boston, Massachusetts
Anholt, Robert H., M.S., University of California, La Jolla
Birchmeier, Walter, Ph.D., University of California, La Jolla
Bradlaw, June A., Ph.D., Food and Drug Administration, Washington, D.C.
Hiscott, John, M.S., New York University, New York
Hsu, Wen-Tah, Ph.D., University of Chicago, Illinois
Huesgen, Adi, Ph.D., Max-Planck-Institut, Tübingen, West Germany
Karess, Roger E., B.S., Rockefeller University, New York, New York
Lockwood, Arthur, Ph.D., New York University, New York
Makan, Nizar R., M.D., Cornell University, Ithaca, New York
Mayo, Kristen A., B.A., University of California, Santa Barbara
Mueller, Christian, Ph.D., University of Berlin, West Germany
Peehl, Donna M., B.S., University of Colorado, Boulder
Scandella, Carl J., Ph.D., State University of New York, Stony Brook
Schollmeyer, Judith E., Ph.D., University of Minnesota, Minneapolis
Smith, Karen K., B.S., Rockefeller University, New York, New York
Villereal, Mitchel L., Oak Ridge National Laboratory, Tennessee
Yefenof, Eitan, B.S., Karolinska Institute, Stockholm, Sweden

SEMINARS

Todaro, G., National Cancer Institute. *Transformations.*
Goldman, R., Carnegie-Mellon University. *Cytoskeleton.*
Botchan, M., Cold Spring Harbor Laboratory. *Recombination and papovaviruses.*
Varmus, H., University of California, San Francisco. *Sarc gene and its products(s).*
Rifkin, D., Rockefeller University. *Proteases.*
Tegtmeyer, P., State University of New York, Stony Brook. *A gene and its product(s).*
Hynes, R., Massachusetts Institute of Technology. *Cell surfaces.*
MacDougall, J., Cold Spring Harbor Laboratory. *Adenovirus.*
Brinkley, B., University of Texas, Houston. *Cytoplasmic microtubule complex.*
Fidler, J., Frederick Cancer Research Center. *Metastasis.*
Folkman, J., Children's Hospital, Boston. *Angiogenesis.*
Green, H., Massachusetts Institute of Technology. *Normal skin in culture.*
Lilly, F., Albert Einstein College of Medicine. *Host genetics of murine tumor viruses.*
Shin, S.-I., Albert Einstein College of Medicine. *Nude mice.*
Smith, H., University of California, Oakland. *Human tumors in culture.*
Sato, G., University of California, San Diego. *Hormones.*

MOLECYLAR CYTOGENETICS, June 12–July 2

INSTRUCTORS

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

ASSISTANTS

Erba, Harry, Yale University, New Haven, Connecticut

The molecular cytogenetics course emphasized the integration of classical and molecular techniques for analysis of chromosome structure and function. A number of visiting scientists joined with the class for discussion of current problems in the field and for experiments utilizing chromosome banding, the isolation of chromatin subunits, in situ hybridization, visualization of transcription by electron microscopy, restriction-enzyme digestion of DNA, nucleic acid sequencing, heteroduplex mapping, and fluorescent antibody studies of the cytoskeleton. As always, we benefited from the many contributions of Barbara McClintock.

Students came from Switzerland, Germany, England, Canada, and various parts of the United States. Their previous research experience ranged from neurobiology to crystallography, adding a wide variety of interests and expertise to the group.

PARTICIPANTS

Baumgartel, D. Mona, Ph.D., San Diego Zoological Society, California
Hayashi, Shizu, Ph.D., University of British Columbia, Canada
Jones, C. Weldon, B.A., Harvard University, Cambridge, Massachusetts
Kay, Alan C., Ph.D., National Cancer Institute, Bethesda, Maryland
Mauron, Alex, M.S., Swiss Institute for Cancer Research, Lausanne
Rosa, Margaret D., Ph.D., Yale University, New Haven, Connecticut

Sato, J. Denry, B.S., Oxford University, England
Schafer, Ulrich, Ph.D., University of Dusseldorf, West Germany
Wigley, Caroline, Ph.D., Imperial Cancer Research Fund Laboratories, London, England

SEMINARS

Bakken, A., University of Washington. *Chromosome structure during mammalian oogenesis.*
Latt, S., Harvard Medical School. *Fluorescent dyes as probes of chromosome structure.*
Noll, M., University of Basel. *The structure of the nucleosome.*
Fujiwara, K., Harvard Medical School. *Probing the cytoskeleton with fluorescent antibodies.*
Lindsley, D.L., University of California, San Diego. *Genetic studies on* Drosophila.
Gall, J.G., Yale University. *Gene amplification in* Tetrahymena.
Pardue, M.L., Massachusetts Institute of Technology. *Structure and function of* Drosophila *polytene chromosomes.*
Davis, R., Stanford Medical School. *Expression of eukaryotic genes in bacteria.*

NERVOUS SYSTEM OF THE LEECH, June 12–July 2

INSTRUCTORS

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

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

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

The final phase of the course consisted of devising and performing original experiments, some of which proved to be of sufficient interest to be pursued in greater detail. For example, the nervous systems of various hitherto unexplored leeches were studied.

PARTICIPANTS

Bearer, Elaine L., M.A., Stanford University, California
Grinvald, Amiram, Ph.D., Yale University, New Haven, Connecticut
Katzel, Les I., B.S., Johns Hopkins University, Baltimore, Maryland
Kramer, Andrew P., B.A., University of California, Los Angeles
Laffer, Cheryl L., B.S., University of Wisconsin, Madison
Nelson, Margaret C., Ph.D., Harvard Medical School, Boston, Massachusetts
Schnapp, Bruce J., Ph.D., University of Connecticut, Storrs
Scott, Sheryl A., Ph.D., McMaster University, Ontario, Canada
Tilney, Lewis G., Ph.D., University of Pennsylvania, Philadelphia
Wu, Chun-Fang, Ph.D., California Institute of Technology, Pasadena

SEMINARS

Sawyer, R., University of California, Berkeley. *Biology and development of leeches.*
Stewart, A.E., Harvard Medical School. *Motoneurons and control of muscle.*
Stent, G., University of California, Berkeley. *Neurons responsible for swimming and the initiation of rhythmical heartbeat.*
Parnas, I., Hebrew University. *Methods for killing individual nerve cells in intact animals.*
Salzberg, B., University of Pennsylvania. *Optical techniques for recording from neurons without electrodes.*

TECHNIQUES FOR STUDYING THE VERTEBRATE CENTRAL NERVOUS SYSTEM, June 12–July 4

INSTRUCTORS

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

ASSISTANT

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

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

PARTICIPANTS

Batson, Deidre E., B.A., University of Bristol, England
Hofbauer, Alois, B.S., University of Freiburg, West Germany
Jan, Yuh Nung, Ph.D., California Institute of Technology, Pasadena
Katz, David M., B.A., State University of New York, Stony Brook
Kennedy, Mary B., Ph.D., Harvard Medical School, Boston, Massachusetts
Nakamura, Richard K., Ph.D., National Institutes of Health, Bethesda, Maryland
Schein, Stanley J., Ph.D., M.D., Albert Einstein College of Medicine, Bronx, New York
Wagner, John A., Ph.D., University of California, San Francisco

SEMINARS

Bizzi, E., Massachusetts Institute of Technology. *Sensory feedback in the control of head and eye movements.*
Moore, G.P., University of Southern California. *Mathematical techniques for analyzing neural connectivity.*
Kirkwood, P., Institute of Neurology, London. *Neural control of respiration.*
Shatz, C., Harvard Medical School. *Visual system abnormalities in a genetic mutant.*
Sherk, H., Massachusetts Institute of Technology. *The role of early experience in the development of the visual cortex.*
Stryker, M., Harvard Medical School. *Metabolic mapping of activity in the visual cortex using the 2-deoxyglucose autoradiographic technique.*
Burke, R., National Institutes of Health. *Motor units, motor neurons, and the control of movement.*
Schiller, P., Massachusetts Institute of Technology. *The role of the superior colliculus in eye movement and vision.*

THE STRUCTURE AND FUNCTION OF SYNAPSES, June 19–July 10

INSTRUCTORS

Rahamimoff, Rami, M.D., Hebrew University Medical School, Jerusalem, Israel
McMahan, Jack U., Ph.D., Harvard Medical School, Boston, Massachusetts
Purves, Dale, M.D., Washington University, St. Louis, Missouri

This course was designed for graduate students and research workers interested in the structure and function of synapses. It consisted of lectures; reading of papers; group discussions; presentation of selected topics by participants; seminars by instructors, faculty, and participants; and demonstrations. After the course ended, nine participants who were uninitiated in electrophysiological techniques stayed on to learn the usage of basic methods.

The topics covered included microscopic anatomy of the nervous system; ionic bases of membrane and action potentials; membrane structure and ionic selectivity; morphology, microphysiology, and biochemistry of the postsynaptic membrane and receptors; excitatory and inhibitory synaptic potentials; physiology and morphology of release of neurotransmitters; electrical synapses and gap junctions; neurotoxins; principles of synaptic pharmacology; transmission in autonomic ganglia; transmission at invertebrate synapses; integrative processes in the nervous system; slow synaptic potentials; immunological studies of acetylcholine receptors, cyclic nucleotides, and neuronal function; structure of central nervous system synapses; biochemistry and

physiology of the adrenergic system; development of the nervous system; denervation, reinnervation, and sprouting in the nervous system; nerve growth factor; the postsynaptic channel and its voltage dependence; and fluctuation analysis in synaptic physiology.

PARTICIPANTS

Bondi, Ardith Y., Ph.D., New York University, New York
Corey, David P., B.A., California Institute of Technology, Pasadena
Curran, Michael J., M.S., University of Toronto, Canada
Gozes, Illana, B.S., The Weizmann Institute, Rehovot, Israel
Greenberg, Allan S., B.A., Purdue University, West Lafayette, Indiana
Hicks, Philip T., B.S., University of British Columbia, Canada
Jan, Lily, Ph.D., California Institute of Technology, Pasadena
Johnson, David A., B.S., University of Connecticut, Storrs
Lansman, Jeffrey B., B.A., Tufts University, Medford, Massachusetts
Lasher, Stuart S., B.S., University of Virginia, Charlottesville
Levine, Richard B., B.S., State University of New York, Albany
Marchand, Claudine, Ph.D., State University of New York, Stony Brook
Miller, Thomas M., B.A., University of California, San Francisco
Orida, Norman K., B.A., University of California, Irvine
Peters, Tim K., M.D., University of Berlin, West Germany
Redmann, Greg A., B.A., Stanford University, California
Salama, Guy, M.S., University of Pennsylvania, Philadelphia
Thomas, William E., M.S., Meharry Medical College, Nashville, Tennessee
Wendon, Linda, B.A., University College London, England

SEMINARS

Hall, Z., University of California, San Francisco. *Acetylcholine receptor: Synthesis and control of distribution.*
——— *Myasthenia gravis and immunological studies of acetylcholine receptor.*
——— *Cyclic nucleotides and neuronal function.*
Nicholls, J., Stanford University. *After effects of signaling.*
——— *Integrative processes in the nervous system.*
McMahan, J.U., Harvard Medical School. *Recycling of vesicles.*
——— *Morphology workshop.*
——— *Development of synapses.*
——— *Reinnervation of muscle.*
Martin, A.R., University of Colorado, Denver. *Statistics of transmitter release. I.*
——— *Statistics of transmitter release. II.*
——— *Electrical synapses: Physiology.*
——— *The effect of acetylcholine on eel electroplaque.*
——— *Principles of transmission in sympathetic ganglion.*
Nichols, J., J. Jansen,* and K. Muller,† Stanford University; *University of Oslo; †Carnegie Institution. *Leech demonstration.*
Blaustein, M., Washington University, St. Louis. *The regulation of intracellular calcium in nerve terminals.*
——— *The physiology of synaptosomes.*
——— *Sodium calcium exchange.*
Goodenough, D., Harvard University. *Morphology of gap junctions and electrical synapses.*
Erulkar, S., University of Pennsylvania. *Neurotoxins.*
——— *Principles of synaptic pharmacology.*
——— *Squid synapses.*
Kuffler, S.W., Harvard University. *Microphysiology of the postsynaptic membrane.*
——— *Slow synaptic potentials in the parasympathetic ganglion.*
Yoshikami, D., Harvard University. *Microphysiology of the postsynaptic membrane at the neuromuscular junction.*
——— *Autonomic ganglia.*
Raviola, E., Harvard University. *Structure of central nervous system synapses.*
——— *Workshop on freeze-fracture.*
Patterson, P., Harvard University. *The adrenergic system.*
——— *Autonomic ganglia tissue.*
Frank, E., Harvard University. *The development of synaptic contacts in tissue culture.*
Stevens, C., Yale University. *The postsynaptic channel and its voltage dependence.*
——— *Selectivity and ion permeation through Mu synaptic channel.*
——— *Fluctuation analysis and single-channel conductance.*
——— *Applications of fluctuation analysis.*
Shimoni, Y., and A. Lev Tov, Hebrew University, *Intracellular recording and stimulation.*
——— *Extracellular recording and stimulation.*
——— *Analysis of synaptic activity.*

ADVANCED BACTERIAL GENETICS, July 5–July 25

INSTRUCTORS

Botstein, David, Ph.D., Massachusetts Institute of Technology, Cambridge
Roth, John, Ph.D., University of Utah, Salt Lake City
Wensink, Peter, Ph.D., Brandeis University, Waltham, Massachusetts

ASSISTANTS

Meyer, Barbara, B.S., Harvard University, Cambridge, Massachusetts
Chumley, Forrest, B.S., University of Utah, Salt Lake City
Guergen, Peter, B.S., Brandeis University, Waltham, Massachusetts

Students in the course carried out complete in vitro recombination experiments in which fragments of *Salmonella* DNA were joined to *E. coli* plasmid vehicles by the terminal transferase method. The in vitro recombinants were analyzed by methods which included colony hybridization to radioactive yeast ribosomal RNA and heteroduplex analysis in the electron microscope and attempted genetic complementation of specific genetic defects in the bacterial hosts.

Students also carried out a variety of genetic experiments with translocatable drug-resistance elements of *S. typhimurium* and *E. coli*. Some of the *E. coli* insertion mutants were used to screen the in vitro recombinants for complementation.

PARTICIPANTS

Berget. Peter B., Ph.D., Massachusetts Institute of Technology, Cambridge
Brefort, Georges A., M.D., Pasteur Institute, Paris, France
Federoff, Howard J., B.A., Albert Einstein College of Medicine, Bronx, New York
Frick, Kevin K., B.A., University of Illinois, Urbana
Guarneros, Gabriel, Ph.D., Institute Politec Nacional, Mexico City
Hennecke, Hauke H., University of Regensburg, West Germany
Hertz, Rachel, M.S., Hebrew University, Jerusalem, Israel
Koch, Arthur L., Ph.D., Indiana University, Bloomington
Kunst, Frank F., M.S., University of Paris, France
Lerner, Terry T., M.A., Rockefeller University, New York, New York
Lillis, Marcella T., B.A., University of Pennsylvania, Philadelphia
Malik, Vedpal S., Ph.D., The Upjohn Company, Kalamazoo, Michigan
Maurelli, Anthony T., B.S., University of Alabama, Birmingham
Neff, Norma F., B.A., University of California, Berkeley
Palva, Tapio E., Ph.D., The Wallenberg Laboratories, Uppsala, Sweden
Schoffl, Friedrich, Ph.D., University of Erlangen, West Germany
Siegel, Eli C., Ph.D., Tufts University, Medford, Massachusetts
Stahl, Franklin, Ph.D., University of Oregon, Eugene
Sullivan, Mark A., B.S., University of Wisconsin, Madison
Vennstrom, Bjorn, Ph.D., Uppsala University, Sweden

SEMINARS

Davis, R., Stanford University. *Quantitative electron microscopy of DNA.*
Simon, M., University of California, San Diego. *Molecular basis of phase variation in* Salmonella *flagella.*
Botstein, D., Massachusetts Institute of Technology. *Genetic studies of translocation of the translocatable element Tn1.*
Stahl, F., University of Oregon, Eugene. *Recombination in bacteriophage lambda.*
Ptashne, M., Harvard University. *Mechanism of action of lambda repressor.*
Brenner, S., Medical Research Council. *Molecular biology of nematodes.*
——— *New in vitro recombination methods.*
Miller, J., University of Geneva. *Genetic studies in* lac *I.*
Kleckner, N., Harvard University. *The genetics of transposition.*

ONCOGENIC VIRUSES, July 5–July 25

INSTRUCTORS

Sharp, Phillip A., Ph.D., Massachusetts Institute of Technology, Cambridge
Spear, Patricia G., Ph.D., University of Chicago, Illinois

GUEST LECTURER

Baltimore, David, Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANT

Wilcox, Kent, Ph.D., University of Chicago, Illinois

Four animal virus groups were discussed during the course and used in laboratory experiments: papovaviruses, adenoviruses, herpesviruses, and retroviruses. Both the biology and molecular biology of the viruses were covered by various speakers in a format that allowed the lecturers to provide background information so that their current work could be understood by students without previous training in the field. Several members of the permanent staff of Cold Spring Harbor Laboratory gave research seminars and demonstrations of specific techniques. The afternoons and part of the evenings were occupied with laboratory work. Topics covered included counting and plating of mammalian cells in culture; transformation of 3T3 cells by Harvey sarcoma virus; SV40 virus and SV40 DNA; transfection of cells with herpes simplex viral DNA; transformation of thymidine-kinase-negative mouse cells to thymidine-kinase-positive cells with herpes simplex DNA; plaque titering of SV40; adenovirus 2 and herpes simplex virus; extraction of adenovirus-2 virions and SV40 and herpesvirus DNAs from infected cells; restriction endonuclease cleavage and gel electrophoresis analysis of viral genomes; nick translation of viral DNA; Southern blotting of cellular and viral DNA; XC assay of Moloney leukemia virus and preparation of an in vitro protein-synthesizing system using rabbit reticulocyte lysate digested with micrococcal nuclease. Brief demonstrations of injection of mammalian cells by microneedles and electron microscope techniques for visualization of DNA were given. In addition to these activities, some students worked on the development of other procedures used in molecular virology.

PARTICIPANTS

Abovich, Nadja B., B.S., Universidad Catolica, Valparaiso, Chile
Biswas, B., Ph.D., Rose Institute, Calcutta, India
Chiang, Tom C.H., Ph.D., University of Texas, Richardson
Cioé, Livia, Ph.D., University of Rome, Italy
d'Auriol, Luc, M.A., Hôpital St. Louis, Paris, France
Flavell, Andrew J., Ph.D., Imperial Cancer Research Fund Laboratories, London, England
Gattoni, Sebastiano, M.D., New York University, New York
Gissmann, Lutz, Ph.D., University of Erlangen, West Germany
Jofre, Juan T., Ph.D., Baylor College, Houston, Texas
Kramer, Barnett S., M.D., National Cancer Institute, Bethesda, Maryland
Miovic, Margaret L., Ph.D., Swarthmore College, Pennsylvania
Murray, Mark J., M.S., University of Oregon, Portland
Ricciardi, Robert P., M.A., University of Illinois, Urbana
Roos, Raymond P., M.D., University of Chicago, Illinois
Sonenberg, Nahum, Ph.D., Roche Institute, Nutley, New Jersey

SEMINARS

Martin, R.G., National Institutes of Health. *Transformation by papovaviruses.*
Weissman, S.M., Yale University. *Structure of the SV40 genome.*
Tegtmeyer, P., State University of New York, Stony Brook. *Genetics and gene products of papovaviruses.*
Philipson, L., University of Uppsala. *Structure of adenovirus virion and viral DNA transcription.*
Levine, A.J., Princeton University. *Early adenovirus gene products.*
Sharp, P.A., Massachusetts Institute of Technology. *Adenovirus transcription.*
Roberts, B., Brandeis University. *In vitro translation and coupled transcription and translation.*
Roizman, B., University of Chicago. *Herpesvirus: Structure, replication, and transcription.*
Spear, P., University of Chicago. *Herpesvirus gene expression in transformed cells.*
Deinhardt, F., Max von Pettenkofer Institute. *Biology of Epstein-Barr viruses.*
Baltimore, D., Massachusetts Institute of Technology. *Molecular biology of RNA tumor viruses.*
Todaro, G., National Cancer Institute. *Evolution and functions of RNA tumor viruses.*
Rowe, W.P., National Institutes of Health. *Biology of RNA tumor viruses.*
Hanafusa, H., Rockefeller University. *Genetics of avian RNA tumors.*
Boettiger, D., University of Pennsylvania. *Transformation of differentiated cells.*
Basilico, Claudio, New York University Medical School. *Genetics and biochemical analysis of somatic animal cells.*

IMMUNOGENETICS AND TUMOR IMMUNOLOGY, July 28–August 13

INSTRUCTORS

Nowinski, Robert, Ph.D., Fred Hutchinson Cancer Center, Seattle, Washington
Eisenman, Robert, Ph.D., Fred Hutchinson Cancer Center, Seattle, Washington

ASSISTANTS

Shaikh, Rashid, B.S., Fred Hutchinson Cancer Center, Seattle, Washington
Doyle, Terri, B.S., Fred Hutchinson Cancer Center, Seattle, Washington

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

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

PARTICIPANTS

Baluch, John D., B.S., University of Pittsburgh, Pennsylvania
Candler, Edrick L., Ph.D., Union Carbide Corporation, Tarrytown, New York
Carry, Paul J., B.S., University of Colorado, Boulder
Celis, Esteban, M.D., Ciudad University, Mexico
Fabian, Ursula, Ph.D., University of Hamburg, West Germany
Lindl, Toni, Ph.D., University of Konstanz, West Germany
Mansion, Sy, M.A., University of Colorado, Denver
Moen, J.E.T., M.D., University of Leiden, The Netherlands
Pfister, Herbert, Ph.D., University of Freiburg, West Germany
Shuman, Stewart, B.A., Albert Einstein College of Medicine, Bronx, New York
Walker, Mary Clare, Ph.D., New York University, New York
Zarling, David A., Ph.D., University of Wisconsin, Madison

SEMINARS

Nowinski, R., Fred Hutchinson Cancer Center. *Introduction to immunogenetic studies of neoplasia.*
Murphy, D., Stanford University. *Major histocompatibility locus.*
McDevitt, H., Stanford University. *Immune response genes.*
Press, J., Brandeis University. *Differentiation of B cells.*
Hood, L., California Institute of Technology. *Immunogenetics and structure of immunoglobulins and histocompatibility antigens.*
Martin, G., University of California, San Francisco. *Teratomas as models of differentiation. I.*
Levine, A., Princeton University. *Teratomas as models of differentiation. II.*
Nowinski, R., Fred Hutchinson Cancer Center. *Basic principles of tumor immunology.*
Krag, S., Johns Hopkins University. *Biochemistry and synthesis of glycoproteins.*
Nowinski, R., Fred Hutchinson Cancer Center. *Expression of viral proteins on the surface of lymphocytes.*
Yamada, K., National Institutes of Health. *Cell surfaces of transformed cells.*
Pollack, R., State University of New York, Stony Brook. *Cell biology of transformed cells.*
Eisenman, R., Fred Hutchinson Cancer Center. *Cell regulation of RNA tumor viruses.*
Gershon, R., Yale University. *Differentiation of T cells.*
Rowe, W., National Institutes of Health. *Leukemogenesis in AKR mice.*
Lilly, F., Albert Einstein College of Medicine. *Genetic influences of H-2 on viral leukemogenesis.*
Waelsch, S., Cornell Medical School. *T locus.*
Linial, M., Fred Hutchinson Cancer Center. *Genetics of RNA tumor viruses.*
Stollar, D., Tufts University. *Antibodies against nucleic acids.*
Levine, A., Princeton University. *Transformation proteins of DNA tumor viruses.*
Livingston, D., Harvard Medical School. *Relationship of T antigen to TSTA of papovavirus.*
Rohrschneider, L., Fred Hutchinson Cancer Center. *Identification of tumor-specific sarcoma antigens on the surface of RSV-transformed cells.*
Lerner, R., Scripps Clinic. *Physiology of leukemia virus protein expression in normal mouse tissues.*

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

INSTRUCTORS

Sherman, Fred, Ph.D., University of Rochester, New York
Fink, Gerald, Ph.D., Cornell University, Ithaca, New York
Petes, Thomas D., Ph.D., University of Chicago, Illinois

ASSISTANT

Johnson, Tina, B.A., Cornell University, Ithaca, New York

This program emphasized the major laboratory techniques used in the genetic analysis of yeast:tetrad analysis, mitotic recombination, and fine-structure mapping. The isolation and characterization of both chromosomal and cytoplasmic mutants were undertaken. Biochemical studies were performed with chromosomal and mitochondrial mutants. Studies on chromosomal and extrachromosomal DNA were pursued by electron microscopy and by digestion with restriction endonucleases.

PARTICIPANTS

Abelson, John N., Ph.D., University of California, San Diego
Beckman, Jacques S., Ph.D., University of California, San Diego
Bedard, Dennis P., Ph.D., Dalhousie University, Nova Scotia, Canada
Foy, James J., M.S., Miami University, Oxford, Ohio
Frezza, Domenico, Ph.D., National Institutes of Health, Research Triangle Park, North Carolina
Hieda, Kotaro, Ph.D., Rikkyo University, Tokyo, Japan
Jazwinski, S. Michal, Rockefeller University, New York, New York
Larimer, Frank W., Ph.D., Oak Ridge National Laboratory, Tennessee
Lopez Calderóne, Isabel, M.S., University of Seville, Spain
Maccecchini, Maria L., M.S., University of Basel, Switzerland
Piepersberg, Wolfgang, Ph.D., University of Regensburg, West Germany
Rabinowitz, Jesse C., Ph.D., University of California, Berkeley
Steege, Deborah A., Ph.D., Duke University, Durham, North Carolina
Taylor, Fred R., B.A., Yale University, New Haven, Connecticut
Walenga, Ronald W., Ph.D., University of Connecticut, Farmington
Willsky, Gail R., Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Hartwell, L.H., University of Washington. *Cell cycle of yeast.*
________ *Control of cell division in yeast.*
Petes, T.D., University of Chicago. *Structure and replication of yeast DNA.*
________ *Genetic and physical analysis of yeast ribosomal DNA.*
Byers, B., University of Washington. *Cytology of the yeast life cycle.*
Tzagoloff, A., Public Health Research Institute, New York City. *Saturation of the mitochondrial genome.*
Abelsen, J., University of California, San Diego. *Cloning of yeast tRNA genes.*
Mackay, V.L., Rutgers University. *Genetic and hormone control of mating in yeast.*
McLaughlin, C.S., University of California, Irvine. *Protein synthesis in yeast.*
Warner, J., Albert Einstein College of Medicine. *Regulation of ribosomal proteins and RNA.*
Mortimer, R.K., University of California, Berkeley. *Genetic mapping in yeast.*
________ *Gene conversion and post meiotic segregation.*
Fink, G.R., Cornell University. *Regulation of gene clusters in yeast.*
________ *The yeast killer factor.*
Jones, E.W., Carnegie-Mellon University. *Proteinase mutants of yeast.*
Lawrence, C., University of Rochester. *Radiation mutagenesis and repair in yeast.*
Sherman, F., University of Rochester. *Nonsense suppression in yeast.*
________ *Regulation of the isocytochromes c.*

EXPERIMENTAL TECHNIQUES IN NEUROBIOLOGY August 7–August 28

INSTRUCTORS

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

The first three days of the course were devoted to lectures and exercises on electronics for cellular neurobiologists given by Ray Kado. The last few days of the course were devoted to individual experimental projects chosen by the students projects that permitted them to try techniques not taught in the course and to use biological preparations that they intended to study upon returning to their own laboratories.

PARTICIPANTS

Bastiani, Michael J., B.A., University of California, Davis
Boyle, Mary B., B.A., Yale University, New Haven, Connecticut
Carlson, Steven S., Ph.D., University of California, San Francisco
Deutsch, James W., Ph.D., University of California, San Francisco
Harris-Warrick, Ronald M., Ph.D., Stanford University, Stanford, California
Hildebrand, John G., Ph.D., Harvard Medical School, Boston, Massachusetts
Kingan, Timothy G., B.A., Oregon State University, Corvallis
Sardet, Christian, Ph.D., Station Zoologique, Villefranche, France
So, Yuen T., B.S., Rockefeller University, New York, New York
Suarez-Isla, Benjamin A., Ph.D., University of Konstanz, West Germany

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

CHROMATIN

Chromosome research has long been one of the main investigative endeavors at Cold Spring Harbor, and numerous past Symposia have been devoted to delving into chromosomal structure and function. The decision to choose this topic once again for this year's meeting reflects the amazing progress made over the last three years on the chromosomal DNA-protein complexes called chromatin. Here the key discovery has been that it has a repeating subunit (the nucleosome) comprised of some two hundred base pairs wrapped around an octamer of histones. With this fact to work from, the chromatin field has broken out of the speculative straightjacket which for so long held it down and has emerged as one of the most exciting areas in biology. And we felt somewhat secure in our anticipation of a full meeting at which many new facts would be presented and from which many new avenues of investigation would lead. The final product, however, was even more exciting than anyone could have hoped. The existence of recombinant DNA and the powerful new DNA sequencing methods have begun to generate results that a few years ago we would have predicted could not emerge until a decade or two of more sustained work. Even more surprisingly, a variety of experimental approaches gave us the bombshell that functional RNA molecules can derive from physically quite separate sections along a DNA molecule. At the end we were both overwhelmed and dazzled, and many participants left feeling they had been part of an historic occasion.

The great breadth of the prospective program meant that substantial help was needed from outside advisors. Invaluable advice was given by Drs. Bruce Alberts, Pierre Chambon, Francis Crick, Gerry Felsenfeld, Joe Gall, Aaron Klug, Roger Kornberg, Mary Lou Pardue, and Harold Weintraub. A total of one hundred and eight formal presentations were given at the week-long gathering. Three hundred and seventy-five participants were in attendance, a Symposium record.

Only with the receipt of substantial outside financial help could we have invited so many speakers. Again we are most indebted to the National Institutes of Health, the National Science Foundation, and the United States Energy Research and Development Administration.

WEDNESDAY EVENING, June 1

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

Opening comments: F.H.C. Crick, Salk Institute

Session 1: Nucleosome structure I: Crystallographic analysis

Chairperson: G. Felsenfeld, National Institutes of Health, Bethesda, Maryland

J.T. Finch and A. Klug, Medical Research Council Laboratory of Molecular Biology, Cambridge, England: X-ray study of crystals of nucleosome cores.

J.F. Pardon, R.I. Cotter, D.M.J. Lilley, D.L. Worcester,* A.M. Campbell,† J.C. Wooley, and B.M. Richards, Searle Research Laboratories, High Wycombe, Bucks, England; *Atomic Energy Research Establishment, Harwell, England; †Biochemistry Department, University of Glasgow, Scotland: Neutron and X-ray scattering studies of chromatin subunits.

THURSDAY MORNING, June 2

Session 2: Nucleosome structure II: Conformational studies

Chairperson: E.M. Bradbury, Portsmouth Polytechnic Institute, England

M. Zama, P.N. Bryan, R.E. Harrington, A.L. Olins, and D.E. Olins, Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee, and Biology Division, Oak Ridge National Laboratory, Tennessee: Conformational states of chromatin.

C.L.F. Woodcock and L.-L.Y. Frado, Department of Zoology, University of Massachusetts, Amherst: Ultrastructure of chromatin subunits during unfolding, histone depletion, and reconstitution.

R.D. Camerini-Otero, B. Sollner-Webb, R. Simon, P. Williamson, M. Zasloff, and G. Felsenfeld, National Institutes of Health, Bethesda, Maryland: DNA folding and histone interactions in the nucleosome.

M. Noll, Department of Cell Biology, Biozentrum University of Basel, Switzerland: Internal structure of the nucleosome—DNA folding in the conserved 140-base-pair core particle.

H.M. Sobell, S.C. Jain, C.-C. Tsai, T.D. Sakore, and S.G. Gilbert, Department of Chemistry and Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, New York: Conformational flexibility in DNA structure as revealed by structural studies of drug intercalation and its implications in understanding the organization of DNA in chromatin.

A. Prunell, B. Baer, and R.D. Kornberg, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Relation of nucleosomes to nucleotide sequences.

H.G. Zachau, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, West Germany: Nuclease cleavage of chromatin.

THURSDAY EVENING, June 2

Session 3: Transcription I: Primary products

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

R. Evans, N. Fraser, S. Goldberg, J. Weber, M. Wilson, E. Ziff, and J.E. Darnell, Molecular Cell Biology Department, Rockefeller University, New York, New York: Localization of transcription units for mRNA by nascent RNA hybridization and UV mapping.

S.M. Berget, A.J. Berk, T. Harrison, and P.A. Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge: Adenovirus mRNAs.

T.R. Broker, L.T. Chow, A. Dunn, R.E. Gelinas, J. Hassell, D.F. Klessig, J. Lewis, and R.J. Roberts, Cold Spring Harbor Laboratory, New York: Novel structure at the 5′ end of Ad2 mRNA.

R.G. Roeder, C.S. Parker, J.A. Jaehning, and V.E.F. Sklar, Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Missouri: Faithful transcription of specific genes in chromatin and nuclear templates by purified eukaryotic RNA polymerases.

R.C. Huang, M.M. Smith, and A.E. Reeve, Department of Biology, Johns Hopkins University, Baltimore,

Maryland: Transcription in vitro of specific genes in isolated mouse myeloma nuclei, chromatin, and DNA.

J.O. Bishop, D.J. Bower, N.D. Hastie, M. Izquierdo, and S. Perlman, Department of Genetics, University of Edinburgh, Scotland: Measurements of genomic expression.

J. Paul and R.S. Gilmour, Beatson Institute for Cancer Research, Glasgow, Scotland: Reconstitution of functional chromatin.

FRIDAY MORNING, June 3

Session 4: Simple systems

Chairperson: D. Nathans, Johns Hopkins University, Baltimore, Maryland

W. Gilbert, Department of Molecular Biology, Harvard University, Cambridge, Massachusetts: Contact between proteins and DNA.

J. Rouvière-Yaniv, Départment de Biologie Moléculaire, Institut Pasteur, Paris, France: Characterization and functional role of a histonelike protein in prokaryotes.

B. Thimmappaya, V.B. Reddy, R. Dhar,* K.N. Subramanian,† B.S. Zain,‡ and S.M. Weissman, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; *National Institutes of Health, Bethesda, Maryland; †University of Illinois Medical School, Chicago; ‡Cold Spring Harbor Laboratory, New York: The structure of the genome of SV40.

A.J. Varshavsky, V.V. Bakayer, and G.P. Georgiev, Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow: On the structure of eukaryotic, prokaryotic, and viral chromatin.

R.L. Rill, D.A. Nelson, and W.R. Beltz, Florida State University, Tallahassee: Studies on isolated chromatin subunits from yeast.

R.M. Lawn, G. Herrick, J. Heumann, and D.M. Prescott, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Structural organization of the gene-sized pieces of DNA in the ciliate *Oxytricha*.

M. Gorovsky, D. Mathis, C. Glover, C. Johmann, M. Samuelson, and J. Keevert, Department of Biology, University of Rochester, New York: Histones, chromatin structure, and gene activity in *Tetrahymena*.

V.G. Allfrey, E.M. Johnson, I.Y.C. Sun, H.R. Matthews,* and E.M. Bradbury, Rockefeller University, New York, New York; *Portsmouth Polytechnic Institute, England: Structural organization and control of the ribosomal genes in *Physarum*.

FRIDAY EVENING, June 3

Session 5: Nucleosome structure III: The core

Chairperson: B. Alberts, University of California, San Francisco

J.O. Thomas, Department of Biochemistry, University of Cambridge, England: The nucleosome "core protein."

R.T. Simpson, J.P. Whitlock, Jr., M. Bina-Stein, and A. Stein, Developmental Biochemistry Section, NIAMDD, National Institutes of Health, Bethesda, Maryland: Histone-DNA interactions in chromatin core particles.

L.C. Lutter, MRC Laboratory of Molecular Biology, Cambridge, England: Studies on the internal structure of the nucleosome core.

A. Mirzabekov, Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow: Organization of histones on DNA in chromatin.

I. Isenberg and S. Spiker, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: The interactions of histones.

J.E. Hearst, Department of Chemistry, University of California, Berkeley: The photochemistry of psoralen derivatives with nucleic acids—A probe to structure.

A. Dieterich, R. Axel, and C.R. Cantor, Departments of Chemistry and Biological Sciences, and Institute for Cancer Research, Columbia University, New York, New York: Fluorescent labeling of nucleosomes.

L. Klevan, M.E. Hogan, N. Dattagupta, and D. Crothers, Departments of Chemistry and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Characterization of nucleosomes and related complexes by rotational relaxation and electric dichroism.

SATURDAY MORNING, June 4

Session 6: Nucleosome structure IV: H1 interactions

Chairperson: D. Crothers, Yale University, New Haven, Connecticut

P. Oudet, J.E. Germond, M. Bellard, and P. Chambon, Institut de Chimie Biologique et Laboratoire de Biologie Moléculaire des Cellules Eucaryotes de CNRS, Strasbourg, France: Nucleosome structure. I. All four histones H2A, H2B, H3, and H4 are required to form a nucleosome, but an H3-H4 subnucleosomal particle is formed with H3-H4 alone.

P. Oudet, C. Spadafora, and P. Chambon, Institut de Chimie Biologique et Laboratoire de Biologie Moléculaire des Cellules Eucaryotes de CNRS, Strasbourg, France: Nucleosome structure. II. Structure of SV40 minichromosome and electron microscopic evidence that a nucleosome could be constituted of two half nucleosomes.

J. Griffith and G. Christiansen, Department of Biochemistry, Stanford University Medical School, California: Salt- and histone-H1-dependent conformations of chromatin probed with the aid of the SV40 minichromosome.

W. Keller, I. Wendel, I. Eicken, and H. Zentgraf, Department of Microbiology, University of Heidelberg, and Institute for Virology, German Cancer Research Center, Heidelberg, West Germany: Studies on SV40 chromatin.

E.M. Bradbury, Biophysics Laboratories, Portsmouth Polytechnic Institute, England: Histone interactions, histone modifications, and chromatin structure.

M. Renz, P. Nehls, and J. Hozier, Max-Planck-Institut für Virusforschung, Tübingen, West Germany: Involvement of histone H1 in the maintenance of the structure of the chromosome fiber.

R.D. Cole, G.M. Lawson, and M.W. Hsiang, Department of Biochemistry, University of California, Berkeley: H1 histone and the condensation of chromatin and DNA.

R. Hardison, J. Gaubatz, and R. Chalkley, Department of Biochemistry, University of Iowa, Iowa City: The organization of H1 on the eukaryotic chromosome.

SATURDAY EVENING, June 4

Session 7: Cloned eukaryotic genes I

Chairperson: J. Sambrook, Cold Spring Harbor Laboratory

B. Mach, S. Longacre, M.-F. Aellen, and F. Rougeon, Department of Pathology, University of Geneva, Switzerland: The use of recombinant plasmid DNA in the study of immunoglobulin genes.

P. Leder, M.H. Edgell, F.I. Polsky, J.G. Seidman, S.M. Tilghman, and D.C. Tiemeier, Laboratory of Molecular Genetics, NICHHD, National Institutes of Health, Bethesda, Maryland: Characteristics of cloned genetic sequences expressed in mouse reticulocytes and plasmacytoma cells.

S. Tonegawa, C. Brack, M. Hirama, N. Hozumi, and G. Matthyssens, Basel Institute for Immunology, Switzerland: Arrangement of immunoglobulin genes studied with DNA clones.

T. Maniatis,[*] A. Efstratiadis,[†] G.K. Sim,[*] N. Rosenthal,[†] C.W. Jones,[†] M. Koehler,[†] B.F. Roberts,[‡] H.M. Kronenberg,[†] L. Villa Komaroff,[†] and F.C. Kafatos,[†]Division of Biology, California Institute of Technology, Pasadena; [†]Cellular and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts; [†]Biology Department, Massachusetts Institute of Technology, Cambridge: The structure and organization of developmentally regulated genes.

E. Ryskov, Y. Ilyian, and G. Georgiev, Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow: Studies on sequence arrangements in the genetic units of eukaryotes.

E.M. Southern and N.J. Hanscomb, MRC Mammalian Genome Unit, Department of Zoology, University of Edinburgh, Scotland: Restriction-site mapping of globin genes in rabbit DNA.

P. Curtis, N. Mantei, J. van den Berg, and C. Weissmann, Institute for Molecular Biology I. University of Zurich, Switzerland: Characterization of putative precursors of α and β globin mRNA from Friend cells.

W. Salser, Molecular Biology Institute, University of California, Los Angeles: Analysis of globin gene copies cloned in bacteria.

SUNDAY MORNING, June 5

Session 8: Transcription II: Regulated systems

Chairperson: M. Birnstiel, University of Zurich, Switzerland

B.W. O'Malley, M.-J. Tsai, H.C. Towle, and S.Y. Tsai, Department of Cell Biology, Baylor College of Medicine, St. Luke's Episcopal Hospital, Houston, Texas: Regulation of gene expression.

K.R. Yamamoto, M.R. Stallcup, J. Ring, and G.M. Ringold, Department of Biochemistry and Biophysics, University of California, San Francisco: Integrated mammary tumor virus genes—Transcriptional regulation by glucocorticoids and specific effects on host gene expression.

R.D. Palmiter and E.R. Mulvihill, Department of Biochemistry, University of Washington, Seattle: Steroid receptor translocation along chromatin.

F.W. Alt, R.E. Kellems, R.T. Schimke, and R. Kaufman, Department of Biological Sciences, Stanford University, California: The regulation of folate reductase synthesis in methotrexate-resistant cultured cells.

P. Feigelson and D. Kurtz, Institute of Cancer Research and Department of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York: Studies on hormonal induction of specific hepatic mRNA species.

H. Busch, N.R. Ballal, R.K. Busch, Y.C. Choi, F.M. Davis, I.L. Goldknopf, M. Rao, and L.I. Rothblum, Department of Pharmacology, Baylor College of Medicine, Houston, Texas: The nucleolus—A model of hierarchical chromatin controls.

R. Baserga, S. Whelly, and T. Ide, Department of Pathology and Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania: Stimulation of ribosomal RNA synthesis in isolated nuclei by partially purified preparations of SV40 T antigen.

H. Weideli, P. Schedl, S. Artavanis-Tsakonas, R. Steward, R. Yuan, and W.J. Gehring, Department of Cell Biology, Biozentrum, University of Basel, Switzerland: Purification of a protein with specific affinity for a defined DNA sequence from unfertilized eggs of *Drosophila* and the cloning of this DNA sequence in bacterial plasmids.

SUNDAY AFTERNOON, June 5

Session 9: Chromosome coiling

Chairperson: H. Swift, University of Chicago, Illinois

A. Worcel, C. Benyajati, and R. Deich, Department of Biochemical Sciences, Princeton University, New Jersey: Higher-order coiling of DNA in chromatin.

J. Sedat and L. Manuelidis, Yale University School of Medicine, New Haven, Connecticut: A direct approach to the structure of eukaryotic chromosomes.

S.M. Cheng, K.W. Adolph, J.R. Paulson, and U.K. Laemmli, Department of Biochemical Sciences, Princeton University, New Jersey: Metaphase chromosome structure—A study of the role of nonhistone proteins.

A. Leth Bak and J. Zeuthen,* Institutes of Medical Microbiology and *Human Genetics, University of Aarhus, Denmark: Higher-order structure of mitotic chromosomes.

M. Bustin, P.D. Kurth,* E.N. Moudrianakis,* D. Goldblatt,† R. Sperling,† and W. Rizzo, National Institutes of Health, Bethesda, Maryland; *Johns Hopkins University, Baltimore, Maryland; †Weizmann Institute of Science, Rehovot, Israel: Immunological probes for chromatin structure.

E.K.F. Bautz, M. Jamrich, and A.L. Greenleaf, Moleculare Genetik der Universität Heidelberg, West Germany: Functional organization of *Drosophila* polytene chromosomes.

H. Weintraub, I.M. Leffak, and R. Grainger,* Department of Biochemistry, Princeton University, New Jersey; *Department of Biology, University of Virginia, Charlottesville: Nonrandom assembly of newly synthesized histones into histone octamers.

K.M. Newrock and L.H. Cohen, Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania: Chromatin remodeling during sea urchin embryogenesis.

R.L. Seale, Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, and Department of Biophysics and Genetics, University of Colorado School of Medicine, Denver: Chromatin replication in vitro.

Session 10: Transcription III: In vivo pathways

Chairperson: C. Weissmann, University of Zurich, Switzerland

M.E. Mirault, M. Goldschmidt-Clermont, L. Moran, A.P. Arrigo, and A. Tissières, Department of Molecular Biology, University of Geneva, Switzerland: The effect of heat shock on gene expression in *D. melanogaster*.

H. Biessmann, S. Wadsworth, B. Levy W., and B.J. McCarthy, Department of Biochemistry and Biophysics, University of California, San Francisco: Correlation of structural changes in chromatin with transcription in the *Drosophilia* heat-shock response.

S.C.R. Elgin, R. De Lorimier, D.W. Miller, A.A. Ribolini, L.A. Serunian, L.M. Silver, and C.E.C. Wu, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Structure and function in *Drosophila* chromatin.

J.J. Bonner, M. Berninger, and M.L. Pardue, Department of Biology, Massachusetts Institute of Technology, Cambridge: Transcription of polytene chromosomes and the mitochondrial genome in *D. melanogaster*.

B. Daneholt, S.T. Case, J. Derksen, M.M. Lamb, L. Nelson, and L. Wieslander, Department of Histology, Karolinska Institutet, Stockholm, Sweden: The 75S RNA transcription unit in Balbiani ring 2.

J.-E. Edström, E. Ericson, S. Lindgren, U. Lönn, and L. Rydlander, Department of Histology, Karolinska Institutet, Stockholm, Sweden: Fate of Balbiani ring RNA in vivo.

W.M. Le Stourgeon, A.L. Beyer, M.E. Christensen, B.W. Walker, S.M. Poupore, and L.P. Daniels, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: The packaging proteins of "core" hnRNP particles and the maintenance of proliferative cell states.

T. Martin, P. Billings, and H. Swift, Department of Biology, University of Chicago, Illinois: Protein core complexes associated with newly synthesized RNA in the nuclei of higher eukaryotes.

MONDAY EVENING, June 6

Session 11: Transcriptionally active chromatin

Chairperson: O.L. Miller, Jr., University of Virginia, Charlottesville

A. Garel and R. Axel, Institute of Cancer Research, Columbia University, New York, New York: The structure of transcribing genes in chromatin.

R. Reeves, Department of Zoology, University of British Columbia, Vancouver, Canada: Structure of *Xenopus* ribosomal gene chromatin during changes in genomic transcription rates.

V.E. Foe, Department of Biochemistry and Biophysics, University of California, San Francisco: Chromatin structure and transcriptional activity—An electron microscopic study of RNA synthesis.

S.L. McKnight,* M. Bustin,† and O.L. Miller, Jr.,* *University of Virginia, Charlottesville; †National Institutes of Health, Bethesda, Maryland: Ultrastructural and antigenic properties of metabolically active chromatin.

W.W. Franke, U. Scheer, M.F. Trendelenburg, H. Zentgraf, and H. Spring, Division of Membrane Biology and Biochemistry, Institute of Experimental Pathology, German Cancer Research Center, Heidelberg, West Germany: Morphology of transcriptionally active chromatin.

M. Bellard, F. Gannon, and P. Chambon, Institut de Chimie Biologique et Laboratoire de Biologie Moléculaire des Cellules Eucaryotes du CNRS, Strasbourg, France: Nucleosome structure. III. Are actively transcribed genes compacted in nucleosomes?

G.H. Dixon, B. Levy W., N.C.W. Wong, E.H. Peters, and D.C. Watson, Division of Medical Biochemistry, Faculty of Medicine, University of Calgary, Canada: The structure and function of low-salt-extractable chromosomal proteins—Association of trout testis H6 with active genes.

J. Bonner, Division of Biology, California Institute of Technology, Pasadena: New insight into control of gene expression.

S.J. Flint, Department of Biochemical Sciences, Princeton University, New Jersey: Sensitivity of viral genes in adenovirus-transformed cell chromatin to deoxyribonuclease I.

TUESDAY MORNING, June 7

Session 12: Cloned eukaryotic genes II

Chairperson: D. Brown, Carnegie Institution of Washington, Baltimore, Maryland

A. Chovnick, M. McCarron, W. Gelbart, J. O'Donnell, S. Clark, and A. Hilliker, Genetics and Cell Biology Section, University of Connecticut, Storrs: Gene organization in *Drosophila*.

M.M. Green, Department of Genetics, University of California, Davis: Directed induction of putative insertion mutants in *D. melanogaster*.

S.S. Potter and C.A. Thomas, Jr., Harvard Medical School, Boston, Massachusetts: Restriction analysis of the organization of *Drosophila* DNA.

P.C. Wensink, Department of Biochemistry and the Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Sequence homology within families of *D. melanogaster* middle repetitive DNA.

G.M. Rubin, Department of Basic Sciences, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Structure of telomeric DNA in *D. melanogaster*.

DJ. Finnegan, G.M. Rubin,* M. Young,† and D.S. Hogness,† MRC Molecular Genetics Unit, University of Edinburgh, Scotland; *Sidney Farber Cancer Institute, Boston, Massachusetts; †Department of Biochemistry, Stanford University School of Medicine, California: Multigene families in the genome of *D. melanogaster*.

D.S. Hogness, Department of Biochemistry, Stanford University School of Medicine, California: Dispersed and tandem topographies of repeated genes.

E.H. Davidson, R.C. Angerer, W.H. Klein, C. Lai, A.S. Lee, W.D. Niles, T.L. Thomas, R.H. Scheller, and R.J. Britten, Division of Biology, California Institute of Technology, Pasadena: Organization and character of interspersed repetitive-sequence families studied in cloned sea urchin DNA fragments.

TUESDAY AFTERNOON, June 7

Session 13: Histone genes

Chairperson: C.A. Thomas, Jr., Harvard Medical School, Boston, Massachusetts

M.L. Birnstiel, W. Schaffner, A. Kressmann, and H.O. Smith, Institute for Molecular Biology, University of Zurich, Switzerland: Sequence organization and transcription in the *Xenopus* oocyte nucleus of cloned histone DNA.

L.H. Kedes, R.H. Cohn, I. Sures, and J. Lowry, Department of Medicine, Stanford Medical School, and Veterans Administration Hospital, Palo Alto, California: Comparative anatomy of histone genes from two sea urchin species.

M. Grunstein, J.E. Grunstein, and T. Platt,* Molecular Biology Institute and Department of Biology, University of California, Los Angeles; *Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Sequence of the histone H4 gene of the sea urchin *S. purpuratus*.

G. Stein, J. Stein,* W. Park, J. Thomson, R. Jansing, S. Detke, D. Kota, A. Lichtler, I. Phillips, and A. Phillips, Department of Biochemistry and Molecular Biology and *Department of Immunology and Medical Microbiology, University of Florida, Gainesville: Nonhistone chromosomal protein fractions and transcription of histone mRNA sequences from chromatin.

R.A. Laskey, B.M. Honda, A.D. Mills, N.R. Morris, A.H. Wyllie, J.E. Mertz, E.M. De Robertis, and J.B. Gurdon, MRC Laboratory of Molecular Biology, Cambridge, England: Chromatin assembly and transcription in eggs and oocytes of *X. laevis*.

WEDNESDAY MORNING, June 8

Session 14: Repeated DNA sequences

Chairperson: J.G. Gall, Yale University, New Haven, Connecticut

W.J. Peacock, A.R. Lohe, W.L. Gerlach, P. Dunsmuir,* E.S. Dennis, and R. Appels, Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia; *The Biological

Laboratories, Harvard University, Cambridge, Massachusetts: Fine structure and evolution of DNA in heterochromatin.

D. Brutlag, M. Carlson, K. Fry, and T.S. Hsieh, Department of Biochemistry, Stanford University, California: DNA sequence organization in the pericentric heterochromatin of *D. melanogaster*.

P.R. Musich, F.L. Brown, and J.J. Maio, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Mammalian repetitive DNA and the subunit structure of chromatin.

R.H. Reeder, H.L. Wahn, P. Botchan, and B. Sollner-Webb, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Ribosomal genes and associated proteins from *Xenopus*.

I.B. Dawid, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: *Drosophila* ribosomal DNA—Relation of the insertion sequence to nonribosomal parts of the genome.

N.V. Fedoroff and D.D. Brown, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: The nucleotide sequence of the repeating unit in *X. laevis* oocyte 5S DNA.

Summary: P. Chambon, Institut de Chimie Biologique, Strasbourg, France: The molecular biology of the eukaryotic genome is coming of age.

SUMMER MEETINGS

PARVOVIRUSES

arranged by
DAVID C. WARD, *Yale University*

48 participants

THURSDAY EVENING, May 12

Welcoming remarks: D.C. Ward, Yale University

Session 1: Biology and parvovirus-cell interactions

Chairperson: P. Tattersall, Imperial Cancer Research Fund, Mill Hill, London, England

G. Margolis and L. Kilham, Departments of Pathology and Microbiology, Dartmouth Medical School, Hanover, New Hampshire: The parvoviruses—Pantropes or selective cytotropes.

H.W. Toolan, Putnam Memorial Hospital Institute for Medical Research, Bennington, Vermont: The maternal role in susceptibility of the embryo and newborn hamster to H-1 parvovirus.

M.D. Hoggan, J.F. Sears, G.F. Thomas, and A. Roy, National Institutes of Health, Bethesda, Maryland: Biological properties of rat virus variants.

P. Tattersall, Imperial Cancer Research Fund, Mill Hill Laboratories, London: Susceptibility to MVM as a function of host-cell differentiation.

P. Linser and R.W. Armentrout, Department of Biological Chemistry, University of Cincinnati Medical Center, Ohio: Binding of MVM to cells in culture.

H.S. Joo, R.H. Johnson, and D.L. Watson, Department of Tropical Veterinary Science, James Cook University of North Queensland, Australia: Serological procedures to determine duration of infection with porcine parvoviruses.

B.A. Federici, Department of Entomology. University of California, Riverside: A parvovirus in larvae of the blackfly *Simulium vittatum*.

L. Wosu, R.H. Johnson, and S. Locarnini, Department of Tropical Veterinary Science, James Cook University of North Queensland, Australia: Attempts to demonstrate parvovirus in fecal specimens from patients with infectious hepatitis.

FRIDAY MORNING, May 13

Session 2: Structure of parvovirus DNA

Chairperson: K. Berns, Department of Microbiology and Immunology, University of Florida, Gainesville

K.I. Berns, K.H. Fife, and I. Spear, Department of Immunology and Medical Microbiology, University of Florida, Gainesville: Fine structure of AAV DNA.

L.M. de la Maza, F.T. Jay, and B.J. Carter, National Institutes of Health, Bethesda, Maryland: Structure of the DNA from incomplete AAV particles.

M.B. Chow and D.C. Ward, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Comparative studies on the DNA genomes of non-defective parvoviruses.

G. Lavelle, S.K. Niyogi, and S. Mitra, Biology Division, Oak Ridge National Laboratory, Tennessee: Double-helical regions in Kilham rat virus DNA.

D.C. Kelly, National Environment Research Council, Unit of Invertebrate Virology, Oxford, England: The fine structure of densonucleosis virus DNA.

N. Blacklow, Department of Medicine and Microbiology, University of Massachusetts Medical School, Worcester: Interaction of AAV with herpesvirus-transformed cells.

FRIDAY AFTERNOON, May 13

Meeting to discuss *Restriction enzyme nomenclature for parvovirus physical maps*

FRIDAY EVENING, May 13

Session 3: Replication of adeno-associated viruses (AAV)

Chairperson: W. Hauswirth, Department of Microbiology and Immunology, University of Florida, Gainesville

W.W. Hauswirth and K.I. Berns, Department of Immunology and Medical Microbiology, College of Medicine, University of Florida, Gainesville: Initiation and termination of AAV DNA replication.

S.E. Straus, E.D. Sebring, and J.A. Rose, Washington University, St. Louis, Missouri: Self-primed replication of AAV DNA.

S.E. Straus, R.M.L. Buller,* and J.A. Rose,* Washington University, St. Louis, Missouri; *National Institutes of Health, Bethesda, Maryland: AAV replication in African green monkey kidney cells.

H.D. Mayor, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas: Complementation of AAV by temperature-sensitive mutants of human adenoviruses and herpesviruses.

H. Handa, K. Shiroki, and H. Shimojo, Department of Tumor Virus Research, Institute of Medical Science, University of Tokyo, Japan: Establishment and characterization of KB cell lines latently infected with AAV-1.

J.F. Young and H.D. Mayor, Baylor College of Medicine, Houston, Texas: The effect of phosphonoacetic acid on the replication of adeno-associated satellite virus.

SATURDAY MORNING, May 14

Session 4: DNA replication of nondefective parvoviruses

Chairperson: S. Rhode, Institute of Medical Research, Putnam Memorial Hospital, Bennington, Vermont

S.L. Rhode, Institute for Medical Research, Putnam Memorial Hospital, Bennington, Vermont: H-1 DNA synthesis.

I.I. Singer and S.L. Rhode III, Institute for Medical Research, Putnam Memorial Hospital, Bennington, Vermont: Immuno-electron microscopic autoradiography of H-1 parvovirus DNA synthesis and protein accumulation.

A.T. Li, G.C. Lavelle, and R.W. Tennant, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, and Biology Division, Oak Ridge National Laboratory, Tennessee: Guanidine hydrochloride extraction of intracellular forms of Kilham rat virus DNA.

L. Salzman, Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: In vitro DNA synthesis with Kilham rat virus.

M. Gunther and B. Revet, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Study of Kilham rat virus nucleoprotein complexes extracted from infected rat cells.

G.S. Hayward, H. Bujard, and M. Gunther,* Institute for Molecular Genetics, University of Heidelberg, Germany; *Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Replicative forms of Kilham rat virus DNA.

G. Siegl and M. Gautschi, Institute of Hygiene and Medical Microbiology, University of Berne, Switzerland: Isolation and characterization of RF and RI DNA molecules of parvovirus LuIII.

D. Ward, M. Chow, and J. Idriss, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Studies on the replication of MVM DNA.

R.C. Bates, C.P. Kuchenbuch, J.T. Patton, and E.R. Stout, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg: DNA polymerase activity in parvovirus-infected cells.

SATURDAY AFTERNOON, May 14

Session 5: Transcription, translation and properties of viral proteins

Chairperson: B. Carter, Laboratory of Experimental Pathology, NIAMDD, National Institutes of Health, Bethesda, Maryland

B.J. Carter, F.T. Jay, C.A. Laughlin, W.J. Cook, and L.M. de la Maza, National Institutes of Health, Bethesda, Maryland: AAV RNA synthesis in vivo and in vitro.

F.B. Johnson, D.A. Vlazny, T.A. Thomson, P.A. Taylor, and M.D. Lubeck, Department of Microbiology, Brigham Young University, Provo, Utah: AAV polypeptides—Molecular similarities.

J.L. Peterson and D.C. Ward, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Comparison of parvovirus capsid proteins—Evidence for posttranslational modification.

D.C. Kelly and N.F. Moore, National Environment Research Council, Unit of Invertebrate Virology, Oxford, England: The structural proteins of densonucleosis virus.

E. Kurstak and P. Tijssen, Comparative Virology Research Group, Université de Montréal, Canada: Study of DNA and proteins of densonucleosis parvovirus.

A. Roy, G.F. Thomas, J.F. Sears, and M.D. Hoggan, National Institutes of Health, Bethesda, Maryland: Use of immuno-electron microscopy to demonstrate antigenic and structural differences between infectious AAV particles banding at different densities in cesium chloride.

SUNDAY MORNING, May 15

Session 6: Virus maturation and morphogenesis

Chairperson: R. Armentrout, Department of Biological Chemistry, University of Cincinnati, Ohio

R. Richards, P. Linser, and R.W. Armentrout, Department of Biological Chemistry, University of Cincinnati Medical Center, Ohio: Assembly of MVM particles in synchronized rat brain cells.

M. Gautschi, P. Reinhard, J.R. Gautschi, and G. Siegl, Institute of Hygiene and Medical Microbiology, University of Berne, Switzerland: Maturation of parvovirus LuIII in Brij-58-lysed cells.

H.P. Müller, M. Gautschi, and G. Siegl, Institute of Hygiene and Medical Microbiology, University of Berne, Switzerland: Defective particles of parvovirus LuIII.

S.L. Rhode, Institute for Medical Research, Putnam Memorial Hospital, Bennington, Vermont: A preliminary report on the defective interfering viruses of H-1.

I.I. Singer and S.L. Rhode III, Institute for Medical Research, Putnam Memorial Hospital, Bennington, Vermont: Immuno-electron microscopy of H-1 parvovirus temperature-sensitive mutants.

S.L. Rhode, I.I. Singer, and J.R. Kongsvik, Institute for Medical Research, Putnam Memorial Hospital, Bennington, Vermont: The abortive infection of human diploid fibroblasts by the parvovirus H-1.

J.R. Kongsvik, M.S. Hopkins, and K.A.O. Ellem, Institute for Medical Research, Putnam Memorial Hospital, Bennington, Vermont: Two populations of virus produced during H-1 infection of synchronized human chondrosarcoma cells.

THE TRANSFORMED CELL

arranged by
ROBERT POLLACK, *State University of New York, Stony Brook*
RICHARDS HYNES, *Massachusetts Institute of Technology*
LAN BO CHEN, *Cold Spring Harbor Laboratory*

131 participants

WEDNESDAY EVENING, May 18

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

Session 1: Tumors—Transformation in vivo

Chairperson: G. di Mayorca, University of Illinois Medical Center, Chicago

V.H. Freedman, A.S. Klein, M. Greenberg, P.K. Canary, and S. Shin, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Use of the nude mouse to assay for cell transformation.

J.K. McDougall, L.B. Chen, and P.H. Gallimore, Cold Spring Harbor Laboratory, New York: In vitro traits of adenovirus-transformed cell lines and their relevance in tumorigenicity in nude mice.

L. Kopelovich,* S. Conlan,† and R. Pollack,† *Memorial Sloan-Kettering Cancer Center, New York, New York; †Microbiology Department, State University of New York, Stony Brook: Defective organization of actin in cultured skin fibroblasts from individuals with an inherited adenocarcinoma.

C.W. Boone and N. Takeichi, NCI, National Institutes of Health, Bethesda, Maryland: Spontaneous neoplastic transformation in vitro is a modified form of foreign-body tumorigenesis.

M.S. Paranjpe and C.W. Boone, NCI, National Institutes of Health, Bethesda, Maryland: Correlation of growth aggressiveness of tumors formed by substrate-attached C3H/10T1/2 cells with degree of loss of density, anchorage, and serum regulation in vitro.

W.C. Topp, J.D. Hall,* D. Rifkin,† A.J. Levine‡ B. Lane, § S. Shin,** and R. Pollack,‡ Cold Spring Harbor Laboratory, New York; *University of Arizona, Tucson; †Rockefeller University, New York, New York; ‡Princeton University, New Jersey; §Department of Pathology and ‡Department of Microbiology, State University of New York, Stony Brook, **Albert Einstein College of Medicine, Bronx, New York: Properties of mouse teratocarcinoma cell lines cloned by SV40 establishment.

S. Silagi, E. Newcomb, and S. Silverstein,* Cornell University Medical College, New York, New York; *Rockefeller University, New York, New York: Loss of tumor-forming ability by malignant melanoma cells mixed with nonmalignant BrdU-grown derivatives.

THURSDAY MORNING, May 19

Session 2A: Transformed cells—Induction and maintenance

Chairperson: R. Hynes, Massachusetts Institute of Technology, Cambridge

N. Bouck and G. di Mayorca, Department of Microbiology, University of Illinois at the Medical Center, Chicago: The mutagenic nature of in vitro transformation by chemicals.

J. Kamine and J.M. Buchanan, Department of Biology, Massachusetts Institute of Technology, Cambridge: Cell-free synthesis of two proteins unique to RNA of transforming virions of Rous sarcoma virus.

C. Prives, Y. Gluzman, M. Revel, and E. Winocour, Virology Department, Weizmann Institute of Science, Rehovot, Israel: The cellular and cell-free synthesis of SV40 T antigen in transformed and lytic state.

D. Kiehn, Department of Microbiology and Immunology, University of Washington, Seattle: Mechanisms of transformation in rat embryo cells.

D. Zouzias and C. Basilico, Department of Pathology, New York University School of Medicine, New York: T-antigen expression in SV40-transformed cells.

B. Steinberg, R. Pollack, K. Nyman, A. Lo, W. Topp,* and M. Botchan,* Department of Microbiology, State University of New York, Stony Brook; *Cold Spring Harbor Laboratory, New York: T-antigen-negative revertants from transformed rat cells carrying one copy of SV40 DNA.

N.J. Maitland and J.K. McDougall, Cold Spring Harbor Laboratory, New York: Biochemical transformation of mouse cells by fragments of herpes simplex virus DNA.

H.L. Ozer and K.K. Jha, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Expression of transformation in cell hybrids.

R. Sager, N. Howell, and P. Hoover, Sidney Farber Cancer Institute, Boston, Massachusetts: Cellular transformation in Chinese hamster cell hybrids and cybrids.

THURSDAY AFTERNOON, May 19

Session 2B: Transformed cells—Induction and maintenance

Chairperson: R. Sager, Sidney Farber Cancer Institute, Boston, Massachusetts

P.B. Fisher, D. Eisenberg, and I.B. Weinstein, Institute for Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York: Interaction between an initiating agent carcinogen, a promoting agent, and adenovirus in the transformation of rat embryo cells.

P.E. Driedger and P.M. Blumberg, Department of Pharmacology, Harvard Medical School, Boston, Massachusetts: Effects of phorbol myristate acetate and related derivatives on chick embryo fibroblasts.

J. Mather and G. Sato, Department of Biology, University of California, San Diego: Hormone requirements of a clonal line of the B-16 mouse melanoma.

R.R. Bürk, Friedrich Miescher-Institut, Basel, Switzerland: Is the progression of transformation the progressive derepression of the production of growth factors?

R. Ross, J. Glomset, E. Raines, B. Kariya, and A. Vogel, Department of Pathology, University of Washington School of Medicine, Seattle: Platelet mitogens and growth control.

C.D. Scher, C.D. Stiles, and H.N. Antoniades, Harvard Medical School and the Center for Blood Research, Harvard University School of Public Health, Boston, Massachusetts: Radioimmunoassay of a human serum growth factor polypeptide which induces cell proliferation.

C.D. Stiles, J.W. Pledger, H. Antoniades, and C.D. Scher, Harvard Medical School and the Center for Blood Research, Harvard University School of Public Health, Boston, Massachusetts: Growth requirement of normal and neoplastic cell cultures for platelet factors.

W.J. Pledger, C.D. Stiles, H. Antoniades, and C.D. Scher, Harvard Medical School and the Center for Blood Research, Harvard University School of Public Health, Boston, Massachusetts: The commitment of BALB/c 3T3 cells to DNA synthesis.

THURSDAY EVENING, May 19

Session 3: Phenotypes of transformed cells–Maintenance

Chairperson: H. Ozer, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

H. Rubin, D. Moscatelli, and J. Kamine, Department of Molecular Biology, University of California, Berkeley: The coordinate response of chick embryo fibroblasts to external stimuli and to viral transformation.

R.D. Dubrow, R. Pollack,* and A.B. Pardee, Sidney Farber Cancer Institute, Boston, Massachusetts; *State University of New York, Stony Brook: Transport rates by 3T3, SV3T3 and revertant lines under various growth conditions.

W. Heiser and E. Englesberg, Biochemistry and Molecular Biology Section, Department of Biological Sciences, University of California, Santa Barbara: Amino acid sensitivity and SV40 transformants—Characteristics of methionine-resistant SV40 3T3 (SVT2).

M.J. Weber, K. Nakamura, and M. Johnson, Department of Microbiology, University of Illinois, Urbana: Functional membrane alterations in cells transformed by Rous sarcoma virus.

D. Paul and H.-J. Ristow, Cell Biology Laboratory, The Salk Institute, San Diego, California: Cell-cycle control by Ca^{++} ions in 3T3 cells and in transformed 3T3 cells.

J. Fenno and E. Gurney, Department of Biology, University of Utah, Salt Lake City: SV40 transformation and cAMP-dependent protein kinases.

D.R. Critchley, M. Vicker, S. Dilks, and J. Ingram, Department of Biochemistry, University of Leicester, England: The effect of cholera toxin on serum-stimulated uridine uptake in mouse fibroblasts.

M. Rieber, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela: On the conditions promoting the expression of nonvirion oncornaviruslike glycoproteins in transformed cells.

FRIDAY MORNING, May 20

Session 4A: Cytoskeleton

Chairperson: D. Rifkin, Rockefeller University, New York, New York

R.W. Tucker and F.R. Frankel, NCI, National Institutes of Health, Bethesda, Maryland: Microtubules and actin cables in neoplastic and non-neoplastic rodent cell lines—Indirect immunofluorescence.

H.S. Smith, A.J. Hiller, E.L. Springer, R.E. Pollack,* and D. Rifkin,† University of California, Berkeley; *State University of New York, Stony Brook; †Rockefeller University, New York, New York: In vitro characterization of human epithelial cells.

K. Weber and M. Osborne, Max Planck Institut für Biologisch Chemie, Goettingen, West Germany: Studies on microtubule system by indirect immunofluorescence.

G.M. Fuller and C.S. Artus, University of Texas, Galveston: Involvement of cAMP and a possible role of calcium in the in vivo control of microtubule assembly.

G. Albrecht-Buehler, Cold Spring Harbor Laboratory, New York: Predetermination of movement in 3T3 cells.

E. Lazarides, B.D. Hubbard, and J.G. Izant, Division of Biology, California Institute of Technology, Pasadena: Immunological and biochemical characterization of the major subunit of the 100-Å filaments from chick muscle cells.

R.D. Goldman and J.M. Starger, Biological Sciences Department, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Rapid isolation and purification of 10-nm filaments from cultured cells.

J.M. Stargér and R.D. Goldman, Biological Sciences Department, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Analysis of 10-nm-filament-associated proteins from cultured cells.

R. Hynes and A. Destree, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge: 100-Å filaments in normal and transformed cells.

P. Cooke, Department of Physiology, University of Connecticut Health Center, Farmington: A filamentous cytoskeleton in vertebrate smooth muscle fibers.

FRIDAY AFTERNOON, May 20

Session 4B: Cytoskeleton

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

V. Mautner and R. Hynes, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge: Surface distribution of LETS protein in relation to the cytoskeleton of normal and transformed NIL8 hamster fibroblasts.

K. Wang, Clayton Foundation Biochemical Institute, Department of Chemistry, University of Texas, Austin: Filamin, molecular properties, interaction with F-actin, and immunocytological localization.

J.F. Ash, D. Louvard, and S.J. Singer, Department of Biology, University of San Diego, California: Cytoskeletal-membrane interaction.

R. Pollack, Department of Microbiology, State University of New York, Stony Brook: Actin-containing cables within normal rat embryo cells require a divalent cation.

W. Gordon and A. Bushnell, Cold Spring Harbor Laboratory, New York: Myosin and 100-Å filament localiza-

tion in a nonmuscle cell type—A combined immunofluorescent electron microscopic study.

R. Schlegel and T. Benjamin, Department of Pathology, Harvard Medical School, Boston, Massachusetts: Morphological alteration of rat cells by wild-type and HR-T mutants of polyoma virus.

M.C. Willingham, K.M. Yamada, S.S. Yamada, J. Pouyssegur, and I. Pasten, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Microfilament bundles and cell shape are dissociable from growth control in cultured fibroblasts.

T. Graf and H. Beug, Max-Planck-Institut für Virusforschung, Biologisch-Medizinische Abteilung, Tübingen, West Germany: Cell transformation induced in enucleated cells infected with a thermosensitive mutant of Rous sarcoma virus.

J.C. Brown and M.L. Nagpal, Department of Microbiology, University of Virginia School of Medicine, Charlottesville: Identification of myosin associated with purified phagosome membranes from mouse L cells.

FRIDAY EVENING, May 20

Session 5: Proteases and cell proliferation

Chairperson: A. Pardee, Sidney Farber Cancer Institute, Boston, Massachusetts

S.T. Rohrlich and D.B. Rifkin, Department of Chemical Biology, Rockefeller University, New York, New York: Patterns of plasminogen activator production in cultured normal embryonic cells.

B.A. Wolf and A.R. Goldberg, Rockefeller University, New York, New York: Elevation of plasminogen activator levels by the *src* gene of Rous sarcoma virus can be either cellularly or virally determined.

R.H. Goldfarb and J.P. Quigley, Department of Microbiology and Immunology, State University of New York, Downstate Medical Center, Brooklyn: Studies on the nature of the membrane-associated form of plasminogen activator—Comparison with the soluble, extracellular form.

V. Mahdavi and R. Hynes, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge: Membrane-bound proteases in normal and transformed cells.

D. Rifkin and R. Pollack,* Rockefeller University, New York, New York; *Department of Microbiology, State University of New York, Stony Brook: The production of plasminogen activator by established cell lines of mouse origin.

I.N. Chou, C. Dietrich, and P.H. Black, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts: Calcium stimulation of plasminogen activator secretion by Swiss 3T3 cells.

M.H. Park, J.G. Burr, and J.M. Buchanan, Department of Biology, Massachusetts Institute of Technology, Cambridge: Binding and internalization of [^{125}I]thrombin to chick embryo fibroblasts.

B.M. Martin and J.P. Quigley, Department of Microbiology and Immunology, Downstate Medical Center, State University of New York, Brooklyn: Thrombin-induced mitogenesis—Binding and uptake of [^{125}I]thrombin in chick fibroblasts.

B.R. Zetter and D. Gospodarowicz, Cancer Research Institute, University of California Medical School, San Francisco: Effects of thrombin, fibroblast growth factor, and epidermal growth factor on endothelial cell proliferation.

D.D. Cunningham and D.H. Carney, Department of Medical Microbiology, College of Medicine, University of California, Irvine: Initiation of chick cell division by trypsin action at the cell surface.

V.B. Hatcher and P.G. Burk, Department of Biochemistry and Medicine, Albert Einstein College of Medicine, and Montefiore Hospital and Medical Center, Bronx, New York: The relationship between surface protease activity and the rate of cell proliferation in normal and transformed cells.

H.B. Bosmann, I. Evans, and A.C. Spataro, Department of Pharmacology and Toxicology and University of Rochester Cancer Center, University of Rochester School of Medicine and Dentistry, New York: Proteolytic activity—Does it have a role in control of cell division?

R. Roblin,* M. Fitzsimmons, T. Rakusanova, and P.H. Black, Infectious Disease Unit, Massachusetts General Hospital, and Departments of Microbiology and Molecular Genetics, Harvard Medical School, Boston: SV40-transformed 3Y1 cells: Mycoplasma curing and resultant properties.

SATURDAY MORNING, May 21

Session 6: Major cell-surface glycoproteins I: Biochemistry

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

M.W. Mosesson, A.B. Chen, and D.L. Amrani, Department of Medicine, Downstate Medical Center, State University of New York, Brooklyn: Heterogeneity of the cold-insoluble globulin of human plasma (Clg), a circulating cell surface protein (LETS).

A. Vaheri, D.F. Mosher, K. Hedman, J. Keski-Oja, M. Kurkinen, O. Saksela, S. Stenman, and J. Wartiovaara, University of Helsinki, Finland: Fibronectin in cultures of normal and transformed adherent cells.

R. Hynes, A. Destree, and I.U. Ali, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge: Extensive disulfide bonding at the cell surface.

L.B. Chen, K. Burridge, P.H. Gallimore, R. Gudor, J.K. McDougall, and M.W. Mosesson,* Cold Spring Harbor Laboratory, New York; *Downstate Medical Center, State University of New York, Brooklyn: Studies on the major cell-surface glycoprotein.

L.B. Chen, A. Murray, A. Bushnell, and J.K. McDougall, Cold Spring Harbor Laboratory, New York: Alteration in extracellular matrix upon oncogenic transformation.

K.M. Yamada, D.H. Schlessinger, D.W. Kennedy, and I. Pastan, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of the transformation-sensitive cell-surface protein of chick embryo fibroblasts.

K.M. Yamada and K. Olden, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Quantitation of a transformation-sensitive cell-surface glycoprotein—Mechanism of decrease after transformation.

S.L. Adams, M.E. Sobel, B.H. Howard, K. Olden, K.M. Yamada, B. de Crombrugghe, and I. Pastan, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Reduction in functional mRNA for CSP and for collagen in Rous sarcoma virus-transformed chick embryo fibroblasts.

D. Becker, R. Kurth, D. Critchley, R.R. Friis, and H. Bauer, Institut für Virologie, Giessen, West Germany, and Imperial Cancer Research Fund Laboratories, London, England: Significance of transformation-associated cell-surface changes as investigated with two groups of temperature-sensitive, transformation-defective Rous sarcoma virus mutants.

E. Wang, and A.R. Goldberg, Rockefeller University, New York, New York: Analysis of concurrent changes of microfilament organization and LETS protein distribution in mammalian cells infected with a temperature-sensitive mutant of Rous sarcoma virus.

SATURDAY AFTERNOON, May 21

Session 7: Major cell-surface glycoproteins II: Reconstitution and secretion

Chairperson: J.M. Buchanan, Massachusetts Institute of Technology, Cambridge

J.A. Weston, K.M. Yamada, and K.L. Hendricks, Department of Biology, University of Oregon, Eugene: Factor(s) in culture supernatants affecting cell social behavior.

I.U. Ali, V. Mautner, and R. Hynes, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge: Effects of addition of LETS protein to transformed cells.

L.I. Gold and E. Pearlstein, Irvington House Institute, Department of Pathology, New York University Medical Center, New York: Studies on the nature and mechanism of cell adhesion factor (CAF)-mediated attachment.

M. Imada, L.M. Fink, and S.M. Clarke, Department of Pathology, University of Colorado Medical School, Denver: Heterogeneity of the 220,000 m.w. proteins of syrian hamster cells as analyzed by two-dimensional electrophoresis.

R. Nairn, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York: The cell-surface glycoproteins of normal, transformed, and variant fibroblasts.

R.C. Hughes, S. Rosen, A. Meager, R. Nairn, and J. Garrett, National Institute for Medical Research, Mill Hill Laboratories, London, England: LETS glycoprotein of normal and transformed fibroblasts.

Y.M. Plesser and F. Doljanski, Department of Experimental Medicine and Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel: The shedding of glucosamine-labeled cell-surface components and the possible role of a large surface glycoprotein in intercellular associations.

E. Ruoslahti and E. Engvall, Division of Immunology, City of Hope National Medical Center, Duarte, California: Binding of fibroblast surface protein, fibronection, to collagen.

C.H. Damsky, D.W. Wylie, and C.A. Buck, The Wistar Institute, Philadelphia, Pennsylvania: Cytoskeletal changes in BHK cells treated with anti-surface-membrane serum.

D.F. Mosher and A. Vaheri, *University of Wisconsin, Madison; *University of Helsinki, Finland: Secretion and catabolism of alpha-2-macroglobulin (α_2M) by cultured fibroblasts.

M.M. Gottesman, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Transformation-dependent secretion of a 33,000 m.w. protein by mouse cells.

SUNDAY MORNING, May 22

Session 8A: Membrane alterations

Chairperson: P.W. Robbins, Massachusetts Institute of Technology, Cambridge

B. Whittenberger and L. Glaser, Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Missouri: Control of DNA synthesis by plasma membranes.

R.J. Mannino, K. Ballmer, and M.M. Burger, Department of Biochemistry, Biozentrum, University of Basel, Switzerland: Cell-surface modulation and growth inhibition of transformed cells.

J.F. Perdue, Lady Davis Institute, Jewish General Hospital, Montreal, Canada: Loss of regulation of insulin binding but not thrombin binding in virus-transformed cells and cultured avian and mammalian tumor cells.

L.A. Smets, The Netherlands Cancer Institute, Amsterdam: Membrane glycopeptides of transformed and neoplastic mouse 3T3 cells.

R.M. Evans and L.M. Fink, Department of Pathology, University of Colorado Medical School, Denver: Identification of transmembrane bridging proteins in plasma membrane of cultured mouse L cells.

J.R. Sheppard, A.J. Faras, and C.M. Moldow, University of Minnesota, Minneapolis: Decreased catecholamine hormone receptors in transformed cells.

R.P.C. Shiu, J. Pouyssegur, and I. Pastan, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous sarcoma virus-transformed chick embryo fibroblasts.

D.E. Wylie, C.H. Damsky, and C.A. Buck, The Wistar Institute, Philadelphia, Pennsylvania: Effect of anti-surface-membrane serum on morphology and growth properties of normal and transformed BHK cells.

P.E. Branton and J. Landry-Magnan, Department of Pathology, McMaster University, Hamilton, Ontario, Canada: Plasma membrane protein phosphorylation and transformation.

H. Bauer, J. Ignajatović, H. Rüosamen, M. Hayami, and R.R. Friis, Institut für Virologie, Geissen, West Germany: Immunological and biochemical studies on tumor cell-surface-associated proteins.

SUNDAY AFTERNOON, May 22

Session 8B: Membrane alterations

Chairperson: J. Weston, University of Oregon, Eugene

A.B. DeLeo, H. Shiku, T. Takahashi, M. John, and L.J. Old, Sloan-Kettering Institute, New York, New York: Cell-surface antigens of murine fibroblasts and sarcoma cells.

E.A. Davidson, V.P. Bhavanandan, and J. Banks, Department of Biological Chemistry, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey: Complex saccharides of cultured mammalian cells.

G.A. Molinaro, E.O. Major, G. Bernhardt, S. Dray, and G. di Mayorca, Department of Microbiology, University of Illinois Medical Center, Chicago: Similar cell-surface antigens on hamster cells transformed by different polyoma viruses.

D. Kyner, P. Zabos, R. Seide-Kehoe, J. Christman, and G. Acs, Departments of Pediatrics and Biochemistry, Mount Sinai School of Medicine, City University of New York, New York: Effect of ethionine on lymphocyte activation.

J.K. Christman, P. Price, and L. Pedrinan, Departments of Pediatrics and Biochemistry, Mount Sinai School of Medicine, City University of New York, New York: Ethionine, hypomethylation of DNA, and expression of globin genes in Friend erythroleukemia cells.

P.H. Atkinson, Departments of Pathology and Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Glycoprotein and protein precursors to plasma membranes in virus-infected and uninfected HeLa cells.

P. Kurth, J. Smart, and V. Bosch, Imperial Cancer Research Fund Laboratories, London: Cell-membrane glycoproteins of endogenous avian oncornaviruses.

M. Kotler, Y. Rosenberg, and B. Friedlender, Laboratory for Molecular Virology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel: Ribonuclease-sensitive DNA polymerase, a membrane-bound enzyme specific for transformed cells.

RNA TUMOR VIRUSES

arranged by
DAVID BALTIMORE, *Massachusetts Institute of Technology*
NAOMI ROSENBERG, *Massachusetts Institute of Technology*

301 participants

WEDNESDAY EVENING, May 25

Session 1: Reverse transcription

Chairperson: J.M. Bishop, University of California, San Francisco

G.G. Peters, F. Harada, and J.E. Dahlberg, Department of Physiological Chemistry, University of Wisconsin, Madison: Interaction of primer and template for RNA-directed DNA synthesis in Moloney murine leukemia virus.

B. Cordell, R. Swanstrom, H.M. Goodman,* and J.M. Bishop, Departments of Microbiology, *Biochemistry and Biophysics, University of California, San Francisco: Transcription of DNA from the genome of avian sarcoma virus may initiate on $tRNA^{trp}$ in the absence of stable binding between primer and enzyme.

M.S. Collett and A.J. Faras, Department of Microbiology, University of Minnesota Medical School, Minneapolis: The nature of RNA-directed DNA transcription at the 5′ end of the Rous sarcoma virus genome.

J. Leis, P. Scheible, and R. Smith, Department of Surgery, Duke University Medical Center, Durham, North Carolina: In vitro transcription of reconstituted 34S Rous sarcoma virus $RNA \cdot tRNA^{trp}$ template primer complexes by the avian oncornavirus DNA polymerase—Demonstration of intra- and interstrand transcription by hybridization and electron microscopic analysis.

R. Joho, A. Palmenberg, M.A. Billeter, and C. Weissmann, Institut für Molekularbiologie I, Universität Zürich, Switzerland: Concordance of 5′ and 3′ terminal sequences in recombinant Rous sarcoma virus RNA issuing from crosses of parents with distinct terminal redundancies.

E. Stoll, M.A. Billeter, A. Palmenberg, and C. Weissmann, Institut für Molekularbiologie I, Universität Zürich, Switzerland: Nucleotide sequence of the termini of AMV RNA—Terminal redundancy extending for 16-19 nucleotides.

E. Rothenberg, R.A. Weinberg, and D. Baltimore, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge: Characterization of infectious DNA synthesized by reverse transcription in vitro.

W.A. Haseltine, J. Coffin,* and A. Maxam,† Sidney Farber Cancer Institute; *Tufts Medical School; †Harvard Medical School, Boston, Massachusetts: A jumping polymerase—Replication and structure of RNA tumor viruses.

D.P. Grandgenett, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: A 32,000-dalton protein from avian retravirus cores possesses endonuclease activity.

S.G. Lee and P.P. Hung, Molecular Virology Laboratory, Abbott Laboratories, North Chicago, Illinois: Isolation of nucleic acid-binding protein from Rous sarcoma virus-transformed cells and its interaction with the RSV genome.

Session 2: Viral DNA: Integration and expression

Chairperson: J. Dahlberg, University of Wisconsin, Madison

P.R. Shank, G. Ringold, S. Hughes, H.J. Kung, K. Yamamoto,* J.M. Bishop, and H.E. Varmus, Departments of Microbiology and *Biochemistry, University of California, San Francisco: Restriction endonuclease mapping of the unintegrated DNA of RNA tumor viruses.

S.H. Hughes, P.R. Shank, H.-J. Kung, J.M. Bishop, and H.E. Varmus, Department of Microbiology, University of California, San Francisco: Integration of avian sarcoma virus DNA into the host genome.

C.J. Collins and J.T. Parsons, Department of Microbiology, University of Virginia, Charlottesville: Integration of avian sarcoma virus DNA sequences.

E.F. Fritsch and H.M. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Absence of complete "+" and "−" strand viral DNAs in stationary chicken embryo fibroblasts infected with avian ribodeoxyviruses.

F. Yoshimura, M. Goldfarb, D. Steffen, J. Hoffmann, and R.A. Weinberg, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge: Restriction endonuclease mapping of the genomes of Harvey sarcoma and Moloney leukemia viruses.

Y. Chien, I. Verma,* and N. Davidson, Department of Chemistry, California Institute of Technology, Pasadena; *Salk Institute, San Diego, California: A heteroduplex study of sequence relations between RNAs of several murine viruses.

C.A. Roberts and I.M. Verma,* Department of Biology, University of California, San Diego; *Tumor Virology Laboratory, Salk Institute, San Diego, California: In vitro synthesis of double-stranded DNA complementary to the genomes of avian and murine RNA tumor viruses.

R. Jaenisch, Heinrich-Pette-Institut, Hamburg, West Germany: Insertion of tumor virus genes into the germ line of mice.

R.J. Rascati and R.W. Tennant, Biology Division, Oak Ridge National Laboratory, Tennessee: Induction of endogenous murine leukemia virus by hydroxyurea and related compounds—Evidence for involvement of DNA damage and repair.

R. Risser, J. Hartley,* and W.P. Rowe,* McArdle Laboratory, University of Wisconsin, Madison; *Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: Genetic control of the frequency of endogenous MuLV induction.

THURSDAY AFTERNOON, May 26

Session 3: Evidence for retroviruses in human tissues

Chairperson: H. Kaplan, Stanford University Medical Center, California

R.J. Huebner, D.C. Fish,* R. Toni,* D.B. Djurickovic,* C.E. Whitmire,† J. Rhim,† R.V. Gilden,* P.R. Hill, and R.W. Hill, NCI, National Institutes of Health, Bethesda, Maryland; *Frederick Cancer Research Center, Frederick, Maryland; †Microbiological Associates, Bethesda, Maryland: Immune protection against cancer in experimental animals and a strategy for protection against cancer in man.

H.S. Kaplan, R.S. Goodenow, A.L. Epstein, S. Gartner, A. Declève, and P.N. Rosenthal, Cancer Biology Research Laboratory, Department of Radiology, Stanford University Medical Center, California: Isolation and characterization of C-type virus from an established human histiocytic lymphoma cell line.

K. Nooter, P. Bentvelzen, J.C. Coolen,* and C. Zurcher,* Radiobiological Institute TNO and *Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands: Isolation of a leukemogenic virus from human leukemic bone marrow by cocultivation procedures.

G. Koch, K. Nooter, P. Bentvelzen, and J.J. Haaijman,* Radiobiological Institute TNO and *Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands: Serological characterization of a putative human C-type oncornavirus by means of the Sepharose bead immunofluorescence assay.

R.G. Smith, S.A. Lee, and R.C. Gallo, NCI, National Institutes of Health, Bethesda, Maryland: Detection by competitive molecular hybridization of type-C viral related sequences in RNA derived from normal and neoplastic human tissues.

P. Jacquemin, W. Saxinger, and R. Gallo, National Institutes of Health, Bethesda, Maryland: Evidence for a specific immunological response against viral reverse transcriptase as evidenced by natural Ig found on the membrane of leukocytes from patients with leukemia.

D. Gillespie, R.C. Gallo, B. Nelkin, W. Prensky,* and D. Strayer, NCI, National Institutes of Health, Bethesda, Maryland, and *Sloan-Kettering Institute, New York, New York: Organization of type-C viral related sequences in human DNA.

F. Wong-Staal, N.R. Miller, M.S. Reitz, E.G. Lewis, D.H. Gillespie, and R.C. Gallo, NCI, National Institutes of Health, Bethesda, Maryland: Distribution of primate type-C viral related sequences in DNA from humans.

THURSDAY EVENING, May 26

Session 4: Genetics and restriction

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

G.M. Cooper, Sidney Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, Massachusetts: Marker rescue with subgenomic fragments of avian leukosis virus DNA.

K. Steimer and D. Boettiger, Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia: A complementation system for Rous sarcoma virus replication genes using absolute mutants.

J.M. Coffin, M. Planitz, and F. Chabot, Department of Molecular Biology and Microbiology, Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts: Relationship between the genomes of exogenous and endogenous avian RNA tumor viruses.

A. Rein, R.H. Bassin, and B.I. Gerwin, NCI, National Institutes of Health, Bethesda, Maryland: A replication-defective variant of Moloney MuLV—Interactions with amphotropic MuLV.

D.V. Faller, J. Rommelaere, and N. Hopkins, Biology Department, Massachusetts Institute of Technology, Cambridge: RNase T_1-resistant oligonucleotides of MuLVs.

W.K. Yang, L.R. Boone,* I.C. Hsu, D.M. Yang, R.W. Tennant, and A. Brown,* Biology Division, Oak Ridge National Laboratory, Tennessee; *Department of Microbiology, University of Tennessee, Knoxville: DNA transfection of N- and B-tropic MuLVs in cell cultures of Fv-1 permissive and nonpermissive genotypes.

G. Duran-Troise, R.H. Bassin, B.I. Gerwin, and A. Rein, NCI, National Institutes of Health, Bethesda, Maryland: Abrogation of Fv-1^b restriction by N-tropic MuLV rendered noninfectious by gamma irradiation or by heating.

S.J. O'Brien and J.M. Simonson, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: Pleiotropic restriction of BALB/c endogenous oncornavirus expression by the Bvr-1 gene in cat-mouse hybrid cells.

R.S. Lemons, S.J. O'Brien, and C.J. Sherr, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: A new gene on human chromosome 6 which controls the replication of baboon type-C virus in human cells.

FRIDAY MORNING, May 27

Session 5: Leukemia viruses and leukemia

Chairperson: S. Aaronson, NCI, National Institutes of Health, Bethesda, Maryland

H. Hiai, P. Morrissey, R. Khiroya, and R.S. Schwartz, Department of Hematology, Tufts-New England Medical Center, Boston, Massachusetts: Expression of xenotropic virus in hairless mice.

A.J. Mayer, A. Bosch, C. Comas, M.L. Duran-Reynals, and F. Lilly, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Resistance to spontaneous leukemia in the (AKR x RF)F_1 mouse.

M. Lieberman, A. Declève, and H.S. Kaplan, Department of Radiology, Cancer Biology Research Laboratory, Stanford University School of Medicine, California: Rescue of RadLV from cultured, nonproducer lymphoma cells of strain C57Bl/Ka mice.

N. Rosenberg, D. Baltimore, and C.D. Scher,* Massachusetts Institute of Technology, Cambridge, and *Harvard Medical School, Boston, Massachusetts: The effect of helper virus on Abelson virus-induced leukemogenesis.

U.R. Rapp, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: Leukemia in inbred strains of mice—Virulence of endogenous ecotropic virus as a determinant of disease incidence.

J.H. Elder, F.C. Jensen, R.A. Lerner, J.W. Hartley,* and W.P. Rowe,* Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California; *Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: Generation of a new leukemogenic virus via recombination between the envelope genes of endogenous ecotropic and xenotropic proviruses.

D.H. Troxler, J.K. Boyars, and E.M. Scolnick, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: The Friend strain of spleen focus-forming virus—A recombinant between mouse type-C ecotropic viral sequences and sequences related to xenotropic virus.

Y. Ikawa and M. Yoshida, Laboratory of Viral Oncology, Cancer Institute, Toshima-ku, Tokyo, Japan: Erythroblastosis-inducing RNA sequences in virions from Friend leukemia virus-induced erythroleukemia cells of a DDD mouse origin.

FRIDAY AFTERNOON, May 27

Session 6: Poster session on retrovirus biochemistry

Reverse transcription

T.Y. Shih, H.A. Young, W.P. Parks, and E.M. Scolnick, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: In vitro transcription of Moloney leukemia virus genes in infected cell nuclei and chromatin—Elongation of chromatin-associated RNA by *E. coli* RNA polymerase.

J. Harel, S.A. Leibovitch, and H. Tapiero, Groupe de Recherche du CNRS, Institute Gustave-Roussy, Villejuif, France: Single-stranded DNA related to transcription of cellular and oncornavirus genes.

S.R. Tronick, W.A. Haseltine,* C.D. Cabradilla, K.C. Robbins, and S.A. Aaronson, Laboratory of RNA Tumor Viruses, NCI, National Institutes of Health, Bethesda, Maryland: *Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Demonstration of 5′-terminal nucleotide sequences of the mammalian helper type-C viral genome in replication-defective mammalian transforming viruses.

B.I. Gerwin and J.G. Levin, National Institutes of Health, Bethesda, Maryland: Properties of MuLV reverse transcriptase assembled into noninfectious virions lacking the 60-70S genome.

A.M. Wu, V.S. Kalyanaraman,* L. Cellei,* and T. Le Bon,* Department of Anatomy (Histology), University of Toronto, Canada; *Department of Biology, Litton Bionetics, Inc., Bethesda, Maryland: Dilemma of molecular weight of mammalian type-C viral reverse transcriptase.

R.C. Sawyer, C.M. Metroka, W.S. Mason, and H. Hanafusa, Rockefeller University, New York, New York, and Institute for Cancer Research, Philadelphia, Pennsylvania: Lack of functional viral reverse transcriptase in normal chicken cells.

Viral DNA integration and expression

T. Tsuruo and M.A. Baluda, Department of Pathology, University of California School of Medicine, Los Angeles: DNA repair and provirus integration.

D.P. Nayak and K. Som, University of California School of Medicine, Los Angeles: Purification of the coding strand of the avian myeloblastosis virus by affinity chromatography and molecular hybridization.

Y. Akiyama, Department of Microbiology, University of Southern California School of Medicine, Los Angeles: Evidence against tandem integration of two different avian sarcoma viruses in the same cell.

P.C. Kimball, Comprehensive Cancer Center and Department of Microbiology, Ohio State University, Columbus: Comparison of RNA components from feline and murine sarcoma-leukemia virus complexes.

W. Drohan, R. Kettmann, D. Colcher, and J. Schlom, Meloy Laboratories, Springfield, Virginia, and NCI, National Institutes of Health, Bethesda, Maryland: Isolation of the mouse mammary tumor virus sequences not transmitted as germinal provirus in the C3H and RIII mouse strains.

Translation and mRNA

E.C. Murphy, Jr. and R.B. Arlinghaus, The University of Texas System Cancer Center, M.D. Anderson Hospital

and Tumor Institute, Houston: In vitro translation of Rauscher leukemia virus 35S RNA in nuclease-treated mouse cell extracts.

S.A. Edwards and H. Fan,* Department of Biology, University of California, San Diego; *Salk Institute for Biological Studies, La Jolla, California: Analysis of nascent polypeptides on polyribosomes precipitated with antibody to p30.

E.M. Scolnick and W.P. Parks, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: Identification of a protein coded for by Harvey sarcoma virus.

U. Olshevsky, I. Kerr,* and D. Baltimore,† Laboratory for Molecular Virology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; *The National Institute for Medical Research, London, England; †Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: In vitro translation of RNA from Moloney murine leukemia virus and Harvey murine sarcoma virus.

G.F. Okasinski and L.F. Velicer, Department of Microbiology and Public Health, Michigan State University, East Lansing: Cell-free translation of feline leukemia virus subunit RNA.

H.-H.M. Dahl, R. McCormick, and B. Ozanne, Imperial Cancer Research Fund Laboratories, London, England: In vitro translation of murine leukemia virus and Kirsten sarcoma virus RNA.

Proteins and morphogenesis

R. Klemenz and H. Diggelmann, Swiss Institute for Experimental Cancer Research, Epalinges-Lausanne, Switzerland: Peptide analyses of envelope glycoproteins of Rous sarcoma virus.

W.J.M. van de Ven and H.P.J. Bloemers, Department of Biochemistry, University of Nijmegen, The Netherlands: Impaired expression of the *env* gene of Rauscher murine leukemia virus.

M.C. Kemp, K.S. Wise, R.T. Acton, and R.W. Compans, Department of Microbiology, University of Alabama, Birmingham: Characterization of minor glycoproteins of MuLVs.

J.-S. Tung and E. Fleissner, Memorial Sloan-Kettering Cancer Center, New York, New York: Two species of type-C viral core polyprotein on AKR mouse leukemia cells.

R. Ascione,* D.K. Haapala,† W.G. Robey,* and G.F. Vande Woude,* *Laboratory of DNA Tumor Viruses and †Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: The site of synthesis and the location of Moloney sarcoma virus-specific polyprotein P60 (gag).

B.K. Pal, M.L. Bryant, and P. Roy-Burman, Departments of Pathology and Biochemistry, University of Southern California School of Medicine, Los Angeles: Phosphoproteins of RNA tumor viruses—Primary structural analysis and virion location of the multiple-charged species.

A.F. Shields, N. Rosenberg, and D. Baltimore, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge: The characterization of defective virions produced by Abelson leukemia virus-transformed lymphoid cells.

J. Forchhammer and G. Turnock,* The Fibiger Cancer Laboratory, Copenhagen, Denmark; *Department of Biochemistry, School of Biological Sciences, University of Leicester, England: Isomers of viral proteins from Moloney-MuLV and M-MSV(MuLV).

J.E. Bubbers, S. Chen, and F. Lilly, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Detection of selectively incorporated H-2 antigens in disrupted, but not intact, Friend virus particles.

P. Dezelee and F. Catala, Foundation Curie-Institut du Radium, Centre Universitaire, Orsay, France: Structural proteins of Schmidt-Ruppin Rous sarcoma virus and transformation thermosensitive mutants.

S.L. Marcus, S.W. Smith, J. Racevskis, and N.H. Sarkar, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of oncornaviral proteins by hydrophobic chromatography.

D. Robertson and R.E. Thach, Department of Biological Chemistry, Washington University, St. Louis, Missouri: Characterization of the proteins of intracisternal A-type particles and extracellular oncornaviruslike particles produced by MOPC-460 cells.

C. Dickson, M. Atterwill, and H. Dahl, Imperial Cancer Research Fund Laboratories, London, England: Polyprotein precursors to the envelope and internal proteins of the mouse mammary tumor virus.

J. Racevskis and N.H. Sarkar, Memorial Sloan-Kettering Cancer Center, New York, New York: Synthesis and processing of precursor polypeptides to mouse mammary tumor virus structural proteins.

J.M. England, B. Dietzschold, and M.S. Halpern, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Antibody-independent detection of virus-specific glycoprotein synthesis in oncornavirus-infected cells.

Session 7: Poster session on retrovirus biology

Leukemia and leukemia viruses

S.K. Datta, N. Manny, C. Andrzejewski, M. Casey, and R.S. Schwartz, Department of Hematology, Tufts-New England Medical Center, Boston, Massachusetts: Relation between virus-inducing loci and autoimmune disease in NZB mice.

K.F. Manly, Department of Medical Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York: Pathogenesis by sublines of an MuLV.

A. Bosch, C. Comas, A. Mayer, M.L. Duran-Reynals, and F. Lilly, Albert Einstein College of Medicine, Bronx, New York: Leukemogenesis in mice by X irradiation and by 3-methylcholanthrene—Expression of endogenous MuLV in irradiated leukemic mice.

R.C. Nowinski, Fred Hutchinson Cancer Research Center, Seattle, Washington: Expression of viral antigens on the cell surface of lymphocytes.

M.S. Halpern and R.R. Friis,* Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania; *Institüt fur Virologie, Fachbereich Humanmedizin, Justus Leibig Universität, Giessen, West Germany: Immune reactivity to endogenous viral glycoprotein is a consequence of the sensitization of chf (+) chickens to the glycoprotein of exogenous avian tumor virus.

P. O'Donnell, E. Stockert, A. DeLeo, N. Famulari, T. Lee, U. Rapp,* and N. Hopkins[T] Memorial Sloan-Kettering Cancer Center, New York, New York; *NCI, National Institues of Health, Bethesda, Maryland: [T]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Studies on the XC^- to XC^+ conversion of N-ecotropic murine type-C viruses.

Virus-cell interaction

P. Moss, N. Sandefur, and G.S. Martin, Department of Zoology, University of California, Berkeley: Transformation of myogenic cells by Rous sarcoma virus.

H.L. Robinson, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Presence of an endogenous non-subgroup-E type-C virus in line 15 chickens.

N.A. Wivel and H.L. Malech, Laboratory of Cell Biology, NCI, National Institutes of Health, Bethesda, Maryland: Studies on the transfer and replication of murine intracisternal A particles.

B.J. Weimann, J. Schmidt, and I.B. Pragnell,* Basel Institute for Immunology, Switzerland; *Beatson Institute for Cancer Research, Glasgow, Scotland: Biochemical studies on intracisternal A-type particles from mouse myelomas.

A. Bernstein and T. Mak, Ontario Cancer Institute, Toronto, Canada: Isolation of Friend spleen focus-forming virus nonproducer NIH/3T3 clones.

B. Moll, J.W. Hartley, and W.P. Rowe, North Shore University Hospital, Manhasset, New York, and NIAID, National Institutes of Health, Bethesda, Maryland: Induction of B-tropic and N-tropic MuLV from B10.BR mouse embryo cell lines.

C. Moroni, P. Monckton, J. Stoye, J. De Lamarter, A. Matter, and G. Schumann, Friedrich Miescher-Institut, F. Hoffmann-La Roche & Co., Ltd., and CIBA-GEIGY, Ltd., Basel, Switzerland: Induction of endogenous C-type viruses from lymphoid cells.

R.P. Monckton and C. Moroni, Friedrich Miescher-Institut, Basel, Switzerland: Serum acts as an inducer of endogenous murine C-type viruses.

G. Vecchio, G. Colletta, F. Fragomele, M. Laurenza, and M.L. Sandomenico, Cattedra di Virologia Oncologica, Istituto di Patologia Generale, II Facolta di Medicina, University of Naples, Italy: Induction of viral polypeptides and viral messenger RNA in Friend erythroleukemic cells.

F.W. George IV, D.W. Krempin, and V. Klement, Departments of Microbiology and Pediatrics, University of Southern California School of Medicine, Los Angeles: Sendai virus-mediated infection of mink cells with ecotropic mouse leukemia virus.

C. Kryceve-Martinerie, S. Barlati,* J.M. Biquard, D. Lawrence, and P. Vigier, Faculté des Sciences, Institut du Radium, Orsay, France; *Laboratorio di Genetica Biochimica ed Evoluzionistica del CNR, Pavia, Italy: Characteristics of the transformation-enhancing factor(s) produced by Rous sarcoma virus-transformed and other transformed cells.

S.P. Staal, J.W. Hartley, and W.P. Rowe, Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: Isolation of transforming viruses from spontaneously arising tumors of high-lymphoma strain mice.

Genetics and restriction

M. Linial, University of Washington School of Medicine and Fred Hutchinson Cancer Research Center, Seattle, Washington: Characterization of two coordinately defective mutants of Rous sarcoma virus.

Y.C. Chen, M.J. Hayman,* and P.K. Vogt,† Department of Micribiology, Texas College of Osteopathic Medicine, North Texas State University; *Imperial Cancer Research Fund, London, England; †Department of Microbiology, University of Southern California, Los Angeles: Mammalian cells transformed by temperature-sensitive mutants of avian sarcoma virus.

K. Toyoshima, M. Nasu, and H. Nomaguchi, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka, Japan: Selection and modification by quail cell passage of avian sarcoma virus.

R.W. Tennant, W.K. Yang, R.J. Rascati, and A. Brown,* Biology Division, Oak Ridge National Laboratory, Tennessee;* University of Tennessee, Knoxville: Factors affecting transfer of Fv-1 locus-specific resistance to mouse leukemia cells.

M.E. Blackstein and T. Mak, Departments of Anatomy (Histology) and Medical Biophysics, University of Toronto, Ontario, Canada: Inherited resistance to N- and B-tropic MuLVs in vitro—Expression of N-tropic viral RNA and integrated sequences of N- and B-tropic viruses in cells of the Fv-1 congenic pair SIM.S and SIM.R.

F. Westenbrink, W.H. Koornstra, P.C. Creemers, J. Brinkhof, J.J. Haaijman,* and P. Bentvelzen, Radiobiological Institute TNO and *Institute for Experimental Gerontology, TNO, Rijswijk, The Netherlands: Epigenetic factors causing differential synthesis of murine mammary tumor virus polypeptides.

Natural history

M. Barbacid, S. Hino, J.M. Krakower, S.R. Tronick, and S.A. Aaronson, Laboratory of RNA Tumor Viruses, NCI, National Institutes of Health, Bethesda, Maryland: Immunologic approaches to the genetic analysis of mammalian type-C RNA viruses.

W.A. Haseltine and D. Kleid,* Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; Stanford Research Institute, Menlo Park, California: A new method of RNA tumor virus classification.

M.L. Bryant, P. Roy-Burman, M.B. Gardner, and B.K. Pal, Departments of Pathology and Biochemistry, University of Southern California School of Medicine, Los Angeles: Genetic sequence homology among ecotropic, xenotropic, and amphotropic murine type-C RNA viruses.

R. Callahan, C.J. Sherr, and G.J. Todaro, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: Immunologic and biochemical characterization of isolates from a new class of murine retroviruses.

P.R. Andersen and D. Boettiger, Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia: Biological and biochemical studies of reptilian C-type viruses.

S. Rasheed and M.B. Gardner, Department of Pathology, University of Southern California School of Medicine, Los Angeles: Isolation of type-C virus from a wild rat.

J.S. Rhim, D.L. Putman, D.K. Park, P. Hill, and R.J. Huebner, Microbiological Associates, Inc., and NCI, National Institutes of Health, Bethesda, Maryland: Expression of common rat tumor antigen in rat cells transformed by guinea pig herpesvirus.

A.R. Davis and D.P. Nayak, Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles: Expression of endogenous retraviral genes in leukemic guinea pig cells.

T.G. Kawakami and L. Sun, Comparative Oncology Laboratory, University of California, Davis: Type-C virus-induced leukemia in gibbons.

S.T. Barker, R.L. Heberling, J.W. Eichberg, and S.S. Kalter, Southwest Foundation for Research and Education, San Antonio, Texas: Expression of baboon endogenous virus in tissues of animals infected with a murine sarcoma-baboon endogenous pseudotype virus.

FRIDAY EVENING, May 27

Session 8: Translation and mRNA

Chairperson: H. Diggelmann, Swiss Institute for Experimental Cancer Research, Epalinges-Lausanne

S.R. Weiss, H.E. Varmus, and J.M. Bishop, Department of Microbiology, University of California, San Francisco: Size and genetic composition of cytoplasmic avian sarcoma virus-specific RNAs.

D.W. Stacey and H. Hanafusa, Rockefeller University, New York, New York: Determination by microinjection of the size of RAV-2 envelope-glycoprotein mRNA.

W.S. Hayward and S.Y. Wang, Rockefeller University, New York, New York: Gene-specific viral RNAs in uninfected and avian sarcoma virus-infected cells.

B. Baker, H. Robinson, H.E. Varmus, and J.M. Bishop, Department of Microbiology, University of California, San Francisco: Regulation of endogenous viral gene expression in uninfected chicken cells.

A.J. Conley and L.F. Velicer, Department of Microbiology and Public Health, Michigan State University, East Lansing: Analysis of intracellular feline leukemia virus-specific RNA.

H. Oppermann, A. Ulrich, S. Weiss, H.E. Varmus, J.M. Bishop, and L. Levintow, Department of Microbiology, University of California, San Francisco: Cells producing avian sarcoma virus contain a polypeptide (pr180) translated from the genes *gag* and *pol*.

J. McGinnis and J. Leis, Department of Surgery, Duke University Medical Center, Durham, North Carolina: Translation of 34S Rous sarcoma virus RNA in vitro into a polymerase-gs antigen precursor polypeptide and processing of 34S RNA with purified RNase III.

K. Beemon and T. Hunter, Tumor Virology Laboratory, Salk Institute, San Diego, California: In vitro translation yields a possible Rous sarcoma virus *src* gene product

R. Palmiter, S. Ripley, and R. Eisenman, Department of Biochemistry, University of Washington, and Fred Hutchinson Cancer Research Center, Seattle, Washington: Annealing with $tRNA^{trp}$ specifically inhibits the in vitro translation of Rous sarcoma virus 35S RNA.

SATURDAY MORNING, May 28

Session 9: Proteins and morphogenesis

Chairperson: E. Fleissner, Memorial Sloan-Kettering Cancer Center, New York, New York

R. Eisenman, R. Shaikh, and W. Mason, Fred Hutchinson Cancer Research Center, Seattle, Washington; Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania: Identification of an endogenous virus polyprotein in uninfected chick fibroblast cells.

K. Bister, M.J. Hayman, P.H. Duesberg,* and P.K. Vogt, Department of Microbiology, University of Southern California School of Medicine, Los Angeles; *Department of Molecular Biology and Virus Laboratory, University of California, Berkeley: The defectiveness of strain MC29 avian leukosis virus.

D.L. Buchhagen and H. Hanafusa, Rockefeller University, New York, New York: Identification and characterization of a precursor to the avian RNA tumor virus glycoprotein.

K. Moelling and M. Hayami* Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, West Germany; Institut für Virologie der Justus-Liebig Universität, Fachbereich Humanmedizin, Giessen, West Germany: Analysis of precursors to the envelope glycoproteins of avian RNA tumor viruses in chicken and quail cells.

R.B. Arlinghaus, J.J. Kopchick, G.A. Jamjoom, and R.B. Naso, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Characterization of intracellular polyprotein precursors of reverse transcriptase in cells infected with Rauscher leukemia virus.

S. Oroszlan and R. V. Gilden, Viral Oncology Program, NCI, National Institutes of Health, Bethesda, and Frederick Cancer Research Center, Frederick, Maryland: Primary structure and immunologic analysis of *env* and gag gene products.

J.W. Gautsch and J.H. Elder, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California: Conservation of the primary structure of the gag gene products of ecotropic, xenotropic, and amphotropic MuLVs—A contrast to the variability of the *env* gene product gp70.

E. Fleissner, H. Snyder, Jr., and E. Stockert, Memorial Sloan-Kettering Cancer Center, New York, New York: The

molecular nature of the Gross surface antigen.

R.B. Luftig and Y. Yoshinaka, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Cleavage of MuLV P70 in vitro can be accompanied by a shift from a concentrically coiled internal strand (immature) to a collapsed (mature) form of the virus core.

Y. Yoshinaka and R.B. Luftig, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Partial characterization of a P70 proteolytic factor that is present in purified virions of Rauscher leukemia virus.

O. Witte and D. Baltimore, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge: Mechanism of formation of pseudotypes between vesicular stomatitis virus and murine leukemia virus.

SATURDAY AFTERNOON, May 28

Session 10: Virus-cell interaction

Chairperson: R. Benveniste, NCI, National Institutes of Health, Bethesda, Maryland

P.M. Pitha, B.F. Fernie, S.P. Staal, D.P. Bolognesi, and W.P. Rowe, Johns Hopkins Oncology Center, Baltimore, Maryland; National Institutes of Health, Bethesda, Maryland; Duke University, Durham, North Carolina: The effect of interferon on MuLV infection.

E. Hunter and G.L. Davis, Department of Microbiology, University of Alabama, Birmingham: Inhibition of Rous sarcoma virus replication by interferon.

J. Périès, M. Canivet, R. Emanoil-Ravicovitch, M.C. Debons-Guillemin, J. Robert, and C.J. Sherr, Laboratoire d'Hématologie Expérimentale, Hopital Saint Louis, Paris, France, and Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: Expression and infectivity of murine type-C viruses in mouse teratocarcinoma cells.

R. Michalides, R. Nusse, and R. van Nie, Department of Genetrics and Virology, Netherlands Cancer Institute, Amsterdam: Identification by nucleic acid hybridization of the gene responsible for the occurrence of pregnancy-dependent mammary tumors in the GR mouse strain.

D.R. Lowy, Dermatology Branch, NCI, National Institutes of Health, Bethesda, Maryland: Glucocorticoid-induced transformation of a mink cell line which contains a sarcoma viral genome.

J.-M. Biquard and P. Dezelee, Fondation Curie-Institut du Radium, Orsay, France: Changes in cellular glycoproteins after treatment of chick embryo fibroblasts with 5-bromodeoxyuridine.

P. Roy-Burman,*† H.L. Niman,† J.R. Stephenson,‡ and M.B. Gardner,* *Departments of Pathology and †Biochemistry, University of Southern California School of Medicine, Los Angeles; ‡Laboratory of RNA Tumor Viruses, NCI, National Institutes of Health, Bethesda, Maryland: Endogenous RD-114 virus genome expression in malignant cat tissues.

W.D. Hardy, Jr., E.E. Zuckerman, E.G. MacEwen, and M. Essex, Laboratory of Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York, and Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts: Evidence that the feline oncornavirus-associated cell-membrane antigen is an FeLV- and FeSV-induced tumor-specific antigen.

R.E. Benveniste and G.J. Todaro, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: Primate retroviruses—Different origins and modes of transmission.

SUNDAY MORNING, May 29

Session 11: Src and avian viral genomes

Chairperson: R. Weinberg, Massachusetts Institute of Technology, Cambridge

D. Spector, K. Smith, H.E. Varmus, and J.M. Bishop, Department of Microbiology, University of California, San Francisco: The DNAs of uninfected vertebrates contain nucleotide sequences related to the transforming gene of avian sarcoma virus.

A.L. Schincariol, Cancer Research Unit, University of Western Ontario, London, Canada: Analysis of sarcoma-specific sequences from murine sarcoma virus growing on thymus-bone marrow cells.

T. Graf, B. Royer-Pokora, and H. Beug, Max-Planck-Institut für Virusforschung, Biologisch-Medizinische Abteilung, Tübingen, West Germany: Transformation parameters in chicken fibroblasts transformed by avian leukosis viruses.

D. Stèhelin and T. Graf,* Virology Unit, INSERM, Lille, and Institut Pasteur de Lille, France,* Max-Planck-Institut für Virusforschung, Tübingen, West Germany: Avian erythroblastosis and myelocytomatosis viruses lack the avian sarcoma virus-transforming gene.

M.M.C. Lai, S. Hu, and P.K. Vogt, Department of Microbiology, University of Southern California School of Medicine, Los Angeles: The occurrence of partial deletion and substitution of *src* gene in the RNA genome of avian sarcoma virus.

E. Wang and A.R. Goldberg, Rockefeller University, New York, New York: Effect of the *src* gene product on microfilament and microtubule organization in mammalian cells infected with a temperature-sensitive mutant of Rous sarcoma virus.

G. Calothy, F. Poirier, and B. Pessac, Fondation Curie-Institut du Radium, Orsay, France: Transformation-defective mutants of Rous sarcoma virus inducing chick embryo neuroretinal cell multiplication.

E. Keshet and H.M. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Pheasant-derived nucleotide sequences in the genome of recombinant avian leukosis viruses with subgroup F specificity.

P.E. Neiman, University of Washington and The Fred Hutchinson Cancer Research Center, Seattle, Washington: Mapping by competitive hybridization of genome sequences which differ between endogenous and exogenous chicken leukosis viruses.

L.B. Crittenden, W. Okazaki, P.E. Neiman, J.M. Sharma, and R.L. Witter, Regional Poultry Research Laboratory, East Lansing, Michigan, and The Fred Hutchinson Cancer Research Center, Seattle, Washington: Occurrence of lymphoid tumors in chickens free of infection with known avian tumor viruses.

THE MOLECULAR BIOLOGY OF YEAST

arranged by
FRED SHERMAN, *University of Rochester*
JAMES BROACH, *Cold Spring Harbor Laboratory*

254 participants

THURSDAY EVENING, August 18

Session 1

Chairperson: V. MacKay, Rutgers University, New Brunswick, New Jersey

E. Ciejek, Y. Jones-Brown, and J. Thorner, Department of Bacteriology and Immunology, University of California, Berkeley: The mode or action of α factor—A synthetic approach.

R. K. Chan, Department of Microbiology, University of Cincinnati College of Medicine, Ohio: A yeast mutant super-sensitive to α factor.

R. Sumrada and T. G. Cooper, University of Pittsburgh, Pennsylvania: Control of protein degradation by α factor, the cell cycle arrest signal, and inhibition of protein synthesis.

J. McCullough and I. Herskowitz, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: Relationship of *S. cerevisiae* and *S. kluyveri* mating factors.

J. E. Haber, B. Garvik, D. D. Wygal, and J. M. Stoler, Department of Biology, Brandeis University, Waltham, Massachusetts: Mutations affecting the efficiency of homothallic mating-type interconversion.

D. B. Finkelstein and M. Udden, Department of Biochemistry, University of Texas Health Science Center, Dallas: α Factor—Cell-cycle arrest and α factor metabolism.

L. M. Melnick and J. Blamire, Department of Biology, Brooklyn College, New York: Regulation of sporulation by functions located on chromosome III.

J. Rine, J. Strathern, J. Hicks, and I. Herskowitz, Institute of Molecular Biology and Department of Biology,

University of Oregon, Eugene: Control of cell type in heterothallic cells.

L. M. Melnick and J. Blamire, Department of Biology, Brooklyn College, New York: The effect of the *dmt* gene on the mating reaction and chromosome III in yeast.

A. K. Hopper and J. Forest, Department of Microbiology, University of Massachusetts Medical Center, Worcester: Mutants of yeast which alter mating-type control of meiosis and spore formation.

A. J. S. Klar, D. N. Radin, and S. Fogel, Department of Genetics, University of California, Berkeley: Switching of a yeast mating-type mutant allele, a^0, in homothallic and heterothallic strains.

L. C. Blair, J. Strathern, J. Rine, J. Hicks, and I. Herskowitz, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: Extensions and tests of the cassette model of homothallism.

FRIDAY MORNING, August 19

Session 2

Chairperson: G. R. Fink, Cornell University, Ithaca, New York

H. L. Klein and S. K. Welch, Department of Genetics, University of Washington, Seattle: Inverted repeated sequences in yeast nuclear DNA.

M. Forte and W. Fangman, Department of Genetics, University of Washington, Seattle: Quantitation of cross-links in yeast chromosomes.

D. Lohr and K. E. Van Holde, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: Evidence for nucleosomal phasing in *S. cerevisiae* nuclear chromatin.

D. Lohr, G. Riedel, and K. E. Van Holde, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: The structure of chromatin in stationary-phase cells of *S. cerevisiae* and comparison to log-phase cells.

S. G. Elliott and C. S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California, Irvine: RNA synthesis during the cell cycle of *S. cerevisiae*.

N. J. Pearson and J. E. Haber, Department of Biology, Brandeis University, Waltham, Massachusetts: Changes in the regulation of ribosome synthesis in growing and sporulating yeast.

C. Gorenstein and J. R. Warner, Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Aspects of the metabolism of ribosomal proteins.

C. Gorenstein and J. R. Warner, Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: The synthesis of mRNA for ribosomal proteins may depend on concurrent synthesis of ribosomal RNA.

D. Ursic, B. Littlewood, and J. Davies, Department of Biochemistry, University of Wisconsin, Madison: A cold-sensitive mutant of *S. cerevisiae* defective in ribosome processing.

J. L. Bennetzen, L. D. Schultz, and B. D. Hall, Departments of Biochemistry and Genetics, University of Washington. Seattle: Nuclear function of yeast RNA polymerase III.

S. G. Oliver, J. R. Ludwig II, and C. S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California, Irvine: Separate controls on tRNA and rRNA synthesis in yeast.

A. K. Hopper and F. Banks, Department of Microbiology, University of Massachusetts Medical Center, Worcester: Mutants of yeast which are conditionally defective in the synthesis of yeast tRNA.

J. W. Gorman, J. D. Young, H. M. Laten, F. H. Webb, and R. M. Bock, Laboratory of Molecular Biology, University of Wisconsin, Madison: Isolation and characterization of eight recessive antisuppressors in *S. cerevisiae*.

M. R. Culbertson and G. R. Fink, Department of Genetics, Development, and Physiology, Cornell University, Ithaca, New York: Frameshift suppression.

FRIDAY AFTERNOON, August 19

Session 3: Poster session

B. G. Adams and I. M. Gladstone, Department of Microbiology and Immunology, University of Washington, Seattle: Genetic basis for the sugar-negative phenotype.

B. B. Chattoo, F. Sherman, and M. Ogur, Department of Radiation Biology and Biophysics, University of Rochester Medical School, New York, and Department of Microbiology, Southern Illinois University, Carbondale: A system for selecting *lys*2 mutants.

D. Fraenkel, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Glycolysis mutants of *S. cerevisiae*.

L. R. Friedman and H. M. Sobell, Department of Chemistry, University of Rochester, New York: Evidence for symmetric and asymmetric hybrid DNA formation during genetic recombination—Unselected tetrad analysis in *S. cerevisiae*.

F. Karst and F. Lacroute, Laboratoire de Génétique Physiologique, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Yeast mutants in the early part of the sterol pathway.

C. W. Lawrence and R. B. Christensen, Department of Radiation Biology and Biophysics, University of Rochester Medical Center, New York: Potential pyrimidine dimer sites and UV mutagenesis.

W. J. Colonna and P. T. Magee, Yale University Medical School, New Haven, Connecticut: Glycogen degrading enzymes: Sporulation-specific gene products in *S. cerevisiae*.

C. A. Michels and S. Dhar, Department of Biology, Queens College, City University of New York, New York: Catabolite-repression-resistant mutants in *S. carlesbergensis*.

A. P. Mitchell and E. W. Jones, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Protease-B mutants of *S. cerevisiae*.

C. Moore, Department of Biology, University of Rochester, New York: Comparisons of growth inhibition by bleomycin and phleomycin among normal and radiation-sensitive strains of *S. cerevisiae*.

B. Ono, J. W. Stewart, and F. Sherman, Department of Radiation Biology and Biophysics, University of Rochester Medical School, New York: Serine-inserting UAA suppressors of yeast.

L. Prakash, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: Defective excision of pyrimidine dimers in nuclear DNA in *rad*7, *rad*14, and *rad*17 mutants of *S. cerevisiae*.

S. Prakash, Department of Biology, University of Rochester, New York: Characterization of mutants of *S. cerevisae* sensitive to the alkylating agent methyl methane sulfonate.

L. Skogerson and U. Somasundarsan, Department of Biochemistry, The Medical College of Wisconsin, Milwaukee: Interaction of cycloheximide with yeast cytoplasmic ribosomes.

B. F. Sloat and J. R. Pringle, Division of Biological Sciences, University of Michigan, Ann Arbor: Studies of a yeast mutant defective in bud formation.

J. S. Swedes and C. S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California, Irvine: Regulation of protein synthesis during energy limitation in *S. cerevisiae*.

FRIDAY EVENING, August 19

Session 4

Chairperson: C. S. McLaughlin, University of California, Irvine

H. M. Fried and G. R. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Electron microscope heteroduplex analysis of yeast killer double-stranded RNA plasmids.

J. Bruenn and W. Kane, Division of Cell and Molecular Biology, State University of New York, Buffalo: Repeated sequences and sequence diversity in yeast double-stranded RNAs.

S. R. de Kloet, G. I. Chang, and J. L. Lilquist, Institute of Molecular Biophysics, Florida State University, Tallahassee: Some properties of double-stranded RNA of yeast.

K. A. Bostian,* J. E. Hopper, L. B. Rowe, and D. J. Tipper, *University of Massachusetts Medical Center, Worcester; Brandeis University, Waltham, Massachusetts: Translational analysis of the double-stranded RNA genome of the killer-associated-viruslike particles of *S. cerevisiae*.

R. B. Wickner, M. J. Leibowitz, and A. Toh-E, Laboratory of Biochemical Pharmacology, NIAMDD, National Institutes of Health, Bethesda, Maryland: Bypass of chromosomal genes normally needed for killer-virus replication.

C. Denis, J. Hopper, R. Menard, and T. Young, Department of Biochemistry, University of Washington, Seattle: Preparative polyacrylamide gel electrophoresis of yeast mRNA.

R. D. Water, J. R. Pringle, and L. J. Kleinsmith, Division of Biological Sciences, University of Michigan, Ann Arbor: Identification of yeast messenger RNAs coding for the synthesis of tubulin and actinlike proteins.

J. E. Hopper and L. B. Rowe, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Molecular expression and regulation of the galactose gene cluster—mRNA sizes and *gal*4 control of inducible mRNAs.

C. E. Sripati and J. R. Warner*, Institut de Biologie Physicochimique, Paris, France;* Department of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Kinetics of formation and decay of yeast messenger RNA.

P. Neiderberger, G. Miozzari, and R. Hütter, Institute of Microbiology, Federal Institute of Technology, Zurich, Switzerland: Growth behavior and RNA synthesis in mutants of *S. cerevisiae* defective in the general control of amino acid biosynthesis.

H. C. Kelker and A. O. Pogo, Laboratory of Cell Biology, The New York Blood Center, New York: Effect of amino acid starvation and addition of cycloheximide on the rate of [^{3}H]uridine incorporation into yeast RNA species.

C. A. Saunders, S. J. Sogin, and H. O. Halvorson, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Cytoplasmic poly(A) in *S. cerevisiae*.

J. F. Harper and P. T. Magee, Yale University Medical School, New Haven, Connecticut: Characteristics of poly(A)-associated RNA in ascospores of *S. cerevisiae*.

SATURDAY MORNING, August 20

Session 5

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

J. R. Cameron and R. W. Davis, Department of Biochemistry, Stanford University School of Medicine, California: Cloning and characterization of *S. cerevisiae* tyrosine tRNA genes.

M. V. Olson, H. M. Goodman, and B. D. Hall, Department of Genetics, University of Washington, Seattle: Identification, cloning, and DNA sequencing of the *sup*4 gene from *S. cerevisiae*.

G. Page, M. Olson, and B. D. Hall, Department of Genetics, University of Washington, Seattle: Isolation and characterization of $tRNA^{ser}$ genes from *S. cerevisiae*.

K. Nath, P. Holmans, and A. P. Bollon, Department of Biochemistry, University of Texas Health Science Center, Dallas: Organization of ribosomal genes on chromosomal DNA.

J. H. Cramer, J. T. Barnitz, F. W. Farrelly, D. D. Womble, and R. H. Rownd, Laboratory of Molecular Biology and Department of Biochemistry, University of Wisconsin, Madison: Organization of ribosomal DNA in yeast.

P. Philippsen, R. A. Kramer, J. Ferguson, M. Thomas, J. R. Cameron, and R. W. Davis, Department of Biochemistry, Stanford University School of Medicine, California: The gene cluster for ribosomal RNA in *S. cerevisiae*.

P. Valenzuela, A. Venegas, G. I. Bell, and W. J. Rutter, Department of Biochemistry and Biophysics, University of California, San Francisco: DNA sequence studies of the yeast ribosomal RNA genes.

T. Petes,* J. Broach,† P. Wensink,‡ L. M. Hereford,‡ G. R. Fink,§ and D. Botstein,* Department of Biology, Massachusetts Institute of Technology, Cambridge, †Cold Spring Harbor Laboratory, New York; ‡Department of Biology and Rosenstiel Center, Brandeis University, Waltham, Massachusetts; §Department of Genetics, Cornell University, Ithaca, New York: Isolation and analysis of recombinant DNA molecules containing yeast DNA.

D. Petes and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: Simple Mendelian inheritance of the reiterated ribosomal DNA genes of yeast.

P. E. Berg, A. Lewin, T. Christianson, A. Lustig, and M. Rabinowitz, Departments of Medicine, Biology, and Biochemistry, University of Chicago, and the Franklin McLean Research Institute, Chicago, Illinois: Cloning of yeast mitochondrial DNA in *E. coli*.

C. P. Hollenberg and H. D. Royer, Max-Planck-Institut für Biologie, Tübingen, West Germany: Expression of cloned *S. cerevisiae* 2-μm DNA in *E. coli* minicells."

G. D. Lauer and L. M. Hereford, Department of Biology and Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Isolation of *S. cerevisiae* histone genes cloned in pMB9.

T. St. John and R. W. Davis, Department of Biochemistry, Stanford University School of Medicine, California: Isolation of yeast DNA fragments showing regulated gene expression.

Session 6: Poster session

J. Adams and E. D. Rothman, Division of Biological Sciences and Department of Statistics, University of Michigan, Ann Arbor: The demography of yeast populations.

H. N. Anathaswamy and R. K. Mortimer, Division of Medical Physics, University of California, Berkeley: Isolation and analysis of X-ray-sensitive mutants.

K. D. Atkinson, T. F. Donahue, A. I. Kolat, and S. A. Henry, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Physiology of inositol-less death in yeast inositol synthetase mutants.

A. P. Bollon, Department of Biochemistry, University of Texas Health Science Center, Dallas: Characterization of *ilv*1 constitutive mutants.

C. Greer and R. Schekman, Department of Biochemistry, University of California, Berkely: Yeast actin.

R. A. Hackel and N. A. Khan,* Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts; *Department of Biology, Brooklyn College, New York: Isolation and characterization of mutants conferring invertase hyperproduction in strain EK-6B of *S. cerevisiae* carrying the *suc*3 gene.

D. P. Morrison, P. J. Hastings, and S.-K. Quah, Department of Genetics, University of Alberta, Edmonton, Canada: The effect of mating type on the expression of mutator mutations in diploids.

R. H. Haynes, F. Eckardt, and S.-J. Teh, Department of Biology, York University, Downsview, Ontario, Canada: Mathematical analysis of mutation induction kinetics in Rad wild-type yeast.

M. Bień, J. Kolodynski, and T. M. Lachowicz, Institute of Microbiology, Wroclaw University, Poland: Respiratory mutation and galactose metabolism in yeast *S. cerevisiae*.

N. E. Trimble and J. G. Little, Department of Biology, York University, Downsview, Ontario, Canada: Thymidylate starvation sensitizes yeast cells to ultraviolet light.

V. L. MacKay and G. P. Livi, Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Influence of the mating-type locus on MMS survival in diploid yeast cells.

J. H. McCusker and J. E. Haber, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Characterization of mutant causing nonrandom mitotic chromosome loss.

D. Mowshowitz, Department of Biology Sciences, Columbia University, New York: Control of α glucosidase levels in yeast strains carrying different *mal* genes.

R. J. Planta, R. C. Brand, and J. Klootwijk, Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands: Processing of ribosomal precursor RNA in yeast.

S.-K. Quah, R. C. Von Borstel, and P. J. Hastings, Department of Genetics, University of Alberta, Edmonton, Canada: Antimutators in yeast.

G. H. Rank and R. P. Van Hoeven,* Department ot Biology, University of Saskatchewan, Canada; *Division of Cell Biology, The Netherlands Cancer Institute, Amsterdam: Separation of high- and low-viscosity vesicles by aggregation at pH 4.

R. Snow, Department of Genetics, University of California, Davis: Genetic evidence for complementation between nonhomologous proteins in *S. cerevisiae*.

E. F. Walton, B. L. A. Carter, and J. R. Pringle,* Department of Genetics, University of Dublin, Ireland; *Division of Biological Sciences, University of Michigan, Ann Arbor: An enrichment method for temperature-sensitive mutants of yeast.

SATURDAY EVENING, August 20

Session 7

Chairperson: L. Hartwell, University of Washington, Seattle, Washington

D. Stinchcomb and R. W. Davis, Department of Biochemistry, Stanford University School of Medicine, California: Yeast gene expression during lytic infection of *E. coli* by λ-yeast hybrids.

K. Struhl and R. W. Davis, Department of Biochemistry, Stanford University School of Medicine, California: Genetic expression of yeast DNA in *E. coli*–isolation and mapping of the structural gene for IGP dehydratase (*his* 3).

M.-L. Bach,*† T. D. Petes,† F. Lacroute,* and D. Botstein,† *Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France;† Department of Biology, Massachusetts Institute of Technology, Cambridge: Cloning of the yeast *ura*3 gene in *E. coli*.

J. I. Stiles, J. W. Szostak,* R. Wu,* and F. Sherman, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York;* Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Detection and restriction analysis of the iso-1-cytochrome *c* gene.

J. W. Szostak, J. I. Stiles, F. Sherman, and R. Wu, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York, and Department of Radiation Biology and Biophysics, University of Rochester, New York: Sequence analysis of the 5′ noncoding region of yeast iso-1-cytochrome *c* mRNA.

D. M. Livingston, Department of Genetrics, University of Washington, Seattle: Control of *S. cerevisae* 2-μm DNA replication by cell-division-cycle genes that control nuclear DNA replication.

U. Wintersberger, Institute for Cancer Research, University of Vienna, Austria: DNA synthesis in isolated yeast nuclei.

K. Nasmyth, Department of Zoology, University of Edinburgh, Scotland: DNA ligase mutants in fission yeast.

G. Viehauser, R. Grün, and E. Wintersberger, Physiologische Chemisches Institut, University of Würzburg, West Germany: Yeast histones—Properties and synthesis in the cell cycle.

B. Shilo and G. Simchen, Department of Genetics, The Hebrew University, Jerusalem, Israel: Environmental regulation of cell-cycle initiation in *S. cerevisiae*.

S. I. Reed and T. Naujack, Department of Genetics, University of Washington, Seattle: Isolation and characterization of cell-cycle "start" mutants of *S. cerevisiae*.

T. J. Zamb and R. Roth, Biology Department, Illinois Institute of Technology, Chicago: The timing of early meiotic events in yeast.

M. A. Resnick, D. H. Williamson, and G. Brunborg,* National Institute for Medical Research, Mill Hill, London, England; *Institute of General Genetics, University of Oslo, Blindern, Norway: Cell-cycle-dependent variations in the repair of DNA double-strand breaks.

SUNDAY MORNING, August 21

Session 8

Chairperson: R. Needleman, Albert Einstein College of Medicine, Bronx, New York

R. Morimoto, A. Lewin, S. Merten, and M. Rabinowitz, Departments of Medicine, Biochemistry, and Biology, University of Chicago, and the Franklin McLean Research Institute, Chicago, Illinois: Mapping of yeast mitochondrial genes and the organization of petite mitochondrial DNA.

D. Hanson, D. H. Miller, and H. R. Mahler, Department of Chemistry, Indiana University, Bloomington: Peptide mapping of mitochondrial translation products of *S. cerevisiae*.

G. Faye and F. Sor, Section Biologie, Fondation Curie-Institut du Radium, Orsay, France: Analysis of mitochondrial ribosomal proteins of *S. cerevisiae*.

F. Cabral, M. Solioz, and G. Schatz, Department of Biochemistry, Biozentrum, University of Basel, Switzerland: Identification of the structural gene for yeast cytochrome oxidase subunit II on mitochondrial DNA.

D. Levens, R. Morimoto, and M. Rabinowitz, Departments of Medicine, Biochemistry, and Biology, University of Chicago, and the Franklin McLean Research Institute, Chicago, Illinois: Transcription complex and partially purified RNA polymerase from yeast mitochondria.

I. Z. Ades and R. A. Butow, Department of Biochemistry, University of Texas Health Science Center, Dallas: Studies on the mechanism of transport of cytoplasmically synthesized proteins into mitochondria of yeast.

R. D. Vincent, D. M. Ellis, and P. S. Perlman, Developmental Biology Program and Department of Genetics, Ohio State University, Columbus: Physical mapping of the structural gene for a mitochondrial protein, *var*1.

R. L. Strausberg and R. A. Butow, Department of Biochemistry, University of Texas Health Science Center, Dallas: Expression of petite mitochondrial DNA in vivo—Zygotic gene rescue.

E. D. Zanders and R. A. Butow, Department of Biochemistry, University of Texas Health Science Center, Dallas: Molecular basis for the polymorphism of the mitochondrial gene *var*1.

D. H. Miller, T. Bilinski, D. Hanson, H. R. Mahler, P. S. Perlman,* and D. M. Ellis,* Department of Chemistry, Indiana University, Bloomington; *Department of Genetics, Ohio State University, Columbus: The characterization of mitochondrial function in mit^- mutants of *S. cerevisiae*.

A. Singh, T. L. Mason, and R. A. Zimmermann, Department of Biochemistry, University of Massachusetts, Amherst: A cold-sensitive cytoplasmic mutant of *S. cerevisiae* with defective synthesis of the 50S subunit of mitochondrial ribosomes.

B. J. Stevens, Laboratoire de Biologie Cellulaire, Université de Paris-Sud, Orsay, France, and Department of Biology, University of Chicago, Illinois: Mitochondrial form and numbers during sporulation.

SUNDAY AFTERNOON, August 21

Session 9

Chairperson: P. S. Perlman, Ohio State University, Columbus

R. Needleman, J. Cohen, and J. Marmur, Albert Einstein College of Medicine, Bronx, New York: Rhodamine 6G resistance—A new yeast mitochondrial gene in the omega region.

B. Dujon, Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: Approaches to the mechanisms of genetic recombination between mitochondrial genes in *S. cerevisiae*.

W. Bandlow, Genetics Institute, University of Munich, West Germany: Control of gene expression by a mitochondrial gene product of mitochondrial and extramitochondrial enzymes.

E. P. Sena, Department of Biology, Case Western Reserve University, Cleveland, Ohio: Molecular and genetic studies on mitochondrial DNA inheritance during mating in *S. cerevisiae*.

M. F. Waxman, J. A. Knight, and P. S. Perlman, Department of Genetics, Ohio State University, Columbus: Nuclear suppressors of mitochondrial drug resistance in bakers' yeast.

B. J. Barclay and J. G. Little, Department of Biology, York University, Downsview, Ontario, Canada: Induction of mutations in the mitochondrial genome of yeast by thymidylate starvation.

S. C. Hixon and E. Moustacchi,* Department of Biochemistry, University of Alabama, Birmingham; *Fondation Curie-Institut du Radium, Orsay, France: Degradation of yeast mitochondrial DNA during post-UV dark liquid holding recovery of the petite mutation.

J. Leff and R. Eccleshall, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Replication of dBrUMP-substituted mitochondrial DNA in yeast.

C. S. Newlon and R. D. Ludescher, Department of Zoology, University of Iowa, Iowa City: Production of petites by strains of yeast defective in DNA synthesis.

B. Y. Rubin and J. Blamire, Department of Biology, Brooklyn College, New York: Analysis of a mutant conditionally deficient in mitochondrial DNA metabolism.

H. S. Cross and H. Ruis, Institut für Allgemeine Biochemie der Universität Wien and Ludwig-Boltzmann-Forschungsstelle für Biochemie, Vienna, Austria: Catalase A deficiency of [*rho*]$^-$, [*mit*]$^-$, and nuclear petite mutants of *S. cerevisiae*.

J. R. Mattoon, D. R. Malamud, A. Brunner, G. Braz, and A. Panek, Department of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland, and Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, Brazil: Chemical and genetic modification of delta-aminolevulinic acid metabolism in yeast.

MONDAY MORNING, August 22

Session 10

Chairperson: R. R. Mortimer, University of California, Berkeley

S. Fogel, R. Mortimer, F. Tavares, and K. Lusnak, Departments of Genetics and Medical Physics, University of California, Berkeley: Gene conversion in yeast—Updated.

D. Campbell and C. Methot, Department of Biology, Holy Cross College, Worcester, Massachusetts: Cold-sensitive gene-conversion-deficient mutants of yeast.

J. F. Lemontt, Biology Division, Oak Ridge National Laboratory, Tennessee: Mutants of *S. cerevisiae* resistant to ultraviolet forward mutation—Defective in fixation or expression of mutagenesis?

V. L. MacKay and J. F. Lemontt,* Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey; *Biology Division, Oak Ridge National Laboratory, Tennessee: Pleiotropic mutations which affect mating and seemingly unrelated processes.

R. Rothstein and F. Sherman,* Department of Biochemistry, Cornell University, Ithaca, New York; *Department

of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: Genetic studies on mutations which affect iso-2-cytochrome c in yeast.

A. Toh-E and Y. Oshima, Department of Fermentation Technology, Faculty of Engineering, Osaka University, Japan: Genetic control of acid phosphatase synthesis.

F. Messenguy and T. G. Cooper,* Institut de Recherches du CERIA, Brussels, Belgium; *Department of Life Sciences, University of Pittsburgh, Pennsylvania: Evidence that the specific and general control of ornithine carbamoyltransferase production occurs at the level of transcription in *S. cerevisiae*.

R. Bigelis, J. Keesey,* and G. R. Fink,* Cold Spring Harbor Laboratory, New York; *Section of Genetics, Development, and Physiology, Cornell University, Ithaca, New York: The *his*4 multifunctional protein of yeast.

T. Cooper, University of Pittsburgh, Pennsylvania: Pleiotropic mutants defective in production of the inducible nitrogen catabolic enzymes.

E. W. Jones and P. G. Szauter, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Biological properties of *pep* mutants.

M. J. Miller, J. Bossinger, C. Herring, N. H. Xuong, and E. P. Geiduschek, Departments of Biology, Physics, and Chemistry, University of California, San Diego: Quantitation of yeast proteins separated by two-dimensional gel electrophoresis.

B. Byers, K. Shriver, and G. Goetsch, Departments of Genetics and Biochemistry, University of Washington, Seattle: Role of yeast spindle-pole bodies in the initiation of microtubule polymerization.

H. Greer and G. R. Fink, Department of Genetics, Development, and Physiology, Cornell University, Ithaca, New York: Unstable genetic elements carrying the *his*4 region.

BACTERIOPHAGE

arranged by
AHMAD I. BUKHARI, *Cold Spring Harbor Laboratory*
THOMAS R. BROKER, *Cold Spring Harbor Laboratory*

246 participants

TUESDAY EVENING, August 23

Session 1: Lambda—Integrative recombination and replication

R. Hoess and A. Landy, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Nucleotide sequence of the attachment site in λb2.

J. Engler and R.B. Inman, Department of Biochemistry and Biophysics Laboratory, University of Wisconsin, Madison: An electron microscope study of site-specific recombination in phage λ.

L. Enquist, R. Weisberg, and H. Nash, National Institutes of Health and NIMH, Bethesda, Maryland: Is a cross-strand exchange an intermediate in coliphage λ*int*-promoted recombination?

L. Enquist and R. Weisberg, National Institutes of Health, Bethesda, Maryland: More *int* protein is required for integrative than for excisive recombination of phage λ.

Y. Kikuchi and H. Nash, NIMH, National Institutes of Health, Bethesda, Maryland: Some properties of purified *int* protein.

A. Kikuchi and R. Weisberg, NICHHD, National Institutes of Health, Bethesda, Maryland: New mutants affecting site-specific recombination.

H. Nash, A. Kikuchi,* R. Weisberg,* H. Miller,† D.I. Friedman,† NIMH and *NICHHD, National Institutes of Health, Bethesda, Maryland; †University of Michigan, Ann Arbor: Biochemical studies of *E. coli* mutants defective in λ integration.

H.I. Miller and D.I. Friedman, Department of Microbiology, University of Michigan, Ann Arbor: Genetics of *E. coli* phage integration host factors.

J.B. Hays and S. Böhmer, Department of Chemistry, University of Maryland, Catonsville: Recombination of bacteriophage λ without phage gene expression.

M.E. Furth, McArdle Laboratory, University of Wisconsin, Madison: Elements of the λ replication system.

B.G. Williams, M.E. Furth,* K.E. Kruger, and F.R. Blattner, Department of Genetics and *McArdle Laboratory, University of Wisconsin, Madison: Oops—A deletion of oop RNA which supports replication of phage λ.

D.D. Moore, K. Denniston-Thompson, K.E. Kruger, M.E. Furth,* and F.R. Blattner, Department of Genetics and *McArdle Laboratory, University of Wisconsin, Madison: DNA sequence of the amino terminus of the O gene of λ.

K. Denniston-Thompson, D.D. Moore, K.E. Kruger, M.E. Furth,* and F.R. Blattner, Department of Genetics and *McArdle Laboratory, University of Wisconsin, Madison: DNA sequence of the λ origin.

D.L. Daniels and F.R. Blattner, Department of Genetics, University of Wisconsin, Madison: Construction of λ phage carrying a nonessential late promoter.

WEDNESDAY MORNING, August 24

Session 2A: Lambda—Regulatory systems

K. Calame and G. Ihler,* Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania; *Department of Medical Biochemistry, College of Medicine, Texas A & M University, College Station: Physical location of ribosome binding sites on λ DNA.

M. Belfort and A. Oppenheim, Department of Microbiological Chemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Analysis of phage λ functions in mutant *E. coli* hosts.

G.N. Gussin, T. Nelson, and D.L. Wulff,* Department of Zoology, University of Iowa, Iowa City; *Department of Molecular Biology and Biochemistry, University of California, Irvine: Formation of stable lysogens by λ*prm* 116*cin*1 phages.

G. Guarneros and T. Hernández, Department of Genetics, Centro de Investigacion del IPN, Mexico: A new class of phage λ cII⁻ pseudorevertants.

M.G. Beher and D.L. Wulff, Department of Molecular Biology and Biochemistry, University of California, Irvine: Effects of the *cin*1 and *cnc*1 mutations on *P* gene expression in phage λ.

K.Z. McCullough and G.R. Smith, Institute of Molecular Biology, University of Oregon, Eugene: Lambda mutants affecting the control of repressor synthesis.

D.I. Friedman, P.K. Tomich, and P.R. Holton, Department of Microbiology, University of Michigan, Ann Arbor: Molecular structure of λ *alt*A phage exhibiting alternating immunities.

E. Rosen, K.M. Yen, and G. Gussin, Department of Zoology, University of Iowa, Iowa City: Characterization of *prm*⁻ mutations in bacteriophage λ.

A. Bailone and R. Devoret, Radiobiologie Cellulaire, Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: Isolation of ultravirulent mutants of phage λ.

N.E. Melechen, G. Go, and H.A. Lozeron, Departments of Microbiology and Biochemistry, St. Louis University School of Medicine, Missouri: Thymineless and spontaneous induction of λ lysogens are affected by the levels of cI repressor.

M. Lieb, Department of Microbiology, University of Southern California School of Medicine, Los Angeles: Heat-sensitive λ repressor retains partial activity during induction.

J.W. Roberts, C.W. Roberts, and N.L. Craig, Department of Biochemistry, Cornell University, Ithaca, New York: Biochemical mechanism of λ induction.

A. Bailone, A. Levine, and R. Devoret, Radiobiologie Cellulaire, Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: Inactivation of the λ repressor in overproducing cells of *E. coli* K12.

C.L. Smith and M. Oishi, Department of Genetics, Public Health Research Institute of the City of New York: DNA degradation and temperate phage repressor inactivation.

M.G. Schechtman and J.W. Roberts, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Activity of late gene regulators of λ and φ82.

Y. Takeda and H. Echols, Department of Molecular Biology, University of California, Berkeley: In vitro studies of the λ *cro* gene product (II).

Session 2B: Lambda—N gene function

M.C. Gawron and J.R. Christensen, Department of Microbiology, University of Rochester, New York: "Early" exclusion of T1 requires λ *N* gene product and host factors involved in *N* gene expression.

D.A. Wilder and H.A. Lozeron, Department of Biochemistry, St Louis University School of Medicine, Missouri: Processing and chemical decay of the *N*-dependent ($1_3'$) RNA transcript of bacteriophage λ.

M. Gottesman, S. Adhya, A. Das, and D. Court, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Control of transcription termination in *E. coli*.

C. Dambly, M. Gottesman,* and S. Adhya,* Department de Biologie Moléculaire, Université Libre de Bruxelles, Rhode-St.-Genèse, Belgium; *NCI, National Institutes of Health, Bethesda, Maryland: Control of gene expression in the λ (*cro*-cII-*O*-*P*-*Q*) operon.

D. Court, D. Wulff,* C. Brady, and M. Rosenberg, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland; *Department of Molecular Biology and Biochemistry, University of California, Irvine: Transcription termination in phage λ at ᵗR1.

M. Rosenberg, D. Court, C. Brady, J.S. Salstrom,* and W. Szybalski,* Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland; *McArdle Laboratory, University of Wisconsin, Madison: The nucleotide sequence for the *N* recognition sites of coliphage λ.

J. Shaw and M. Pearson, Department of Medical Genetics, University of Toronto, Ontario, Canada: The N protein of bacteriophage λ and its synthesis in host mutants defective in *N*-gene expression.

N.C. Franklin and G.N. Bennett, Department of Biological Sciences, Stanford University, California: DNA sequencing of the *N* gene of bacteriophage λ.

WEDNESDAY EVENING, August 24

Session 3: P1 and Mu

S. Austin, N. Sternberg, L. Rosner,* and M. Yarmolinsky, Basic Research Program, Frederick Cancer Research Center, Frederick, Maryland; *NIAMDD, National Institutes of Health, Bethesda, Maryland: Miniplasmids of bacteriophage P1.

N. Sternberg, M. Yarmolinsky, and S. Austin, Basic Research Program, Frederick Cancer Research Center, Frederick, Maryland: The control of P1-P7 immunity—A model for *ant* action.

N. Sternberg, Basic Research Program, Frederick Cancer Research Center, Frederick, Maryland: An initial characterization of λ-P1 hybrids.

J.R. Scott, R.H. Chesney, and D. Vapnek,* Department of Microbiology, Emory University, Atlanta, Georgia; *Department of Microbiology, University of Georgia. Athens: Integrative suppression of *E. coli dna Ats* by phages P1 and P7.

R.J. Mural and D. Vapnek, Program in Genetics and Departments of Zoology and Microbiology, University of Georgia, Athens: Transcription of bacteriophage P1.

R. Kahmann and D. Kamp, Cold Spring Harbor Laboratory, New York: Characterization of the Mu modification function *(mom)*.

D. Kamp and R. Kahmann, Cold Spring Harbor Laboratory, New York: Inversion of the G segment of bacteriophage Mu is a site-specific recombination event.

D. Kamp, J.M. Scott, R. Kahmann, and L.T. Chow, Cold Spring Harbor Laboratory, New York: Inversion of the G segment of phage Mu by phage P1.

L.T. Chow, D. Kamp, and R. Kahmann, Cold Spring Harbor Laboratory, New York: Identification of two insertion sequences in nondefective Mu substitution mutants.

K. O'Day, D. Schultz, and M. Howe, Department of Bacteriology, University of Wisconsin, Madison: A search for integration-deficient mutants of bacteriophage Mu.

K. O'Day, D. Schultz, W. Ericsen, L. Rawluk, and M. Howe, Department of Bacteriology, University of Wisconsin, Madison: Correction and refinement of the genetic map of bacteriophage Mu.

G.S. Gill, R.A. Hull, and R. Curtiss III, Department of Microbiology, University of Alabama, Birmingham: Physical and genetic characterization of the DNA of a Mu-like phage D108.

M. Magazin and B. Allet, Département de Biologie Moléculaire, Université de Géneve, Switzerland: In vitro synthesis of Mu proteins.

H. Khatoon and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: Turning on genes with Mu.

B.T. Waggoner and M.L. Pato, National Jewish Hospital and Research Center and University of Colorado Medical Center, Denver: Early replication of Mu prophage DNA.

L. Ambrosio, A.I. Bukhari, F. de Bruijn, M.S. DuBow, and E. Ljungquist, Cold Spring Harbor Laboratory, New York: Studies on Mu DNA replication and integration.

THURSDAY MORNING, August 25

Session 4: Genetics/Transcription

P. Youderian and J. King, Massachusetts Institute of Technology, Cambridge: Isolation and characterization of ϕ80 amber mutants.

B.M. Scher, M.F. Law, and A.J. Garro, Department of Microbiology, Mt. Sinai School of Medicine, New York: Structural analysis of the *Bacillus* phage ϕ105 genome.

J.M. Cregg and C.R. Stewart, Department of Biology, Rice University, Houston, Texas: Terminal redundancy of "high frequency of recombination" cistrons of *B. subtilis* phage SPO1.

G.M. Weinstock and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: Genetic and physical studies of bacteriophage P22 using translocatable drug-resistance elements.

S. Hilliker, M. Gottesman, and S. Adhya, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: The role of *Salmonella* phage P22 gene-24 product in *E. coli*.

M.M. Susskind, Department of Microbiology, University of Massachusetts Medical School, Worcester: A new regulatory gene of phage P22.

H.H. Prell, Department of Molecular Genetics, Gesellschaft für Strahlem Forschung, Göettingen, West Germany: The role of ant product of *Salmonella* phage P22 in transactivation of prophages PX1 and L.

L. Souza, J. Geisselsoder, A. Hopkins, and R. Calendar, Molecular Biology Department, University of California, Berkeley: Physical mapping of the satellite phage P4 genome.

J.D. Harris and R. Calendar, Molecular Biology Department, University of California, Berkeley: Transcriptional regulation of satellite phage P4 gene expression.

C. Talkington, G. Lee, N. Hannett, and J. Pero, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Restriction-fragment analysis of the transcriptional specificity of phage SPO1-modified RNA polymerase.

R. Zivin, S.C. Falco, K. Vanderlaan, J. Stambouly, and L.B. Rothman-Denes, Department of Biophysics and Theoretical Biology, University of Chicago, Illinois. Analysis of bacteriophage N4 transcription.

B.A. Hesselbach and D. Nakada, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: I protein—T7 phage gene-2-coded inhibitor of *E. coli* RNA polymerase.

I.I. Martinez, J.D. Harris, and R. Calendar, Molecular Biology Department, University of California, Berkeley: The map location of an *E. coli* gene affecting the sigma subunit of DNA-dependent RNA polymerase.

THURSDAY AFTERNOON, August 25

Informal poster session

THURSDAY EVENING, August 25

Session 5: Morphogenesis: P22 and lambda

E. Jackson, C. Andres, and F. Laski, Department of Microbiology, University of Michigan, Ann Arbor: Mutants which alter specificity of P22 DNA packaging.

A.R. Poteete and D. Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge: Purification of P22 gene-2 product.

P.B. Berget and A.R. Poteete, Department of Biology, Massachusetts Institute of Technology, Cambridge: Purification and properties of *Salmonella* phage P22 baseplate protein.

S. Casjens, Microbiology Department, University of Utah Medical Center, Salt Lake City: Control of P22 gene-9 protein synthesis.

J.L. Bryant, Jr. and J. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: 9-Aminoacridine-sensitized photoinactivation of bacteriophage P22.

T. Hohn and B. Hohn, Department of Microbiology, Biozentrum, University of Basel, Switzerland: A protease specific for cutting bacteriophage λ scaffold protein.

H. Murialdo, Department of Medical Genetics, University of Toronto, Ontario, Canada: Early intermediates in λ prohead assembly.

H. Murialdo and A. Becker, Department of Medical Genetics, University of Toronto, Ontario, Canada: Genetic analysis of λ prohead assembly in vitro.

S. Benchimol, H. Murialdo, M. Gold, and A. Becker, Department of Medical Genetics, University of Toronto, Ontario, Canada: Studies of the λ F1 gene product in DNA packaging and maturation in vitro.

J. Yochem, R. Fisher, M. Sunshine, and M. Feiss, Department of Microbiology, University of Iowa, Iowa City: Analysis of the *groP-C* region of *E. coli*.

K. Krizsanovich-Williams, K. Chen, J. Donelson, and M. Feiss, Departments of Microbiology and Biochemistry, University of Iowa, Iowa City: *cos*$^{-}$ Lambda prophage—isolation and properties.

J.O. Thomas, N. Sternberg,* and R. Weisberg,* Department of Biochemistry, New York University Medical School, New York; *NICHHD, National Institutes of Health, Bethesda, Maryland: Altered arrangement of the DNA in injection-defective λ.

P. Granboulan, Department of Microbiology, University of Basel, Switzerland, and Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: Light minor proteins of λ.

FRIDAY MORNING, August 26

Session 6: Morphogenesis: P2, P4, and T phage

D.W. Bowden and R. Calendar, Department of Molecular Biology, University of California, Berkeley: Partial purification and characterization of P2 "Ter" components.

D. Shore, C. Diana, and R. Goldstein, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Genetic and biochemical studies of a capsid-size determination mutant of satellite phage P4.

C. Diana, D. Shore, and R. Goldstein, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Diploid and triploid progeny of the capsid-size direction mutant of satellite phage P4—An analysis of the DNA packaging substrate and mature tandem DNA.

Y. Yamada, J. Silnutzer, and D. Nakada, Department of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: A host mutant which blocks T7 phage assembly.

B. Kemper, Institut für Genetik der Universität Köln, Weyertal, West Germany: Purification and preliminary characterization of a gene-49-controlled nucleolytic activity of bacteriophage T4.

L.W. Black, Department of Biological Chemistry, University of Maryland Medical School, Baltimore: Studies on the mechanism of DNA packaging into phage T4 heads.

C.L. Hsiao and L.W. Black, Department of Biochemistry, University of Maryland, Baltimore: The role of gene 40 in bacteriophage T4 head morphogenesis.

L. Onorato, B. Stirmer, and M. Showe, Department of Microbiology, Biozentrum, University of Basel, Switzerland: Expanded and unexpanded preheads produced by mutants in T4 head genes.

J. Tschopp and P.R. Smith, Departments of Biophysical Chemistry and Microbiology, Biozentrum, University of Basel, Switzerland: Extra long T4 tails produced under in vitro conditions.

W. Earnshaw, J. King, S.J. Harrison,* and F.A. Eiserling,† Department of Biology, Massachusetts Institute of Technology, Cambridge, *Department of Chemistry, Harvard University, Cambridge, Massachusetts; †Department of Molecular Biology, University of California, Los Angeles: Orientation of DNA packing in T4 giant phage.

P.B. Berget and J.A. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: Characterization of T4 baseplate precursors.

S.C. Kayman and J.A. King, Department of Biology, Massachusetts Institute of Technology, Cambridge: Suppressors of temperature-sensitive gene-53 product in phage T4D.

A. Musafi, M. Baylor, and H. Fong, New York University Medical School, New York, and Biology Department, State University of New York, Stony Brook: Conservation of some proteins of T-even phage.

FRIDAY AFTERNOON, August 26

Session 7: T phages—Nucleotide and nucleic acid metabolism

H.R. Warner, B.K. Duncan, T.J. Mozer, and R.B. Thompson, Department of Biochemistry, University of Minnesota, St. Paul: Synthesis and properties of bacteriophage containing uracil in their DNA.

E. Kutter, B. Guttman, R. Schenck, B. Morton, R. Laiken, and E. Mosier, Evergreen State College, Washington: Physiological effects of *alc* mutations.

C.G. Goff, Biology Department, Haverford College, Pennsylvania: Genetics of T4 proteins affecting *E. coli* RNA polymerase after infection.

K. Sirotkin and L. Snyder, Department of Microbiology, Michigan State University, East Lansing: The biological role of a T4-induced DNA phosphate shuttling enzyme.

D.H. Hall, K. Trofatter,* and D.L. Russel,* School of Biology, Georgia Institute of Technology, Atlanta; *Department of Biochemistry, Duke University Medical Center, Durham, North Carolina: Suppressors of mutations in gene 63 of bacteriophage T4.

T.J. Snopek, W.B. Wood,* and N.R. Cozzarelli, Department of Biochemistry, University of Chicago, Illinois; *Division of Biology, California Institute of Technology, Pasadena: T4 RNA ligase is gene-63 product, the protein that promotes tail-fiber attachment to the phage baseplate.

W.M. Huang, Department of Microbiology, University of Utah Medical Center, Salt Lake City: Initiation proteins of DNA replication in T-even bacteriophage-infected *E. coli* cells.

A.W. Kozinski, Department of Human Genetics, University of Pennsylvania, Philadelphia: The gene-32 protein—A factor protecting the integrity of the replicative fork and assuring unbiased replication of both parental DNA strands.

G. Mosig, S. Bock, W. Ream, and C. Hornaday, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Multiple interactions of phage-T4 gene 32.

B.R. Baser and J.S. Wiberg, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, New York: Separation of the two roles of protein 45 of bacteriophage T4D—Temperature-insensitive DNA replication in vivo by a triple mutant of T4 synthesizing a temperature-sensitive g45 protein.

P.D. Sadowski, L. Roberts, and R. Sheldon, Department of Medical Genetics, University of Toronto, Ontario, Canada: Properties of the in vitro phage T7 recombination reaction.

J.J. Donegan and R. Sternglanz, Department of Biochemistry, State University of New York, Stony Brook: Host- and phage-mediated resistance to nalidixic acid (NAL) and novobiocin during coliphage T7 infection.

H.R. Revel and I. Leilausis, Division of Biology, California Institute of Technology, Pasadena: Corrected location of the rII gene on the genetic map of bacteriophage T4.

SATURDAY MORNING, August 27

Session 8: RNA phages, Transducing phages, and other phage systems

A.B. Jacobson, Department of Microbiology, State University of New York, Stony Brook: Electron microscopic mapping of loops on single-stranded RNA from the bacteriophage MS2.

A. Rimon and R. Haselkorn, Department of Biophysics and Theoretical Biology, University of Chicago, Illnois: RNA synthesis during bacteriophage $\phi 6$ infection.

E. Goldman, W.M. Holmes, and G.W. Hatfield, Department of Medical Microbiology, University of California, Irvine: RNA-phage-specific protein synthesis in vitro with $tRNA^{leu}$ isoaccepting species—Codon-anticodon recognition.

A.R. Shaw and D.J. McCorquodale, Department of Biochemistry, Medical College of Ohio, Toledo: Mutants of BF23 with deletions in their teminally redundant regions.

J.N. Reeve and G. Mertens, Max-Planck-Institut für Molekulare Genetik, Berlin, West Germany: Early development of bacteriophages SPO1 and SP82G in minicells of *B. subtilis*.

J.A. Mayo and M. Ehrlich, Department of Microbiology, Louisiana State University Medical Center, and Department of Biochemistry, Tulane Medical Center, New Orleans: Not all thymine-containing phage DNAs can be labeled with radioactive thymidine.

F. Murillo, S. Martin, C. Manoil, and D. Kaiser, Department of Biochemistry, Stanford University Medical School, California: The use of bacteriophage to study cell-cell interactions in the development of myxobacteria.

C.K. Dasgupta and A. Guha, Department of Microbiology, Erindale College, University of Toronto, Ontario, Canada: Isolation and characterization of the biotin genes of *E. coli* K12.

K.N. Kreuzer and N.R. Cozzarelli, Department of Biochemistry and the Committee on Genetics, University of Chicago, Illinois: Isolation of λ specialized transducing phages for the *nalA* region of *E. coli*.

A.M. Carothers, S. Palchaudhuri, T. Eckhardt, and E. McFall, Department of Microbiology, New York University School of Medicine, New York: Isolation of a λ*pdsd* phage and its use for physical mapping of the D-serine deaminase operon.

C.A. Irwin, G. Fletcher, J.M. Henson, and J.R. Walker, Department of Microbiology, University of Texas, Austin: Characterization of λ phages which carry the cell division-cell envelope genes near minute two on the *E. coli* map.

L.D. Borchert and H. Drexler, Department of Microbiology and Immunology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina: T1 genes which affect transduction.

J.A. Mayo, H.-W. Ackermann, and L. Berthiaume, Department of Microbiology, Louisiana State University Medical Center, New Orleans; Faculty of Medicine, Laval University, Quebec, Canada; Institut Armand-Frappier, Lavaldes-Rapides, Quebec, Canada; Bacteriophage Subcommittee, ICTV: Standardization of bacteriophage descriptions—Preliminary suggestions.

SINGLE-STRANDED DNA PHAGES

arranged by
DAVID T. DENHARDT, *McGill University*

135 participants

SATURDAY EVENING, August 27

Session 1: In vitro replication

Chairperson: D.T. Denhardt, McGill University, Montreal, Quebec, Canada

S. Wickner, National Institutes of Health, Bethesda, Maryland: Conversion of single-stranded phage DNA to double-stranded replicative forms in vitro.

S.L. Rowen, R. McMacken, and A. Kornberg, Department of Biochemistry, Stanford University School of Medicine, California: Initiation of replication by *dnaG* primase.

L.J. Reha-Krantz and J. Hurwitz, Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: The *dnaB*-gene product of *E. coli*.

D. Capon and M. Gefter, Department of Biology, Massachusetts Institute of Technology, Cambridge: Initiation of φX174 replicative form synthesis in vitro.

S. Eisenberg, J.F. Scott, and A. Kornberg, Department of Biochemistry, Stanford University, California: An enzyme system for replication of duplex circular φX174 RF and synthesis of circular single-stranded viral DNA.

C. Sumida-Yasumoto, Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: φXRFI DNA-dependent synthesis of φXRFI and φX174 viral DNA in vitro.

K. Geider, V. Berthold, M. Abdel-Monem, and H. Hoffman-Berling, Max-Planck-Institut für Medizinische Forschung, Heidelberg, West Germany: Functions of DNA-binding and unwinding proteins.

M.L. Bayne and L.B. Dumas, Department of Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois: In vitro DNA synthesis on the isolated strands of phage f1 RF DNA.

Session 2: In vivo replication I

Chairperson: I. Tessman, Purdue University, West Lafayette, Indiana

P.D. Baas, W.R. Teertstra, W. Keegstra, and H.S. Jansz, Institute of Molecular Biology and Laboratory for Physiological Chemistry, State University, Utrecht, The Netherlands: Bacteriophage ϕX174 RF DNA replication in vivo.

K. Koths and D. Dressler, Harvard University, Cambridge, Massachusetts: An electron microscopic analysis of the ϕX DNA replication cycle.

A. Sugino, C.L. Peebles, K.N. Kreuzer, and N.R. Cozzarelli, Departments of Biochemistry, Biophysics, and Theoretical Biology, and Committee on Genetics, University of Chicago, Illinois: Mechanism of action of nalidixic acid and the interrelationship of the *nalA*-gene product, DNA gyrase, and a novel nicking-closing enzyme.

K. Horiuchi, Rockefeller University, New York, New York: f1 RFIV in vivo.

S. Dasgupta and S. Mitra, University of Tennessee Graduate School of Biomedical Science, Oak Ridge, and Biology Division, Oak Ridge National Laboratory, Tennessee: Structure of nascent RF DNA of coliphage M13.

B.E. Kessler-Liebscher, W.L. Staudenbauer, and P.H. Hofschneider, Max-Planck-Institut für Biochemie, Munich, West Germany: Replication of M13 duplex DNA in plasmolysed cells.

C. Hours and D. Denhardt, Department of Biochemistry, McGill University, Montreal, Quebec, Canada: ϕX174 RF DNA as a probe of *E. coli* DNA metabolism.

A. Taketo and K. Kodaira, Department of Biochemistry, Kanazawa University School of Medicine, Ishikawa, Japan: Properties of microvirid phages with special reference to their host-factor requirements.

T. Komano, H. Sakai, Y. Mano, K. Watabe, and Y. Takahashi, Department of Agricultural Chemistry, Kyoto University, Japan: Requirement for *dnaH*, *dnaI*, and *dnaP* functions in the growth of ϕX174 and ϕX174 DNA synthesis in vivo.

C.E. Dowell, Department of Microbiology, University of Massachusetts, Amherst: Change in a requirement for DNA synthesis in host-range mutants of bacteriophages St1 and ϕX174.

SUNDAY EVENING, August 28

Session 3: In vivo replication II

Chairperson: D. Dressler, Harvard University, Cambridge, Massachusetts

F.C. Wheeler, E. Johnson, and R. Benzinger, Department of Biology, University of Virginia, Charlottesville: Abnormal forms of fd phage and fd phage DNA.

M. Ohsumi, G.F. Vovis, and N.D. Zinder, Rockefeller University, New York, New York: Isolation and characterization of f1 transducing particles.

J. Chen and D. Ray, Molecular Biology Institute, University of California, Los Angeles: Replication of bacteriophage M13 and its miniphage particles in *E. coli polA* ex1.

M. Farber and D.S. Ray, Molecular Biology Institute, University of California, Los Angeles: Clear mutants of phage M13 affecting DNA replication.

V. Enea, J. Bergelson, and N.D. Zinder, Rockefeller University, New York, New York: Interference-resistant mutants of f1.

A. Razin, A. Friedmann, and J. Friedman, Department of Cellular Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel: The role of DNA methylation in excision of one-genome-long ϕX174 DNA.

T.J. Lerner and P. Model, Rockefeller University, New York, New York: RF synthesis late in f1 infection.

R.K. Poddar, C.K. Dasgupta, U. Bandopadhyaya, and A.R. Thakur, Biophysics Section, Physics Department, University College of Science, Calcutta, India: Repair of lesions on single-stranded DNA of phage ϕX174.

J. Das and J. Maniloff, Department of Microbiology, University of Rochester Medical Center, New York: Replication of a single-stranded DNA mycoplasma virus—In vivo synthesis of virus-specific proteins.

Session 4: DNA sequence studies

Chairperson: R.N.H. Konings, University of Nijmegen, The Netherlands

G.N. Godson and J.C. Fiddes, Medical Research Council Laboratory of Molecular Biology, Cambridge, England: Sequencing G4 DNA.

D. Hourcade, J. Sims, and D. Dressler, Harvard University, Cambridge, Massachusetts: The site-specific initiation of a DNA fragment.

A.D.M. van Mansfeld, S.A. Langeveld,* P.J. Weisbeek,* P.D. Baas,† H.S. Jansz,† and G.A. van Arkel,* Institute of Molecular Biology, *Laboratory for Molecular Cell Biology, †Laboratory for Physiological Chemistry, State University, Utrecht, The Netherlands: Nucleotide sequence at the replication origin of ϕX174 RF.

K. Sugimoto, H. Sugisaki, T. Takeya, T. Okamoto, and M. Takanami, Institute for Chemical Research, Kyoto University, Japan: Sequence in a region covering genes X, V, VII, and VIII of bacteriophage fd.

J.V. Ravetch, K. Horiuchi, and N.D. Zinder, Rockefeller University, New York, New York: Nucleotide sequence of a region related to the initiation of bacteriophage f1 DNA replication.

J.A. Lautenberger, C.A. Hutchison III, M.H. Edgell, S. Linn,* and D. Lackey,* Department of Bacteriology, University of North Carolina, Chapel Hill; *Department of Biochemistry, University of California, Berkeley: Localization of the nucleotide sequence recognized by the *E. coli* B modification enzyme on the replicative form of ϕX174sB-1.

J.H. Spencer, E. Rassart, F. Grosveld, and B. Goodchild, Department of Biochemistry, McGill University, Montreal, Quebec, Canada: Location and sequence of the *B*-gene promoter in S13.

B.L. Smiley and R.C. Warner, Department of Molecular Biology and Biochemistry, University of California, Irvine: Heteroduplex analysis and comparison of G4 and ϕX174 genomes.

J. French, I. Tessman, and E.S. Tessman, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Genetics of G4.

C.A. Hutchison III, M.B. Edgell, M. Smith, S. Gillam, P. Jahnke, and E. Trip, Department of Bacteriology and Immunology, University of North Carolina, Chapel Hill, and Department of Biochemistry, University of British Columbia, Vancouver: Techniques for specific alteration of the ϕX174 nucleotide sequence.

MONDAY AFTERNOON, August 29

Session 5: Transcription/Translation/Proteins

Chairperson: H.S. Jansz, State University, Utrecht, The Netherlands

T.J. Pollock and I. Tessman, Purdue University, West Lafayette, Indiana: Radiological mapping of functional transcription units of bacteriophages ϕX174 and S13.

T.J. Pollock, I. Tessman, and E.S. Tessman, Purdue University, West Lafayette, Indiana: Multiple products of genes *C*, *A*, and *G* of phage ϕX174 and read-through of the TGA codon.

T.J. Pollock and E.S. Tessman, Purdue University, West Lafayette, Indiana: Two sets of the four gene-*C* products of ϕX174 and an assembly function of the gene-*C* product.

M.F. Shemyakin, E.A. Stukacheva, E.L. Kapitza, I.I. Patrushev, and M.M. Shemyakin, Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow: Studies on ϕX174 genome expression in vitro.

P.D. Baas, W. Keegstra, W.R. Teertstra, and H.S. Jansz, Institute of Molecular Biology and Laboratory for Physiological Chemistry, State University, Utrecht, The Netherlands: R loops in bacteriophage ϕX174 RF DNA.

J.G.G. Schoenmakers, R.N.H. Konings, and L. Edens, Institute of Molecular Biology, University of Nijmegen, The Netherlands: Mapping of promoters on the bacteriophage M13 genome.

M. LaFarina and P. Model, Rockefeller University, New York, New York: Transcription in f1-infected *E. coli*.

P. Model, R.E. Webster, C. McGill, and M. Rementer, Duke University Medical Center, Durham, North Carolina: Rockefeller University, New York, New York: Gene-V protein affects the synthesis of gene-II protein.

P.J. Weisbeek, W.E. Borrias, and G.A. van Arkel, Laboratory for Molecular Cell Biology, State University, Utrecht, The Netherlands: The gene *A* of ϕX174.

L. Dubeau and D. Denhardt, Department of Biochemistry, McGill University, Montreal, Quebec, Canada: Purification and properties of the ϕX174 gene-*A* protein.

E.S. Tessman and P.K. Peterson, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Genetic recombination of phage S13 in a host which overproduces the *recA* protein.

TUESDAY MORNING, August 30

Session 6: Structure and morphogenesis

Chairperson: D.S. Ray, University of California, Los Angeles

D.A. Marvin, European Molecular Biology Laboratory, Heidelberg, West Germany: Structure of the filamentous phage virion.

L.A. Day, R.L. Wiseman, F.-C. Chen, and G. Koopmans, Public Health Research Institute, New York, New York: DNA packing in three filamentous viruses, fd, Pf1, and Xf.

B. Chamberlain, R. Grant, J. Woolford, T. Yen, and R. Webster, Department of Biochemistry, Duke University Medical Center, Durham, North Carolina: Interaction of the f1 and fd coat protein with membranes.

K. Hodgson, T. Lin, W. Konigsberg, and R. Webster, Department of Biochemistry, Duke University Medical Center, Durham, North Carolina; Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Isolation and characterization of a small protein from the f1 filamentous bacteriophage.

N.L. Incardona, Microbiology Department, University of Tennessee Health Science Center, Memphis: Genetic selection technique for eclipse mutants of ϕX174.

H. Fujisawa and M. Hayashi, Department of Biology, University of California, San Diego: Assembly of bacteriophage ϕX174—Identification of a virion capsid precursor and proposal of a model on the functions of bacteriophage gene products during morphogenesis.

R. Mukai and M. Hayashi, Department of Biology, University of California, San Diego: Synthesis of infectious ϕX174 bacteriophage in vitro.

J. Messing, B. Gronenborn, B. Müller-Hill, and P.H. Hofschneider, Max-Planck-Institut für Biochemie, Munich, and Institut für Genetik, Universität zu Köln, West Germany: The filamentous phage M13 as a receptor molecule for in vitro recombined DNA.

SV40, POLYOMA, AND ADENOVIRUSES

arranged by
TERRI GRODZICKER, *Cold Spring Harbor Laboratory*
MICHAEL BOTCHAN, *Cold Spring Harbor Laboratory*

279 participants

WEDNESDAY EVENING, August 31

Session 1: SV40 and polyoma—Genetics

Chairperson: J. Mertz, University of Wisconsin, Madison

L. Crawford, C. Cole, and P. Tegtmeyer, Department of Biochemistry, Stanford University Medical Center, California, and Department of Microbiology, State University of New York, Stony Brook: Proteins of the SV40 early region.

N. Bouck, N. Beales, G. di Mayorca, T.E. Shenk,* C. Cole,† and P. Berg,† Department of Microbiology, University of Illinois Medical Center, Chicago; *University of Connecticut Health Center, Farmington; †Department of Biochemistry, Stanford University Medical School, California: Transformation-defective viable deletion mutants of SV40.

M.J. Sleigh, W.C. Topp, and J. Sambrook, Cold Spring Harbor Laboratory, New York: Viable deletion mutants of SV40 restricted in their ability to induce transformation.

M. Fried, Y. Ito, and M. Griffiths, Imperial Cancer Research Fund Laboratories, London, England: T antigens of early-region deletion mutants of polyoma virus.

Y. Ito and J.R. Brocklehurst, Department of Cell Regulation, Imperial Cancer Research Fund Laboratories, London, England: NG-18 fails to induce virus-specific proteins in the plasma membrane of infected 3T6 cells.

J.Y. Chou, Section on Developmental Enzymology, NICHHD, National Institutes of Health, Bethesda, Maryland: Human placental cells transformed by tsA mutants of SV40 and grown at a restrictive temperature behave like normal placental cells.

M. Fluck and T. Benjamin, Pathology Department, Harvard Medical School, Boston, Massachusetts: Properties of ts-a/A mutants of polyoma and SV40 in cell transformation.

B. Steinberg, R. Pollack, K. Nyman, A. Lo, W. Topp,* and M. Botchan,* Department of Microbiology, State University of New York, Stony Brook; *Cold Spring Harbor Laboratory, New York: T-antigen-negative revertants from transformed rat cells carrying one copy of SV40 DNA.

D. Zouzias and C. Basilico, Department of Pathology, New York University School of Medicine, New York: Regulation of viral functions in SV40-transformed mouse cells.

THURSDAY MORNING, September 1

Session 2: SV40 and polyoma—Proteins

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

T.D. Kempe, W.G. Beattie, and W. Konigsberg, Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Correlation of the nucleotide and protein sequences for major structural protein of SV40 virus.

S.G. Siddell and A.E. Smith, Imperial Cancer Research Fund Laboratories, London, England: Polyoma virus has three late mRNAs, one for each virion protein.

J. Silver, B. Schaffhausen, and T.L. Benjamin, Department of Pathology, Harvard Medical School, Boston, Massachusetts: The size of T antigen in wild-type polyoma virus and an hr-t deletion mutant.

C.A.F. Edwards and R.G. Martin, Laboratory of Molecular Biology, NIAMDD, National Institutes of Health, Bethesda, Maryland: Turnover of phosphorylated T antigen in SV40-transformed CHL cells.

C. Lawrence, K. Mann, T. Hunter, and G. Walter, Tumor Virology Laboratory, Salk Institute, San Diego, California: Characterization of SV40-specific mRNA from HeLa cells infected with nondefective Ad2-SV40 hybrid viruses.

E. Paucha and A.E. Smith, Imperial Cancer Research Fund Laboratories, London, England: Cell-free synthesis of 94K and 17K T antigens.

C. Prives and Y. Beck, Department of Virology, Weizmann Institute of Science, Rehovot, Israel: Characterization of SV40 T-antigen polypeptides.

D.M. Livingston, D.G. Tenen, A.P. Modest, A. Maxam, and W. Gilbert, Sidney Farber Cancer Institute, Harvard Medical School, Boston, and The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Chemical and DNA binding properties of extensively purified SV40 T antigen.

R. Tjian, G. Fey, and A. Graessmann,* Cold Spring Harbor Laboratory, New York; *Freie Universität, Berlin, West Germany: SV40 T antigen and its related proteins from adenovirus-SV40 hybrids.

THURSDAY AFTERNOON, September 1

Session 3: Poster session on SV40 and polyoma

N.H. Acheson, Department of Virology, Swiss Institute for Experimental Cancer Research, Epalinges, Lausanne: Extent of transcription of the E strand of polyoma DNA during the early phase of infection.

P. Beard, Department of Virology, Swiss Institute for Experimental Cancer Research, Epalinges, Lausanne: Movement of histones on DNA.

M. Bina-Stein, NCI, National Institutes of Health, Bethesda, Maryland: Folding of DNA by arginine-rich histones.

S. Bratosin, O. Laub, and Y. Aloni, Weizmann Institute of Science, Rehovot, Israel: Characterization of transcriptional complexes of SV40 and determination of the initiation sites for transcription.

R.B. Carroll, D.S. Greenspan, S.M. Goldfine, and W.F. Mangel,* Department of Pathology, New York University Medical Center, New York; *Department of Biochemistry, University of Illinois, Urbana: Immunogenicity and multiple isoelectric focusing forms of SV40 T antigen and comparison of the tryptic fingerprints of T antigens synthesized in vivo and in vitro.

C. Chang, S.W. Luborsky, and P.T. Mora, Macromolecular Biology Section, NCI, National Institutes of Health, Bethesda, Maryland: Biological and biochemical properties of SV40-induced TSTA.

N. Chiu and N.P. Salzman, Laboratory of Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland: The metabolism of SV40 RNA in cultured BSC-1 cells studied with a rapid uridine pulse-chase.

B. Cogen and W. Eckhart, Tumor Virology Laboratory, Salk Institute, San Diego, California: Virus-specific early RNA in cells infected by temperature-sensitive mutants of polyoma virus.

H.J. Edenberg, S. Anderson, and M.L. DePamphilis, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Identification of the DNA polymerases involved in SV40 DNA replication.

G. Fey, A. Bothwell,* and A. Graessmann,† Cold Spring Harbor Laboratory, New York; *Massachusetts Institute of Technology, Cambridge; †Freie Universität, Berlin, West Germany: Helper function is provided by a small carboxy-terminal domain of SV40 T antigen.

M. Fiori, M.M. Pater, and G. di Mayorca, Department of Microbiology, University of Illinois Medical Center, Chicago: BK virus genome in transformed cells and in human tumors.

W.R. Folk and J.E. Bancuk, Department of Biological Chemistry, University of Michigan, Ann Arbor: Polyoma DNA synthesis in ts-a-transformed BHK 21 cells.

A.H. Fried, Institute für Virusforschung, Deutsches Krebsforschungszentrum, Heidelberg, West Germany: Relative rates of synthesis of the mRNA and non-mRNA sequences from the L strand of SV40 DNA late after infection.

M. Green, J.K. Mackey, and W.S.M. Wold, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Absence of BK virus DNA from DNA extracted from human tumors and malignant cell lines.

P. Hale and J. Lebowitz, Department of Microbiology, University of Alabama Medical Center, Birmingham: In vitro transcription of SV40 DNA by *E. coli* RNA polymerase—Effect of chemical modification of DNA on binding of RNA polymerase.

J.R. Hartman, O. Laub,* Y. Aloni,* and E. Winocour, Departments of Virology and *Genetics, Weizmann Institute of Science, Rehovot, Israel: Transcription of the host DNA sequences in substituted SV40.

R. Kamen, J. Favaloro, and M. Fried, Imperial Cancer Research Fund Laboratories, London, England: In vivo transcription of the defective polyoma virus D50.

G. Ketner and T.J. Kelly, Jr., Departments of Biology and Microbiology, Johns Hopkins University, Baltimore, Maryland: The structure of integrated SV40 DNA in transformed cells.

M. Botchan, S. Weirich, and W. Topp, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: The integration of SV40 DNA and its rescue from rat chromosomal DNA.

H. Manor, Z. Lev, and R. Kamen,* Department of Biology, Technion, Haifa, Israel; *Department of Molecular Virology, Imperial Cancer Research Fund Laboratories, London, England: Mapping of polyoma-specific RNA sequences within poly(A)-containing giant RNA molecules isolated from polyoma-infected cells.

R.C. Moyer, M.P. Moyer, G. Lipotich, H. Hurtado, and D. Davis, Thorman Cancer Research Laboratory, Trinity University, San Antonio, Texas: Maintenance of SV40 DNA fragments in permissive monkey cells.

L.W. Mozes and V. Defendi, Department of Pathology, New York University School of Medicine, New York: The effect of interferon on SV40 T-antigen production.

B. Otto and E. Fanning, Fachbereich Biologie, University of Konstanz, West Germany: Replication enzymes in SV40 nucleoprotein complexes.

C. Prives, Y. Gluzman, and E. Winocour, Weizmann Institute of Science, Rehovot, Israel: Cellular and cell-free synthesis of T antigen in permissive and transformed cells.

S. Riva, M.A. Cline, and P.J. Laipis, Department of Biochemistry, University of Florida, Gainesville: Effect of SV40 infection on DNA polymerase activity in monkey cells.

G. Sauer, K. Bosslet, and W. Waldeck, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Heidelberg, West Germany: A new transforming papovavirus (HD virus).

A. Sen and G.J. Todaro, Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: DNA binding specificity of proteins purified from BALB/3T3 cells transformed by DNA tumor viruses and by chemical carcinogens.

D.T. Simmons, K.K. Takemoto, and M.A. Martin, Laboratory of Biology of Viruses and Laboratory of Viral Diseases, National Institutes of Health, Bethesda, Maryland: Relationships between papovavirus tumor antigens.

T. Takano, Department of Microbiology, Keio University School of Medicine, Tokyo, Japan: Expression of baboon endogenous virus genome in human diploid cells transformed by SV40.

W.C. Topp, Cold Spring Harbor Laboratory, New York: Transformation of rat cells by restriction-endonuclease-generated fragments of SV40.

H. Türler and C. Salomon, Department of Molecular Biology, University of Geneva, Switzerland: Polyoma T antigen.

R. Yang and R. Wu, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: BK virus DNA—Physical maps and sequences analysis.

THURSDAY EVENING, September 1

Session 4: Genome structure

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

B. Thimmappaya, V.B. Reddy, R. Dhar, K.N. Subramanian,* B.S. Zain,† and S.M. Weissman, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; *University of Chicago Medical Center, Illinois; †Cold Spring Harbor Laboratory, New York: Nucleotide sequence of the early region of SV40 DNA.

V.B. Reddy, J. Pan, M. Celma, R. Dhar, K.N. Subramanian,* and S.M. Weissman, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; *University of Chicago Medical Center, Illinois: Nucleotide and mRNA sequences from the late region of SV40 DNA.

T. Friedmann, Medical Research Council Laboratory of Molecular Biology, Cambridge, England, and Department of Pediatrics, University of California, San Diego: Nucleotide sequences around the origin of replication and start of mRNA transcription in polyoma DNA.

H. Rosenberg, M.F. Singer,* and M. Rosenberg, Laboratory of Molecular Biology and *Laboratory of Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: $\sim 3.5 \times 10^8$ Base pairs of the monkey genome—Nucleotide sequence, characterization, and occurrence in defective SV40.

M.W. Gutai and D. Nathans,* Department of Medical Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York; *Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Nucleotide sequences at recombinant joints of SV40 variants.

F.J. O'Neill and D. Carroll, Veterans Administration Hospital, Research Service, and Department of Microbiology, University of Utah, Salt Lake City: Characterization of defective SV40 DNA generated and amplified in infected human glioblastoma cells, A172.

T. Shenk, Department of Microbiology, University of Connecticut Health Center, Farmington: Construction of a viable variant of SV40 which contains two functional origins of DNA replication.

S. Goldberg and V. Defendi, Department of Pathology, New York University School of Medicine, New York: Phenotypic and genotypic changes in doubly transformed cells.

A.J. van der Eb, Sylvius Laboratories, University of Leiden, The Netherlands: Recent studies on adenovirus and SV40 transformation genes.

FRIDAY MORNING, September 2

Session 5: Adenoviruses: Transcription

Chairperson: M. Green, St. Louis University, Missouri

S.M. Berget, A.J. Berk, and P.A. Sharp, Center for Cancer Research. Massachusetts Institute of Technology, Cambridge: Spliced segments at the 5′ termini of Ad2 late mRNA.

D.F. Klessig, Cold Spring Harbor Laboratory, New York: Two adenovirus mRNAs have a common 5'-terminal leader sequence which is encoded at least 10 kilobases upstream from the main coding regions for these messengers.

A.R. Dunn and J.A. Hassell, Cold Spring Harbor Laboratory, New York: Evidence for homology between individual adenovirus mRNAs and multiple discrete areas of the viral genome.

L.T. Chow, R.E. Gelinas, T.R. Broker, and R.J. Roberts, Cold Spring Harbor Laboratory, New York: 5' Leader sequences on late adenovirus mRNAs.

G. Kitchingman, S.-P. Lai, H.C. Meissner, and H. Westphal, NICHHD, National Institutes of Health, Bethesda, Maryland: Nonadjacent sequences of virion DNA are linked in early cytoplasmic adenovirus transcripts.

A.J. Berk, S.M. Berget, and P.A. Sharp, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Biosynthesis of the Ad2 early mRNAs.

N.W. Fraser and E. Ziff, Department of Molecular Cell Biology, Rockefeller University, New York, New York: Analysis of 3'-terminal regions of late Ad2 mRNAs.

R.M. Evans, N.W. Fraser, and E. Ziff, Department of Molecular Cell Biology, Rockefeller University, New York, New York: Promotion of transcription of Ad2.

M. McGrogan and H.J. Raskas, Departments of Pathology and Microbiology, Washington University School of Medicine, St. Louis, Missouri: Two regions of the Ad2 genome specify families of late mRNA containing common sequences.

FRIDAY AFTERNOON, September 2

Session 6: Poster session adenoviruses

M. Arens and T. Yamashita, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: In vitro termination of adenovirus DNA synthesis by a soluble replication complex.

J. Arrand, Division of Virology, National Institute of Medical Research, London, England: Analysis of adenovirus mutants using DNA infection techniques.

S. Bachenheimer, Department of Bacteriology and Immunology, University of North Carolina, Chapel Hill: A tentative intermediate in the processing of adenovirus nuclear precursor RNA into cytoplasmic mRNA.

D.H. Coombs and G.D. Pearson, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: Filter-binding assay for the adenovirus covalent DNA-protein complex.

F. Eggerding, D. Spector, M. McGrogan, and H.J. Raskas, Departments of Pathology and Microbiology, Washington University School of Medicine, St. Louis, Missouri: Effect of protein-synthesis inhibitors on viral mRNAs synthesized early in Ad2 infection.

M.S. Farber and S.G. Baum, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Transcription and translation of adenovirus mRNA in nonpermissive infection of monkey cells.

S.J. Flint and H. Weintraub, Princeton University, New Jersey: An altered subunit configuration associated with actively transcribed DNA of the integrated adenovirus genome.

E. Frolova, E. Zalmanzon, and E. Lukanidin, Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow: Transcription of fragments of viral DNA in Ad5-transformed cell lines.

K. Fujinaga, K. Sekikawa, and S. Ojima, Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College, Japan: Analysis of Ad7 genome by restriction endonuclese *Hin*dIII.

K. Fujinaga, S. Yano, Y. Sawada, S. Ojima, K. Shiroki, and H. Shimojo, Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College, and Department of Tumor Virus Research, Institute of Medical Science, University of Tokyo, Japan: Transforming DNA segments of Ad12.

D. Galloway, E. Lukanidin,* W. Topp, and J. Sambrook, Cold Spring Harbor Laboratory, New York; *Academy of Sciences of the USSR, Moscow: Transformation of rat cells by the hybrid virus Ad2^{++}HEY.

R.F. Gesteland, N. Wills, J.B. Lewis, and T. Grodzicker, Cold Spring Harbor Laboratory, New York: Nonsense mutants of Ad2^{++}ND1.

M.L. Harter and J.B. Lewis, Cold Spring Harbor Laboratory, New York: Comparison of adenovirus-specific early proteins synthesized in human cells and in vitro.

D. Kühn and W. Doerfler, Institute of Genetics, University of Cologne, West Germany: Integration patterns of Ad12 DNA in transformed hamster cells.

J.K. Mackey, M. Green, and W.S.M. Wold, Institute for Molecular Virology, St. Louis University School of

Medicine, Missouri: Grouping of human adenovirus serotypes according to DNA and transforming DNA fragment homology.

I. Mak, J. Smiley, S. Mak, and F. Graham, Biology Department and Pathology Department, McMaster University, Hamilton, Ontario, Canada: Transformation of rat cells by DNA fragments of human Ad12 DNA.

J.L. Manley and R.F. Gesteland, Cold Spring Harbor Laboratory, New York: Characterization of ribosome-protected regions of Ad2 late mRNAs.

M.B. Mathews and U. Pettersson,* Cold Spring Harbor Laboratory, New York; *Biomedical Center, Uppsala, Sweden: The gene for polypeptide IX.

T. Mullenbach, D. Groff, and E. Daniell, Department of Molecular Biology, University of California, Berkeley: Adenovirus DNA replication—Generation of defective genomes of serotypes 2, 3, and 5 in permissive and nonpermissive cells.

K. Nass and G.D. Frenkel, Department of Microbiology and Immunology, Albany Medical College, New York: Adenovirus-induced inhibition of cellular DNase III.

B.M. Paterson, E.L. Kuff, B.E. Roberts,* and M.B. Matthews,† National Cancer Institute, Bethesda, Maryland; *Brandeis University, Waltham, Massachusetts; †Cold Spring Harbor Laboratory, New York: Mapping messenger RNAs to adenovirus DNA by hybrid arrested cell-free translation.

U. Pettersson and M.B. Matthews,* Biomedical Center, Uppsala, Sweden; *Cold Spring Harbor Laboratory, New York: Virus-associated RNAs—Enumeration and mapping of their genes.

E. Lukanidin,* T. Grodzicker, J. Lewis, D. Galloway, and J. Sambrook, *Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow; Cold Spring Harbor Laboratory, New York: Revertants of host-range mutants of $Ad2^{+}ND1$.

J. Schick, E. Fanning, and W. Doerfler, Institute of Genetics, University of Cologne, West Germany: Factors affecting the amount of integrated viral DNA sequences in cells productively infected with Ad2.

A. Sergeant and H.J. Raskas, Departments of Pathology and Microbiology, Washington University School of Medicine, St. Louis, Missouri: Chromatinlike organization of the Ad2 genome early in the lytic cycle.

K. Shiroki, H. Handa, H. Shimojo, S. Yano, S. Ojima, and K. Fujinaga, Department of Tumor Virus Research, Institute of Medical Science, University of Tokyo, and Department of Molecular Biology, Cancer Research Institute, Sapporo, Japan: Establishment and characterization of rat cell lines transformed by restriction endonuclease fragments of Ad12 DNA.

M.J. Sleigh and J. Sambrook, Cold Spring Harbor Laboratory, New York: Interserotypic recombination—A mechanism for adenovirus evolution?

J. Weber and J. Hassell,* Département de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada; *Cold Spring Harbor Laboratory, New York: Mapping of Ad2 mutants and structural genes.

FRIDAY EVENING, September 2

Session 7: SV40 and polyoma—Transcription

Chairperson: L. Crawford, Imperial Cancer Research Fund Laboratories, London, England

Y. Aloni, R. Dhar, O. Laub,* M. Hurowitz,* and G. Khoury, Laboratory of DNA Tumor Viruses, NCI, National Institutes of Health, Bethesda, Maryland; *Weizmann Institute of Science, Rehovot, Israel: A novel mechanism for RNA maturation—The leader sequences of SV40 mRNA are not transcribed adjacent to the coding sequences.

S. Lavi and Y. Groner, Virology Department, Weizmann Institute of Science, Rehovot, Israel: The capped 5′ terminus of SV40 16S mRNA is coded by DNA sequences not adjacent to VP-1 coding region.

J.P. Ford and M.T. Hsu, Rockefeller University, New York, New York: Mapping the 5′ ends of late SV40 mRNA using a new technique.

J.E. Mertz, J.B. Gurdon,* and E.M. De Robertis,* McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; *Laboratory of Molecular Biology, Medical Research Council, Cambridge, England: Characterization of the viral products synthesized in *Xenopus* oocytes injected with SV40 DNA.

T. White, L. Villarreal, C. Cole, and P. Berg, Department of Biochemistry, Stanford University Medical Center, California: Molecular weights of SV40 RNAs and a specific map position for nuclear polyadenylation.

M. Grigoryan, P. Chumakov, E. Lukanidin, and G. Georgiev, Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow: SV40 gene expression in transformed cells.

E. Birkenmeier, N. Chiu, M. Radonovich, and N.P. Salzman, Laboratory of Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland: Control of early and late SV40 RNA synthesis without concurrent viral DNA synthesis.

R. Condit, A. Cowie, R. Kamen, and M. Fried, Imperial Cancer Research Fund Laboratories, London, England: Transcription of a defective polyoma virus genome.

C.-K.J. Shen and J.E. Hearst, Department of Chemistry, University of California, Berkeley: SV40 DNA—The identification of symmetric sequences and the stabilization of transcription complexes by psoralen photochemical crosslinking.

SATURDAY MORNING, September 3

Session 8: Adenoviruses—Genetics and proteins

Chairperson: J. Williams, Carnegie-Mellon University, Pittsburgh, Pennsylvania

N. Jones and T. Shenk, Department of Microbiology, University of Connecticut Health Center, Farmington: Isolation of deletion and substitution mutants in Ad5.

E. Frost and J. Williams,* Institute of Virology, Glasgow, Scotland; *Department of Biological Sciences, Mellon Institute, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Mapping temperature-sensitive and host-range mutations of Ad5 by marker rescue.

N.J. Lassam, F.L. Graham, and S.T. Bayley, Department of Biology, McMaster University, Hamilton, Ontario, Canada: Production of virus-specific proteins by host-range mutants of Ad5.

W.S.M. Wold, Y.-H. Jeng, and M. Green, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Studies on Ad2-induced early and transformation proteins.

J.H. Lupker, A. Davis, H. Jochemsen, and A.J. van der Eb, Sylvius Laboratories, University of Leiden, The Netherlands: Identification and partial characterization of "transforming" proteins of Ad5.

W.C. Russell, G.E. Blair, D.K. Rekosh, and C.H. Shaw, Division of Virology, National Institute for Medical Research, London, England: Studies on regulatory mecnanisms in adenovirus infection.

H. Handa, K. Shiroki, and H. Shimojo, Department of Tumor Virus Research, Institute of Medical Science, University of Tokyo, Japan: A helper factor(s) for growth of adeno-associated virus in cells transformed by Ad12.

A. Mirza and J. Weber, Département de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada: Core structure and decapsidation of a temperature-sensitive mutant of Ad2.

R.M.L. Buller and J.A. Rose, Laboratory of Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland: Mechanism of host restriction of adeno-associated virus replication in African green monkey kidney cells.

SATURDAY AFTERNOON, September 3

Session 9: Adenoviruses—Replication

Chairperson: T.J. Kelly, Jr., Johns Hopkins University, Baltimore, Maryland

J. Arrand and R.J. Roberts,* Imperial Cancer Research Fund Laboratories, London, England; *Cold Spring Harbor Laboratory, New York: The nucleotide sequences of the termini of Ad2 DNA.

R. Lechner and T.J. Kelly, Jr., Johns Hopkins University School of Medicine, Baltimore, Maryland: Electron microscopy of replicating Ad2 DNA molecules.

B. Weingärtner, T. Reiter, and E.-L. Winnacker, Institute of Biochemistry, University of Munich, West Germany: Two terminal initiation sites for Ad2 DNA replication.

J.S. Sussenbach, P. Steenbergh, and T. Kuijk, Laboratory for Physiological Chemistry, State University, Utrecht, The Netherlands: Initiation of adenovirus DNA replication.

A.J. Robinson and G.D. Pearson, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: Replicating adenovirus molecules contain terminal protein.

L.M. Kaplan and M.S. Horwitz, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Selective extraction of Ad2 DNA from infected nuclei—A soluble system for the replication of viral DNA.

C. Tibbetts, Department of Microbiology, University of Connecticut School of Medicine, Farmington: Expression of the Ad7 genome in cells infected by virions and incomplete particles.

E. Fanning and W. Doerfler, Institute of Genetics, University of Cologne, West Germany: Nature and occurrence of high-molecular-weight DNA in productively infected cells.

J.L. Corden and G.D. Pearson, Department of Biochemistry and Biophysics, Oregon State University, Corvallis: Adenovirus chromatin.

SUNDAY MORNING, September 4

Session 10: SV40 and polyoma—Replication

Chairperson: M. Fried, Imperial Cancer Research Fund Laboratories, London, England

M.T. Hsu and R. Fernandez-Munoz, Rockefeller University, New York, New York: Isolation and characterization of SV40 nucleoprotein complexes isolated from SV40-infected CV-1 cells.

M.A. Waqar, M.J. Evans, D. Kowalski, Y. Tsubota, B.I. Milavetz, and J.A. Huberman, Department of Medical Viral Oncology and Enzymology Laboratory, Roswell Park Memorial Institute, Buffalo, New York: Enzymes associated with replicating SV40 chromosomes.

E. Shelton, K. Goh, J. Kang, P. Wassarman, and M. DePamphilis, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Structure and replication of SV40 chromatin.

J. Reiser, L.V. Crawford, and G.R. Stark, Department of Biochemistry, Stanford University Medical Center, California: Association of SV40 T antigen with SV40 chromatin.

M.K. Hobish and V. Pigiet, Johns Hopkins University, Baltimore, Maryland: Polyoma chromatin replication in vitro—Template specificity of *Xenopus* replication factors.

F. Birg, R. Kamen,* and M. Fried, INSERM, Marseille, France; *Imperial Cancer Research Fund Laboratories, London, England: Characterization of the viral DNA in polyoma-virus-transformed rat cells.

S. Gattoni, D. Zouzias, and C. Basilico, Department of Pathology, New York University School of Medicine, New York: Nonintegrated viral DNA in rat cells transformed by polyoma virus.

P. Bourgaux, L. Delbecchi, K. Yu, and D. Bourgaux-Ramoisy, Département de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada: Mouse embryo cell line carrying an inducible polyoma virus genome.

M.A. Israel, H. Chan, and M.A. Martin, Laboratory of Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland: Biologic activity of polyoma DNA in animals.

DIFFERENTIATION OF NORMAL AND NEOPLASTIC HEMATOPOIETIC CELLS

arranged by
BAYARD CLARKSON, *Memorial Sloan-Kettering Cancer Center*
PAUL A. MARKS, *Columbia University*
JAMES TILL, *University of Toronto*

129 participants

TUESDAY EVENING, September 6

Opening: J.D. Watson, Cold Spring Harbor Laboratory

Introductory remarks: J. Till, University of Toronto

Session 1: Ontogeny of hematopoietic development and stem cells I

Chairperson: J. Till, Ontario Cancer Institute, University of Toronto, Canada

N. Le Douarin, Institut d'Embryologie du CNRS et du Collège de France, Nogent-sur-Marne, France: Ontogeny of hematopoietic organs studied in avian embryo interspecific chimeras.

I. Weissman, V. Papaioannou,* and R. Gardner,* Department of Pathology, Stanford University Medical Center, California; *Department of Zoology, Oxford University, England: Fetal hematopoietic origins of the adult hematolymphoid system.

G.R. Johnson and D. Metcalf, Cancer Research Unit, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia: In vitro clonal analysis of fetal hepatic hematopoiesis.

T.M. Dexter, Paterson Laboratories, Christie Hospital, and Holt Radium Institute, Withington, Manchester, England: In vitro analysis of self-renewal and commitment of hematopoietic stem cells.

J.E. Till, S. Lan, R. Buick, J.E. Curtis, and E.A. McCullock, Ontario Cancer Institute, Toronto, Canada: Approaches to the evaluation of human hematopoietic stem-cell function.

WEDNESDAY MORNING, September 7

Session 2: Stem cells II and committed erythroid precursor cells

Chairperson: P.A. Marks, Columbia University, New York, New York

R.A. Phillips, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Canada: Stem-cell heterogeneity—Pluripotent and committed stem cells of the myeloid and lymphoid systems.

J.W. Byron, Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore: Histamine H_2 receptor and the colony-forming unit—spleen.

A.A. Axelrad and D.L. McLeod, Division of Histology, Department of Anatomy, University of Toronto, Canada: Regulation of population size of erythropoietic progenitor cells.

G. Van Zant and E. Goldwasser, Department of Biochemistry and Franklin McLean Memorial Research Institute, University of Chicago, Illinois: The competitive effects of erythropoietin and colony stimulation factor on hematopoietic cells.

N.N. Iscove and L.J. Guilbert,* Basel Institute for Immunology, Switzerland; *Friedrich Miescher-Institut, Basel, Switzerland: Regulation of proliferation and maturation at early and late stages of erythropoiesis.

C.J. Gregory and A.C. Eaves, British Columbia Cancer Foundation, Vancouver, Canada: In vitro studies of erythropoietic progenitor cell differentiation.

D. Housman, R. Geller, R. Levenson, and J. Gusella, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Clonal analysis of in vitro differentiation of murine erythroleukemia cells.

R.A. Rifkind, E. Fibach, R.C. Reuben, H. Yamasaki, I.B. Weinstein, M. Terada, U. Nudel, Y. Gazitt, and P.A. Marks, Department of Medicine and Human Genetics and Development, Columbia University, New York, New York: Erythroleukemia cells—Commitment to differentiate and the role of the cell surface.

WEDNESDAY EVENING, September 7

Session 3: Erythrocyte differentiation and regulation

Chairperson: V.M. Ingram, Massachusetts Institute of Technology, Cambridge

P.A. Marks, R.A. Rifkind, A. Bank, M. Terada, R. Reuben, R. Breslow, E. Fibach, U. Nudel, Y. Gazitt, and J. Salmon, Cancer Research Center, Columbia University College of Physicians and Surgeons, New York, New York: Induction of murine erythroleukemia cells to differentiate—Cell-cycle-related events in expression of erythroid differentiation.

J.W. Adamson, J.E. Brown, and W.J. Popovic, Hematology Research Laboratory, Veterans Administration Hospital, and University of Washington, Seattle: Modulation of in vitro erythropoiesis.

A. Bernstein and D.L. Mager, Ontario Cancer Institute, Toronto, Canada: Early membrane events in Friend-cell erythroid differentiation.

J.R. Neumann, M.G. Riggs, H.K. Hagopian, L.A. Swartz, and V.M. Ingram, Department of Biology, Massachusetts Institute of Technology, Cambridge: Chromatin changes in Friend erythroleukemia and HeLa cells during treatment with DMSO and n-butyrate.

H. Eisen, S. Sassa,* D. Granick,* and F. Keppel,† Institut Pasteur, Paris, France; *Rockefeller University, New

York, New York; †University of Geneva, Switzerland: Two interdependent developmental programs expressed in induced Friend cells.

A. Nienhuis, D. Axelrod, J. Barker, E. Benz, Jr., R. Croissant, D. Miller, and N. Young, Clinical Hematology Branch, NHLBI, National Institutes of Health, Bethesda, Maryland: Regulation of the individual globin genes.

M. Obinata, R. Kameji, Y. Uchiyama, and Y. Ikawa, Laboratory of Viral Oncology, Cancer Institute, Tokyo, Japan: Sequential gene expression during induced differentiation of cultured Friend erythroleukemia cells.

THURSDAY MORNING, September 8

Session 4: Granulocyte and monocyte differentiation and regulation

Chairperson: M. Moore, Memorial Sloan-Kettering Cancer Center, New York, New York

A.W. Burgess, D. Metcalf, and S. Russell, Cancer Research Unit, Walter and Eliza Hall Institute of Medical Research, Parkville, Australia: Regulation of hematopoietic differentiation and proliferation by colony stimulating factors.

M.J. Cline, T. Olofsson, M. Frölich, D.W. Golde, and S. Herman, Department of Medicine, University of California School of Medicine, Los Angeles: Inhibitors of granulopoiesis.

G.B. Price and R.L. Krogsrud, Ontario Cancer Institute, Toronto, Canada: Use of molecular probes for detection of human hematopoietic progenitors.

Z.A. Cohn, Laboratory of Cellular Physiology and Immunology, Rockefeller University, New York, New York: The differentiation and activation of mononuclear phagocytes.

M.A.S. Moore, Memorial Sloan-Kettering Cancer Center, New York, New York: Regulatory interactions in normal and leukemic myelopoiesis.

P.L. Greenberg and B. Mara, Department of Medicine, Stanford Medical Center, and Veterans Administration Hospital, Palo Alto, California: Microenvironmental influences on granulopoiesis in acute myeloid leukemia.

L. Sachs, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: Control of normal cell differentiation and malignancy in myeloid leukemia.

THURSDAY EVENING, September 8

Session 5: Lymphocyte differentiation and regulation

Chairperson: H. Cantor, Harvard Medical School, Boston, Massachusetts

H. Cantor, Department of Medicine, Harvard Medical School, Boston, Massachusetts: Lymphocyte differentiation and regulation of the immune system.

G. Sundharadas, C.E. Hayes, M.L. Sopori, P.R. Narayanan, B.J. Alter, M.L. Bach, and F.H. Bach, Immunobiology Research Center, and Departments of Medical Microbiology, Pediatrics, Medical Genetics, and Surgery, University of Wisconsin, Madison: Differentiation of precursor cytotoxic T lymphocytes following alloantigenic stimulation.

G. Goldstein, Ortho Pharmaceutical Corporation, Raritan, New Jersey: Polypeptides regulating lymphocyte differentiation.

A.E. Silverstone, N. Rosenberg, V.L. Sato,* M.P. Scheid,† E.A. Boyse,† and D. Baltimore, Massachusetts Institute of Technology, Cambridge; *The Biological Laboratories, Harvard University, Cambridge, Massachusetts; †Memorial Sloan-Kettering Cancer Center, New York, New York: Tumor models for murine lymphoid progenitor cells.

J.L. Strominger, Department of Biochemistry and Molecular Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Cell surface antigens of human lymphocytes.

F. Melchers, Basel Institute for Immunology, Switzerland: B-lymphocyte development and growth regulation.

J.W. Uhr, J. Cambier, J. Kettman, F. Ligler, S. Strober,* I. Zan-Bar,* and E. Vitetta, Southwestern Medical School, Dallas, Texas; *Stanford University Medical School, Palo Alto, California: IgD and B-cell differentiation.

FRIDAY MORNING, September 9

Session 6: Viruses, transformation, and differentiation

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

W.P. Rowe, Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: The virology of AKR lymphomagenesis.

E.M. Scolnick, D.H. Troxler, T.Y. Shih, H.A. Young, and D. Linemeyer, NCI, National Institutes of Health, Bethesda, Maryland: A molecular model for the origin and nature of transforming genes of mammalian RNA sarcoma and leukemia viruses.

R.A. Lerner, J.H. Elder, and F.C. Jensen, Scripps Clinic and Research Foundation, La Jolla, California: The multigene family of endogenous proviruses encoding virion and differentiation antigens—Recombination between family members during neoplasia.

J. Rommelaere and N. Hopkins, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Recombinational origin of mink cell focus viruses as indicated by RNA fingerprinting and oligonucleotide mapping.

I. Weissman and M. McGrath, Department of Pathology, Stanford University Medical Center, California: A receptor-mediated model of viral leukemogenesis—Hypothesis and experiments.

R.A. Steeves and F. Lilly, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: The effect of $FV\text{-}2^r$ gene on spleen focus-forming virus and on embryonic development.

W.D. Hardy, Jr., E.E. Zuckerman, E.G. MacEwen, and M. Essex, Laboratory of Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York, and Department of Microbiology, Harvard University, Boston, Massachusetts: A feline leukemia and sarcoma-virus-induced tumor-specific antigen.

FRIDAY EVENING, September 9

Session 7: Viruses, transformation, and differentiation II

Chairperson: E.M. Scolnick, NCI, National Institutes of Health, Bethesda, Maryland

T. Graf, H. Beug, W. Meyer-Glauner and B. Royer-Pokora, Max-Planck-Institut für Virusforschung, Biologisch-Medizinische Abteilung, Tübingen, West Germany: Transformation of chick bone marrow cells by avian erythroblastosis virus—A new model system for normal and neoplastic erythroid differentiation.

S.D. Waksal, Department of Pathology and Cancer Research Center, Tufts School of Medicine, Boston, Massachusetts: Differentiation and leukemic transformation of thymus-derived lymphocytes.

N.M. Teich and T.M. Dexter,* Imperial Cancer Research Fund, London; *Paterson Laboratory, Manchester, England: The influence of murine leukemia virus infection upon hematopoietic differentiation of bone marrow cultures.

R. Gallo, R. Ruscetti, and R. Gallagher, NCI, National Institutes of Health, Bethesda, Maryland: Human leukocytes and type-C viral markers—Complex association signals the need for improved biological study systems.

H.S. Kaplan, Cancer Biology Research Laboratory, Department of Radiology, Stanford University Medical Center, California: Studies of an RNA virus isolated from a human histiocytic lymphoma cell line.

SATURDAY MORNING, September 10

Session 8A: Cytogenetics and expression of cell-surface antigens

Chairperson: J.D. Rowley, University of Chicago, Illinois

J.D. Rowley, Department of Medicine, University of Chicago, Illinois: Nonrandom involvement of chromosomal segments in human hematologic malignancies.

A. Skoultchi, S. Benoff, S. Bruce, P. Lin, H. Lonial, and J. Pyati, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Genetic control of hemoglobin production by murine erythroleukemic cells.

· I. Trowbridge, Salk Institute, San Diego, California: Genetic analysis of lymphocyte surface ynthesis.
ıor Roosevelt Institute for Cancer Research, Denver, Colorado: The role of cAMP and the cellular system in cancer.

. ırrow architecture and microenvironment

.M. Fliedner, University of Ulm, West Germany

and W. Calvo, Department of Clinical Physiology, University of Ulm, West Germany: ıetic stem-cell seeding of a cellular matrix—A principle of initiation and regeneration of :sis.
·son Laboratories, Christie Hospital, and Holt Radium Institute. Manchester, England: Cellular ctural factors influencing the proliferation of hematopoietic stem cells.
/, NCI, National Institutes of Health, Bethesda, Maryland: Orderly cell proliferation and ·on in relation to kinetic heterogeneity—Mouse bone marrow as an experimental model.

SATURDAY AFTERNOON, September 10

:linical-pathological relationships and differentiation of human hematopoietic tumors I

H.S. Kaplan, Stanford University School of Medicine, Palo Alto, California

·/, R. Pahwa, B. Dupont, and R.A. Good, The Marrow Transplantation Unit, Memorial tering Cancer Center, New York, New York: The use of fetal liver as a source of lymphoid stem ransplantation and immunologic reconstitution of severe combined immunodeficiency.
/, Leukaemia Unit, Medical Research Council, Royal Postgraduate Medical School, London, Cell characterization in leukemia by surface markers, cytochemistry, and ultrastructure.
ːs, G. Janossy, and J. Minowada, Department of Membrane Immunology, Imperial Cancer Research ıboratories, London, England: Antigenic and enzymatic phenotypes of human leukemic cells and hed leukemic cell lines.
r, R. Mertelsmann, and D. Filippa, Memorial Sloan-Kettering Cancer Center, New York, New York: ıymphoid neoplasias of T and null cell types.
nann, J.L. Preud'homme, and C.J. Brouet, Laboratory of Immunochemistry, Hôpital Saint-Louis, Paris, ce: Membrane markers in human lymphoid malignancies—Clinical-pathological correlations and ght into the differentiation of normal and neoplastic cells.
appaport, G.A. Pangalis, and B.N. Nathwani, Department of Anatomic Pathology, City of Hope National Medical Center, Duarte, California: The evolution of malignant lymphomas in morphologically non-neoplastic immunoproliferative diseases.

SUNDAY MORNING, September 11

Session 10: *Clinical-pathological relationships and differentiation of human hematopoietic tumors II*

Chairperson: K. Lennert, University of Kiel, West Germany

K. Lennert, E. Kaiserling, and H.-K. Müller-Hermelink, Department of Pathology, University of Kiel, West Germany: Malignant lymphomas—Models for differentiation and cooperation of lymphoreticular cells.
A.L. Epstein, S. Gartner, and H.S. Kaplan, Cancer Biology Research Laboratory, Department of Radiology, Stanford University Medical Center, California: In vitro cultivation and characterization of human malignant lymphomas.
R. Lukes, Department of Pathology, University of Southern California School of Medicine, Los Angeles: Immunological approach to malignant lymphomas.
P.J. Fialkow, Medical Service, Veterans Administration Hospital, and Departments of Medicine and Genetics, University of Washington, Seattle: Stem-cell and clonal origin of human hematopoietic neoplasms studied with G-6-PD markers.

Summary: B. Clarkson, Memorial Sloan-Kettering Cancer Center, New York, New York

POX VIRUSES

arranged by
JOSEPH KATES, *State University of New York, Stony Brook*
SAMUEL DALES, *University of Western Ontario, Canada*

54 participants

WEDNESDAY EVENING, October 12

Informal get-together and planning session

THURSDAY MORNING, October 13

Session 1: DNA Structure

C.V. Cabrera and M. Esteban, Department of Microbiology, Rutgers Medical School, Piscataway, New Jersey: A procedure for purification of intact DNA from vaccinia virus.

L.G. Gafford, E. Bruce Mitchell, Jr., and C.C. Randall, University of Mississippi Medical Center, Jackson: A comparison of sedimentation behavior of three poxvirus DNAs.

M. Soloski, E. Esteban, and J.A. Holowczak, Rutgers Medical School, Piscataway, New Jersey: Evidence for proteins linked to the termini of vaccinia DNA molecules.

A. Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey: Analysis of vaccinia DNA by reassociation kinetics.

G. Jaureguiberry, Institut de Recherche en Biologie Moléculaire du CNRS, Paris, France: Cleavage of vaccinia virus DNA by restriction endonuclease isolation of the cross-links.

R.J. McCarron, C.V. Cabrera, M. Esteban, W.T. McAllister, and J.A. Holowczak, Rutgers Medical School, Piscataway, New Jersey: Structure of vaccinia DNA—Analysis of the viral genome by restriction endonucleases.

THURSDAY AFTERNOON, October 13

Session 2: Replication

M. Esteban, C. Cabrera, and J.A. Holowczak, Department of Microbiology, Rutgers Medical School, Piscataway, New Jersey: Proposed model for vaccinia DNA replication.

B.G. Pogo, Public Health Research Institute of the City of New York, New York: The mode of replication of vaccinia virus DNA.

W.R. Bauer, E.G. Ressner, and J. Kates, Microbiology Department, State University of New York, Stony Brook: Properties of a topoisomerase and a superhelix-binding protein isolated from vaccinia virions.

A. Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey: The nature of the vaccinia-specific DNA and RNA found in the nucleus and cytoplasm of vaccinia-infected HeLa cells.

R. Goorha, G. Murti, and A. Granoff, St. Jude Children's Hospital, Memphis, Tennessee: The nucleus is a site of frog virus 3 DNA and RNA synthesis.

E. Katz, Chanock Centre for Virology, Hebrew University, Hadassah Medical School, Jerusalem, Israel: Biochemical and maturational events during the growth of vaccinia virus in the presence of isatin β-thiosemicarbazone.

W.H.R. Langridge, Boyce Thompson Institute, Yonkers, New York: In vitro entomopoxvirus protein synthesis in permissive cells.

Session 3: Transcription

D. Willis, R. Goorha, and A. Granoff, St. Jude Children's Hospital, Memphis, Tennessee: Transcriptional and posttranscriptional regulation of gene expression by frog virus 3.

M. Nowakowski,* J. Kates,† and W. Bauer,† *Downstate Medical Center, State University of New York, Brooklyn; †State University of New York, Stony Brook: Isolation of two DNA-binding proteins from the intracellular replication complex of vaccinia virus.

W.K. Joklik and J.R. Nevins, Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Isolation from infected cells and characterization of a DNA-dependent RNA polymerase and a poly(A) polymerase specified by vaccinia virus.

E. Paoletti, Division of Laboratories and Research, New York State Department of Health, Albany, New York: Under standard in vitro RNA polymerase reaction vaccinia virus rapidly depletes ATP.

M. Esteban, C.V. Cabrera, and J.A. Holowczak, Rutgers Medical School, Piscataway, New Jersey: Transcription of vaccinia DNA by *E. coli* RNA polymerase—Visualization and physical location of RNA transcripts.

C.V. Cabrera, M. Esteban, R. McCarron, W.T. McAllister, and J.A. Holowczak, Rutgers Medical School, Piscataway, New Jersey: Vaccinia virus transcription—Hybridization of mRNA to restriction fragments of vaccinia DNA.

R.F. Boone and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: Sequence complexity and relative abundance of vaccinia virus mRNAs synthesized in vivo and in vitro.

FRIDAY AFTERNOON, October 14

Session 4: Transcription/Translation/Immunological taxonomy

A. Gershowitz and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: Formation of high-molecular-weight RNA by vaccinia virus cores in the presence of β,γ- analogs of ATP.

E. Barbosa and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: Purification and characterization of an mRNA (nucleoside-2')-methyl transferase from vaccinia virions.

G. Monroy, E. Spencer, and J. Hurwitz, Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Characteristics of the reaction catalyzed by GTP:RNA guanylyl transferase isolated from vaccinia virus.

J.A. Cooper and B. Moss, NIAID, National Institutes of Health, Bethesda, Maryland: Translation of vaccinia virus mRNA coupled to transcription in vitro.

I. Horak and M. Kaerlein, Institut für Virologie und Immunbiologie der Universität Würzburg, West Germany: Vaccinia virus-induced modification of ribosomes in HeLa cells.

M. Schrom and R. Bablanian, Downstate Medical Center, State University of New York, Brooklyn: Inhibition of protein synthesis by vaccinia virus.

J.J. Esposito, J.F. Obijeski, and J.H. Nakano, Center for Disease Control, Atlanta, Georgia: Differentiation of variola, monkeypox, and vaccinia viruses by serological and biochemical assays.

SATURDAY MORNING, October 15

Session 5: Mutants, culture growth/Effects of virus on host function

S. Dales and G. McFadden, Department of Bacteriology and Immunology, University of Western Ontario, Canada: Biogenesis of vaccinia—Isolation of conditional lethal mutants and electron microscopic characterization of their phenotypically expressed defects.

R. Drillien, Laboratory of Virology, University of Strasbourg, France: Phenotypic characterization of two vaccinia virus ts mutants.

R. Raghow, St. Jude Children's Hospital, Memphis, Tennessee: Heat-inactivated frog virus 3.

R. Drillien, Laboratory of Virology, University of Strasbourg, France: Cell killing by frog virus 3.

A.-M. Aubertin, Laboratory of Virology, University of Strasbourg, France: Properties of proteins solubilized from Fv-3 particles—Inhibition of cellular DNA synthesis.

IN-HOUSE SEMINARS

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.

1976–1977

October

22nd Dr. Ron Reeder, Carnegie Institution of Washington, Baltimore, Maryland: *Studies on* Xenopus *18S and 28S transcription.*

November

12th Dr. John Coffin, Tufts University, Boston, Massachusetts: *Studies on RNA tumor viruses.*

15th Drs. Z. Goldberg and G. Weber, Rockefeller University, New York, New York: *Studies on adenovirus transcription.*

23rd Dr. Nina Fedoroff, Carnegie Institution of Washington, Baltimore, Maryland: *Sequence analysis of cloned 5S gene sequences from* Xenopus.

30th Dr. Ken Yamada, National Cancer Institute, Bethesda, Maryland: *Studies on the LETS protein.*

December

4th and 5th *Special seminar on developmental genetics of* Drosophila

Dr. W. Gelbart, Harvard University, Cambridge, Massachusetts
Dr. Jerry Rubin, Stanford University, California
Dr. John Postlethwaite, University of Oregon, Eugene
Dr. Michael Ashburner, University of Cambridge, England
Dr. Walter Gehring, University of Basel, Switzerland

7th Dr. Harold Weintraub, Princeton University, New Jersey: *Studies on chromatin.*

14th Dr. Keith Bachman, Harvard University, Cambridge, Massachusetts: *Studies on phage lambda genetics.*

February

3rd Dr. Peter Rae, Yale University, New Haven, Connecticut: *Studies on unusual bases in algae DNA.*

March

10th Dr. Karl Illmense, The Jackson Laboratories, Bar Harbor, Maine: Drosophila *and the problem of determination.*

29th Dr. Larry Gold, University of Colorado, Boulder: *The T4 gene-32 protein: A unique solution to a special problem.*

April

7th Dr. George Kuo, Yale University: *Maternal contributions to embryogenesis in* Drosophila.

22nd Dr. David Livingston, Sidney Farber Cancer Center, Boston, Massachusetts: *Recent studies on the SV40 T antigen.*

28th Dr. Clark Tibbetts, University of Connecticut, Farmington: *Genome structure and transcription of the adenoviruses.*

May

17th Dr. Jonathan Seidman, National Institutes of Health, Bethesda, Maryland: *The genes for IGG light chains cloned in bacterial plasmids.*

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, 184 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 is now mainly supported by Laboratory funds. They are listed below, with their laboratory sponsors and topics of research. We gratefully acknowledge the generosity of Central General Hospital, Plainview, New York, which provided additional support.

Vicki Lynn Brawley, University of California, Berkeley
Supervisor: N. Harter
Characterization of adenovirus early protein

Carol Clewans, Reed College
Supervisor: A.I. Bukhari
Mapping of a new gene controlling the synthesis of an unusual growth factor in *E. coli*

Jason S. Fisherman, Yale University
Supervisor: R. Roberts
In situ assays for restriction endonucleases

Robert Frank Hanich, Harvard University
Supervisor: R. Tjian
Big T and little t in deletion mutants of SV40

Cynthia L. Sammis, Wells College
Supervisor: L.B. Chen
Studies on the synthesis of LETS protein

Forrest Anne Spencer, Smith College
Supervisor: R. Kahmann
Microinjection into *X. leavis* oocytes

Eve Wolinsky, Massachusetts Institute of Technology
Supervisor: E. Cheng
Degradation of nonsense fragments of *E. coli*

Gary Yellen, Harvard University
Supervisor: J. Broach

Two-dimensional separation of DNA restriction fragments

Olney Fellowship

Iris Isabella Martinez, University of California, Berkeley
Supervisor: D. Zipser

The expression of cloned yeast DNA in *E. coli* minicells

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 the spring, summer, and fall, a total of 404 students participated in this program. When weather permitted, most of the courses were held outdoors on Laboratory grounds or at Uplands Farm Nature Preserve of the Nature Conservancy. The Laboratory has built and equipped classroom/laboratories at Uplands Farm for the study of field specimens collected by the students.

This spring a Long Island Field Geology course was conducted, which included trips to such sites as the Montauk Peninsula, North and South Shores of Long Island, and the State University of New York at Stony Brook. The instructor was Steven Englebright, Curator of Geology, SUNY at Stony Brook.

In the fall, Observational Astronomy was offered as an introduction to all facets of celestial observation. Photography as related to astronomy was emphasized, making use of our fully- equipped darkroom. Telescopes for the observation sessions were provided by Toy Town/Sports Town of Huntington. Equipment and technical assistance were provided by the Astronomical Society of Long Island and the Long Island Observers Association.

This summer a series of one-day Marine Biology Workshops was conducted on Long Island Sound aboard a 65-foot schooner, the R/V Tradewind, chartered from Schooner, Inc., of New Haven, Connecticut. The vessel was equipped with a variety of instrumentation and staffed by a captain, mate, and marine biologists. Students participated in the biological studies and in the actual sailing of the Tradewind.

Program director: Sanford Kaufman, M.S., M.P.A., biology teacher, Hewlett High School

INSTRUCTORS

Linda Bovich, M.S., science teacher, Rocky Point High School
Donn Dunn, M.S., art and photography teacher, Hewlett High School
Steven Englebright, M.S., curator of geology, SUNY at Stony Brook
Fred Maasch, M.Ed., biology teacher, Islip High School
James Romansky, M.S., biology teacher, Bay Shore High School
Edward Tronolone, M.S., science teacher, Lynbrook High School

COURSES

General Nature Study
Advanced Nature Study
Elementary Geology
Advanced Geology
Bird Study
Seashore Life
Long Island Field Geology
Vertebrate Biology
Reptiles and Amphibians
Aquatic Biology
Marine Biology
Nature Photography I and II
Fresh Water Life I and II
Observational Astronomy

LABORATORY STAFF

DECEMBER 1977

DIRECTOR
J.D. Watson

ADMINISTRATIVE DIRECTOR
William R. Udry

ASSISTANT DIRECTOR FOR RESEARCH
Raymond Gesteland

RESEARCH SCIENTISTS

Guenter Albrecht-Buehler
Michael Botchan
James Broach
Thomas Broker
Ahmad Bukhari
Keith Burridge
Lan Bo Chen
Louise Chow
George Fey
Richard Gelinas
Terri Grodzicker
Regine Kahmann
Dietmar Kamp
James Lewis
Thomas Maniatis
Michael Mathews
James McDougall
Richard Roberts
Joseph Sambrook
William Topp
Sayeeda Zain
David Zipser

VISITING SCIENTISTS

John Atkins
Ken Jones

POSTDOCTORAL FELLOWS

Ramunas Bigelis
Yih-Shyun Edmund Cheng
Michael DuBow
Ashley Dunn
Jeffrey Engler
Denise Galloway
Thomas Gingeras
Yakov Gluzman
Marian Harter
John Hassell
Hajra Khatoon
William Kilpatrick
Elisabeth Ljungquist
Walter Schaffner
Daniela Sciaky
Merilyn Sleigh
Jeffrey Strathern
Robert Tjian
Marcus Zabeau

GRADUATE STUDENTS

Daniel Klessig
Norman Maitland
James Manley
Gek-Kee Sim
Louise Silver

RESEARCH ASSISTANTS

Linda Ambrosio
Joseph Bonventre
Peter Bullock
Anne Bushnell
Angela Calasso
Chai-Yuh Cheng
Christine Copple
Frans de Bruijn
Barbara Doretsky
Laurel Garbarini
Geraldine Gavin
Ronni Greene
Robert Gudor
Barbara Hoffman
Lois Jordon
Randi Kelch
Robert Lancaster
Marjorie Lazarus
Sheila Levings
Anita Lewis
Bernice Lieberman
Theodore Lukralle
Carolyn McGill
Robert McGuirk
Karen Messina
Phyllis Myers
Rosemary Oliveros
Diana O'Loane

Rebecca Pashley
Elaine Paul
Patricia Reichel
John Scott
Leslie Smith
Caro-Beth Stewart
Margaret Wallace
Susanne Weirich
Ingrid Wendel
Norma Wills
Steven Young

SUPPORTING STAFF

Robert Borruso
Sallie Chait
Louisa D'Alessandro
Bruce DeTroy
Steven Eagels
Agnes Fisher
Maria Hedges
Marie Moschitta
Carilyn Mutt
Joyce Schneider
Madeline Szadkowski
Deborah Whitfield
Jane Wohlers
Robert Yaffe

ADMINISTRATIVE AND GENERAL STAFF

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Randy Clark
Joan Cook-Carpenter
Guy Cozza
Barbara Eggers
Ellen Farrant
Pam Freund
Terrie Goeke
Martin Henle
James Herb
Louis Iammuno
William Keen
Gladys Kist
Steve Kron
John Maroney
Susan Messina
Agnes Meyer
Dorothy Nilsson
Eileen Oates
Donna Walsh
Jeanne Wright

PUBLICATIONS DEPARTMENT

Paul Bethge
Virginia Cleary
Nancy Ford
Roberta Salant
Annette Zaninovic

LIBRARY STAFF

Carol Dana Chapin
Sarah Jaime Chapin
Susan Gensel
Deborah Gibson
Laura Hyman
Kenneth McElwain
Elizabeth Roberts

BUILDINGS AND GROUNDS DEPARTMENT

Robert Anderson
John Bach
Barry Becker
Karl Berg
Carol Caldarelli
Vincent Carey
Barbara Cuff
Carmen Dewan
Joseph Ellis
Stephen Fusaro
Robert Grundel
Douglas Haskett
Mary Hill
Robert Klipera
Julius Kulmayer
William Leyden
Thomas Lyden
Alfred Nickel
Tom O'Connell
Alfred Pfeiffer
Jack Richards
Lane Smith
Jerry Sorrentino
James Stanley
Owen Stewart
Hans Trede

GENETICS RESEARCH UNIT CARNEGIE INSTITUTION OF WASHINGTON

Alfred Hershey
Barbara McClintock

FINANCIAL STATEMENT

BALANCE SHEET
year ended October 31, 1977
with comparative figures for 1976

ASSETS

	1977	1976
CURRENT FUNDS		
Unrestricted		
Cash	$ 269,437	$ (68,573)
Marketable securities	—	85,903
Accounts receivable	89,366	66,739
Inventory of books	121,937	212,839
Prepaid expenses	68,704	33,921
Due from restricted fund	135,569	233,382
Total unrestricted	685,013	564,211
Restricted		
Grants receivable	2,396,218	728,974
Total restricted	2,396,218	728,974
Total current funds	3,081,231	1,293,185
ENDOWMENT FUNDS		
Robertson Research Fund		
Cash	96,492	82,144
Accrued interest	45,400	63,165
Marketable securities (quoted market 1977—$8,122,321; 1976—$8,290,395)	7,814,204	7,486,241
Total Robertson Research Fund	7,956,096	7,631,550
Olney Memorial Fund		
Cash	550	18,728
Marketable securities (quoted market $20,951)	21,510	—

LIABILITIES AND FUND BALANCES

	1977	1976
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 68,291	$ 49,097
Due to plant funds	417,178	293,468
Due to Banbury Educational Center	—	42,951
Fund balance	199,544	178,695
Total unrestricted	685,013	564,211
Restricted		
Due to unrestricted funds	135,569	233,382
Fund balance	2,260,649	495,592
Total restricted	2,396,218	728,974
Total current funds	3,081,231	1,293,185
ENDOWMENT FUNDS		

Total Olney Memorial Fund	22,060	18,728			
Total endowment funds	7,978,156	7,650,278	Fund balance	7,978,156	7,650,278
PLANT FUNDS			**PLANT FUNDS**		
Investment	84,508	85,037			
Due from unrestricted fund	270,362	293,468			
Land and improvements	719,545	595,954			
Buildings	5,171,784	2,315,592			
Furniture, fixtures, and equipment	792,690	614,980			
Books and periodicals	365,630	365,630			
Construction in progress	24,136	17,211			
	7,428,655	4,287,872			
Less allowance for depreciation and amortization	1,143,102	867,187			
Total plant funds	6,285,553	3,420,685	Fund balance	6,285,553	3,420,685
BANBURY EDUCATIONAL CENTER			**BANBURY EDUCATIONAL CENTER**		
Cash	4,178	59,424			
Accrued interest	18,181	23,790			
Marketable securities (quoted market 1977—$1,530,097; 1976—$1,510,003)	1,515,548	1,480,814			
Due from unrestricted fund	—	42,951	Fund balance		
Total operating funds	1,537,907	1,606,979	Operating	1,537,907	1,606,979
Land	772,500	772,500			
Buildings	384,234	245,000			
Furniture, fixtures, and equipment	147,887	113,343			
Construction in progress	—	3,954			
	1,304,621	1,134,797			
Less allowance for depreciation	45,211	14,221			
Total plant funds	1,259,410	1,120,576	Plant	1,259,410	1,120,576
Total Banbury Educational Center	2,797,317	2,727,555	**Total Banbury Educational Center**	2,797,317	2,727,555
Total—All funds	$20,142,257	$15,091,703	**Total—All funds**	$20,142,257	$15,091,703

CURRENT REVENUES, EXPENDITURES, AND TRANSFERS
year ended October 31, 1977
with comparative figures for 1976

COLD SPRING HARBOR LABORATORY

	1977	1976
REVENUES		
Grants	$2,133,568	$1,918,414
Indirect cost allowance on grants	957,464	783,683
Contributions		
Unrestricted	37,529	170,799
Restricted	25,000	—
Long Island Biological Association	194,000	—
Robertson Research Fund contribution	325,000	300,000
Summer programs	183,744	175,658
Laboratory rental	20,554	19,754
Marina rental	38,000	35,430
Investment income	10,430	10,363
Publications sales	383,196	289,698
Dining hall	238,300	220,783
Rooms and apartments	151,601	143,823
Other sources	8,234	11,762
Total revenues	4,706,620	4,080,167
EXPENDITURES		
Research*	2,014,119	1,722,024
Summer programs*	342,544	371,888
Library	100,927	82,927
Operation and maintenance of plant	638,316	626,047
General and administrative	497,863	446,570
Publications sales*	303,829	225,863
Dining hall*	211,647	190,354
Total expenditures	4,109,245	3,665,673

BANBURY EDUCATIONAL CENTER

	1977	1976
REVENUES		
Endowment income	$ 107,291	$ —
Contributions (including land, buildings, and furnishings in 1976)	15,000	1,230,843
Rooms and apartments	12,750	11,646
Appropriation from Cold Spring Harbor Laboratory	61,043	—
Total revenues	196,084	1,242,489
EXPENDITURES		
Operation and maintenance of plant	56,665	65,361
General and administrative	12,545	13,301
Capital plant	169,825	—
Total expenditures	239,035	78,662
Excess (deficit) of revenues over expenditures	($42,951)	$1,163,827

TRANSFERS		
Plant funds	515,483	403,464
Banbury Educational Center	61,043	—
Total transfers	576,526	403,464
Total expenditures and transfers	4,685,771	4,069,137
Excess of revenues over expenditures and transters	$ 20,849	$ 11, 030

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

NOTE: Copies of the complete financial statements, prepared by our independent auditors, Peat, Marwick, Mitchell & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

GRANTS

November 1, 1976–December 31, 1977

NEW GRANTS

Grantor	Principal investigator and program	Total award	Duration of grant
National Institutes of Health	Contract—advanced bacterial genetics workshop	$ 35,500	6/29/77– 6/30/78
	Dr. Watson—Cancer Research Center	13,185,000	1/1/77–12/31/81
	Dr. Watson—summer workshops	478,750	4/1/77– 3/31/82
	Dr. Galloway—fellowship	28,000	12/1/76–11/30/78
	Dr. Chow—research	236,000	4/1/77– 3/31/80
	Dr. Bukhari—research	121,000	9/9/77– 8/31/80
	Dr. Broach—research	144,320	8/1/77– 7/31/79
	Dr. Zain—research	35,896	9/30/77– 7/31/79
	Contract—neurobiology training	48,600	5/16/77–10/31/77
	Construction grant—animal facility	1,411,000	9/15/77–
National Science Foundation	Dr. Roberts—research	90,000	4/1/77– 9/30/80
	Dr. Zain—research	65,000	8/15/77– 1/31/80
	Dr. McDougall—research	75,000	8/1/77– 1/31/80
	Dr. Broker—single-stranded DNA phages conference	4,000	8/1/77– 1/31/78
	W. Udry—Symposium support	10,000	6/1/77– 5/31/78
Rita Allen Foundation	Dr. Watson—hematopoiesis meeting	10,000	9/6/77– 9/11/77
American Cancer Society	Dr. Gingeras—fellowship	10,500	1/1/77–12/31/77
	Dr. Lewis—research	45,500	7/1/77– 6/30/78
American Heart Association	Dr. Chen—research	12,000	7/1/77– 6/30/78
Jane Coffin Childs Memorial Fund for Cancer Research	Dr. Gluzman—fellowship	24,000	9/16/77– 9/15/79
Energy Research and Development Administration	W. Udry—Symposium support	8,000	5/1/77– 4/30/78
Hoffman-LaRoche, Inc.	Dr. Watson—hematopoiesis meeting	5,000	9/6/77– 9/11/77
Interboro Leukemia Organization	Dr. Botchan—equipment support	5,500	5/1/77–12/31/77
International Union Against Cancer	Dr. Watson—parvovirus meeting	6,691	5/12/77– 5/15/77
Merck & Co.	Dr. Watson—hematopoiesis meeting	2,000	9/6/77– 9/11/77
Muscular Dystrophy Association	Dr. Burridge—research	18,900	7/1/77– 6/30/78
	Dr. Chen—research	20,000	7/1/77– 6/30/78
	Dr. Gordon—fellowship	13,500	1/1/77–12/31/77
	W. Udry—synaptic transmission workshop	5,000	6/19/77– 7/10/77
Damon Runyon-Walter Winchell Cancer Fund	Dr. Sciaky—fellowship	14,490	3/1/77– 2/28/78
Helen Hay Whitney Foundation	Dr. Chen—fellowship	12,250	1/1/77–12/31/77

CONTINUING GRANTS

Grantor	Principal investigator and program	Total award	Duration of grant
National Institutes of Health	Dr. Watson—general research support	$ 46,495	4/1/76– 3/31/77
	Contract—advanced bacterial genetics workshop	25,325	7/1/76– 6/30/77
	Dr. Watson—herpes virus workshop	22,000	6/30/76– 6/29/77
	Dr. Watson—conference on origins of human cancer	60,000	6/30/76– 6/29/77
	Dr. Maniatis—research subcontract from Harvard	26,850	7/1/76–12/31/76
	Dr. Bukhari—career development	150,000	5/1/75– 4/30/80
	Dr. Hassell—fellowship	26,000	10/1/75– 9/30/77
	Dr. Watson—Cancer Research Center	7,789,000	1/1/72–12/31/76
	Dr. Watson—Symposium support	96,000	4/1/74– 3/31/79
	Dr. Zipser—research	356,000	5/1/74– 4/30/79
	Dr. Gesteland—summer workshops	190,620	4/1/74– 3/31/77
	Dr. Watson—neurobiology training	152,172	5/1/74– 4/30/77
National Science Foundation	Dr. Bukhari—research	120,000	8/1/76– 1/31/79
	Dr. Albrecht-Buehler—research	10,000	6/1/76– 1/30/77
	Dr. Bukhari—conference on DNA insertions	3,000	3/15/76– 2/28/77
	Dr. Zain—research	12,100	3/1/76– 5/31/77
	Dr. Watson—herpes virus workshop	5,000	5/15/76– 4/30/77
	Dr. Albrecht-Buehler—research	76,300	7/1/75–12/31/77
	Dr. Gesteland—research	135,000	1/1/75– 7/31/78
	Dr. Roberts—research	50,000	6/15/74–11/30/76
	Dr. Zipser—research	160,000	1/1/74– 8/31/78
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Volkswagen Foundation	Dr. Watson—training support	73,085	1/1/71–12/31/76

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

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

Methods of contributing to Cold Spring Harbor Laboratory

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities

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

(2) If you wish to send stock directly to the Laboratory, either (a) endorse the certificate(s) by signing your name on the back, leave the space for the transferee's name blank, have your signature guaranteed on the certificate(s) by your bank or broker, and send the certificate(s) by *registered mail* to the Laboratory, or (b) send unsigned certificate(s) with a covering letter and send under separate cover a stock power executed in blank, with signature guarantee, for each certificate, and also a copy of the covering letter (use first-class mail). Depreciated securities should be sold to establish a tax loss, then the contribution to the Laboratory should be made by check.

Bequests

Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as a beneficiary ensures that a bequest will be utilized as specified for continuing good.

Appreciated real estate or personal property

Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Conversion of private foundation to "public" status on termination

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

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

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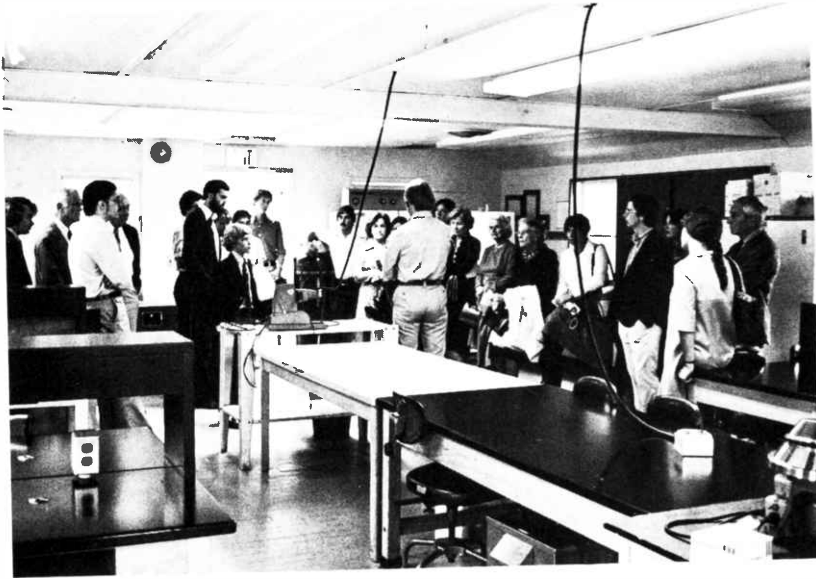

LIBA tour of laboratory grounds

The Laboratory was founded in 1890 by several local philanthropists and the Brooklyn Institute of Arts and Sciences. The first chairman of the Board of Managers of the Laboratory was Eugene G. Blackford, who served from 1890 until his death in 1904. William J. Matheson succeeded him, serving until 1923.

In that year, when the Brooklyn Institute of Arts and Sciences withdrew from Cold Spring Harbor, the local supporters of the research formalized their efforts by incorporating as the Long Island Biological Association. Colonel T.S. Williams became the first Chairman of the new group. Jointly with the Carnegie Institution of Washington, LIBA continued to support and direct the research at Cold Spring Harbor Laboratory.

In 1962 the Laboratory was reorganized as an operating organization and LIBA relinquished its management responsibilities. During the past 15 years, LIBA's chief function has been to widen the interest of the community in the Laboratory and to help support it financially.

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